Handbook of Oncology and Immunology

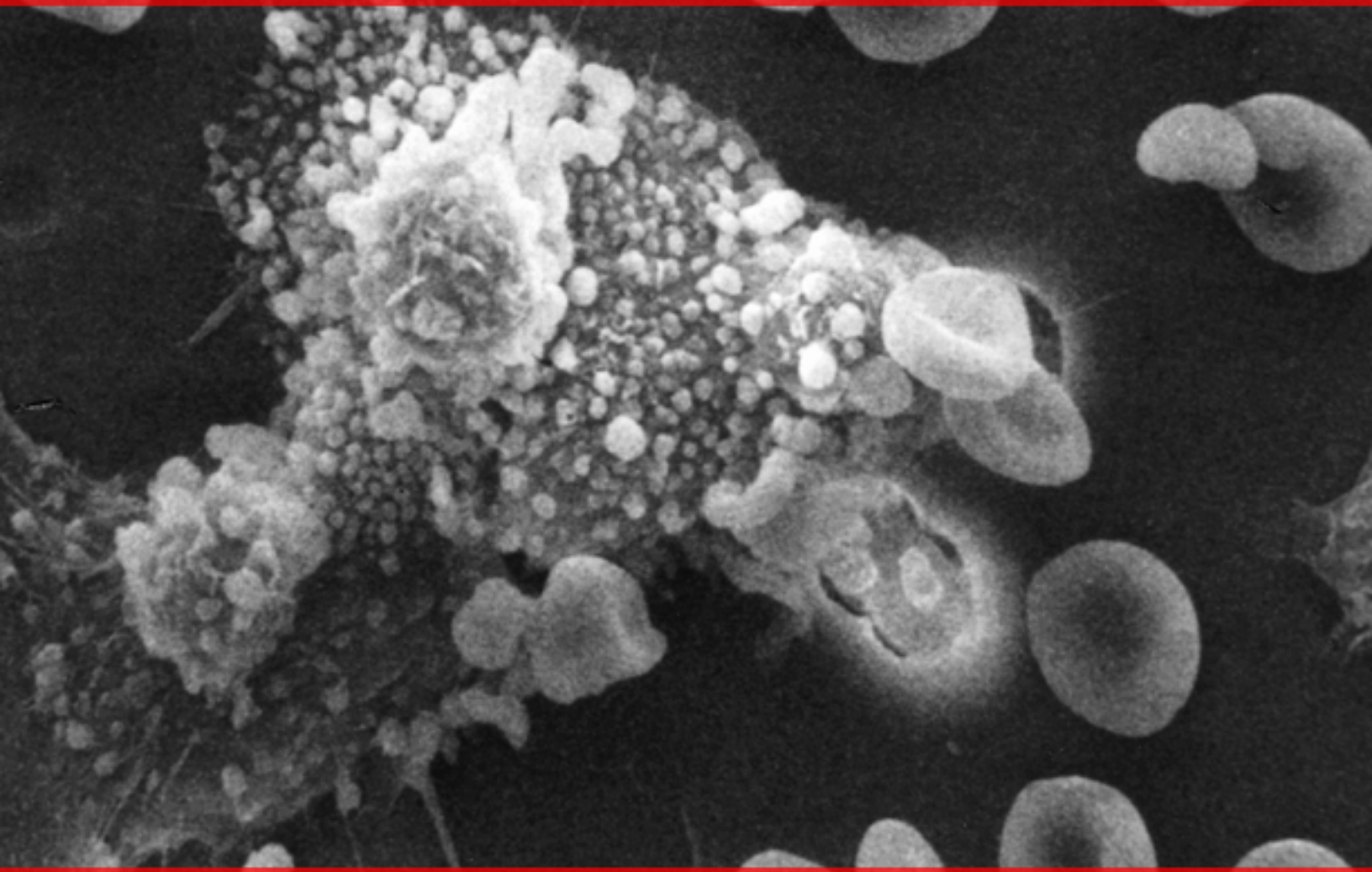

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Table of Contents

Chapter 1 - Oncology

Chapter 2 - Radiation Therapy

Chapter 3 - Surgical Oncology and Gynecologic Oncology

Chapter 4 - Photodynamic Therapy

Chapter 5 - Chemotherapy

Chapter 6 - Post-Chemotherapy Cognitive Impairment

Chapter 7 - Proton Therapy

Chapter 8 - Cancer Immunotherapy

Chapter 9 - Hyperthermia Therapy

Chapter 10 - Adjuvant Cancer Therapy

Chapter 11 - Alternative Cancer Treatments

Chapter 12 - Experimental Cancer Treatment

Chapter 13 - Cholangiocarcinoma

Chapter 14 - Immunology

Chapter 15 - Immunotherapy

Chapter 16 - Reproductive Immunology and Immune Tolerance in Pregnancy

Chapter 17 - Allergen Immunotherapy

Chapter 18 - Allergen

Chapter 19 - Antimicrobial Peptides

Chapter 20 - Artificial Induction of Immunity

Chapter 21 - Immune System

Chapter 22 - Immunologic Adjuvant

Chapter 23 - ELISA

Chapter 24 - Cancer Immunology and Cancer Immunoprevention

Chapter 25 - Coombs Test

Chapter 26 - Autoimmunity

Chapter 1

Oncology

Oncologist	
Occupation	
Names	Doctor, Medical Specialist
Type	Specialty
Activity sectors	Medicine
Description	
	Doctor of Medicine
Education required	Medical residency Fellowship (medicine)
Fields of employment	Hospitals, Clinics

Oncology (from the Ancient Greek *onkos* (ὄγκος), meaning bulk, mass, or tumor, and the suffix *-logy* (-λογία), meaning "study of") is a branch of medicine that deals with tumors (cancer). A medical professional who practices oncology is an *oncologist*.

Oncology is concerned with:

- The diagnosis of any cancer in a person
- Therapy (e.g., surgery, chemotherapy, radiotherapy and other modalities)
- Follow-up of cancer patients after successful treatment
- Palliative care of patients with terminal malignancies
- Ethical questions surrounding cancer care
- Screening efforts:
    - of populations, or
    - of the relatives of patients (in types of cancer that are thought to have a hereditary basis, such as breast cancer)

Diagnosis

The most important diagnostic tool remains the medical history: the character of the complaints and any specific symptoms (fatigue, weight loss, unexplained anemia, fever of unknown origin, paraneoplastic phenomena and other signs). Often a physical examination will reveal the location of a malignancy.

Diagnostic methods include:

- Biopsy, either incisional or excisional;
- Endoscopy, either upper or lower gastrointestinal, bronchoscopy, or nasendoscopy;
- X-rays, CT scanning, MRI scanning, ultrasound and other radiological techniques;
- Scintigraphy, Single Photon Emission Computed Tomography, Positron emission tomography and other methods of nuclear medicine;
- Blood tests, including Tumor markers, which can increase the suspicion of certain types of tumors or even be pathognomonic of a particular disease.

Apart from in diagnosis, these modalities (especially imaging by CT scanning) are often used to determine operability, i.e. whether it is surgically possible to remove a tumor in its entirety.

Generally, a "tissue diagnosis" (from a biopsy) is considered essential for the proper identification of cancer. When this is not possible, "empirical therapy" (without an exact diagnosis) may be given, based on the available evidence (e.g. history, x-rays and scans.)

Occasionally, a metastatic lump or pathological lymph node is found (typically in the neck) for which a primary tumor cannot be found. This situation is referred to as "carcinoma of unknown primary", and again, treatment is empirical based on past experience of the most likely origin.

Therapy

It completely depends on the nature of the tumor identified what kind of therapeutical intervention will be necessary. Certain disorders will require immediate admission and chemotherapy (such as ALL or AML), while others will be followed up with regular physical examination and blood tests.

Often, surgery is attempted to remove a tumor entirely. This is only feasible when there is some degree of certainty that the tumor can in fact be removed. When it is certain that parts will remain, curative surgery is often impossible, e.g. when there are metastases elsewhere, or when the tumor has invaded a structure that cannot be operated upon without risking the patient's life. Occasionally surgery can improve survival even if not all tumour tissue has been removed; the procedure is referred to as "debulking" (i.e. reducing the overall amount of tumour tissue). Surgery is also used for the palliative

treatment of some of cancers, e.g. to relieve biliary obstruction, or to relieve the problems associated with some cerebral tumors. The risks of surgery must be weighed up against the benefits.

Chemotherapy and radiotherapy are used as a first-line radical therapy in a number of malignancies. They are also used for adjuvant therapy, i.e. when the macroscopic tumor has already been completely removed surgically but there is a reasonable statistical risk that it will recur. Chemotherapy and radiotherapy are commonly used for palliation, where disease is clearly incurable: in this situation the aim is to improve the quality of and prolong life.

Hormone manipulation is well established, particularly in the treatment of breast and prostate cancer.

There is currently a rapid expansion in the use of monoclonal antibody treatments, notably for lymphoma (Rituximab), and breast cancer (Trastuzumab).

Vaccine and other immunotherapies are the subject of intensive research.

Palliative care

Approximately 50% of all cancer cases in the Western world can be treated to remission with radical treatment. For paediatric patients, that number is much higher. A large number of cancer patients will die from the disease, and a significant proportion of patients with incurable cancer will die of other causes. There may be ongoing issues with symptom control associated with progressive cancer, and also with the treatment of the disease. These problems may include pain, nausea, anorexia, fatigue, immobility, and depression. Not all issues are strictly physical: personal dignity may be affected. Moral and spiritual issues are also important.

While many of these problems fall within the remit of the oncologist, palliative care has matured into a separate, closely allied speciality to address the problems associated with advanced disease. Palliative care is an essential part of the multidisciplinary cancer care team. Palliative care services may be less hospital-based than oncology, with nurses and doctors who are able to visit the patient at home.

Ethical issues

There are a number of recurring *ethical questions* and *dilemmas* in oncological practice. These include:

- What information to give the patient regarding disease extent/progression/prognosis.
- Entry into clinical trials, especially in the face of terminal illness.
- Withdrawal of active treatment.
- "Do Not Resuscitate" orders and other end of life issues.

These issues are closely related to the patients' personality, religion, culture, personal, and family life. The answers are rarely black and white. It requires a degree of sensitivity and very good communication on the part of the oncology team to address these problems properly.

Progress and research

There is a tremendous amount of research being conducted on all frontiers of oncology, ranging from cancer cell biology to chemotherapy treatment regimens and optimal palliative care and pain relief. This makes oncology a continuously changing field.

Therapeutic trials often involve patients from many different hospitals in a particular region. In the UK, patients are often enrolled in large studies coordinated by Cancer Research UK (CRUK), Medical Research Council (MRC), the European Organisation for Research and Treatment of Cancer (EORTC) or the National Cancer Research Network (NCRN).

Specialties

There are several sub-specialties within oncology. Moreover, oncologists often develop an interest and expertise in the management of particular types of cancer.

Oncologists may be divided on the basis of the type of treatment provided.

- Radiation oncology: treatment primarily with radiation, a process called radiotherapy.
- Surgical oncology: surgeons who specialize in tumor removal.
- Medical oncology: treatment primarily with drugs, e.g. chemotherapy
- Interventional oncology: interventional radiologists who specialize in minimally invasive image guided tumor therapies.
- Gynecologic oncology: focuses on cancers of the female reproductive system.
- Pediatric oncology: concerned with the diagnosis and treatment of cancer in children

In the United Kingdom and several other countries, oncologists may be either *clinical* or *medical oncologists*. The main difference is that clinical oncologists deliver radiotherapy, while medical oncologists do not. (This difference does not apply in North America: the terms, *clinical oncologist* and *medical oncologist* are used interchangeably.)

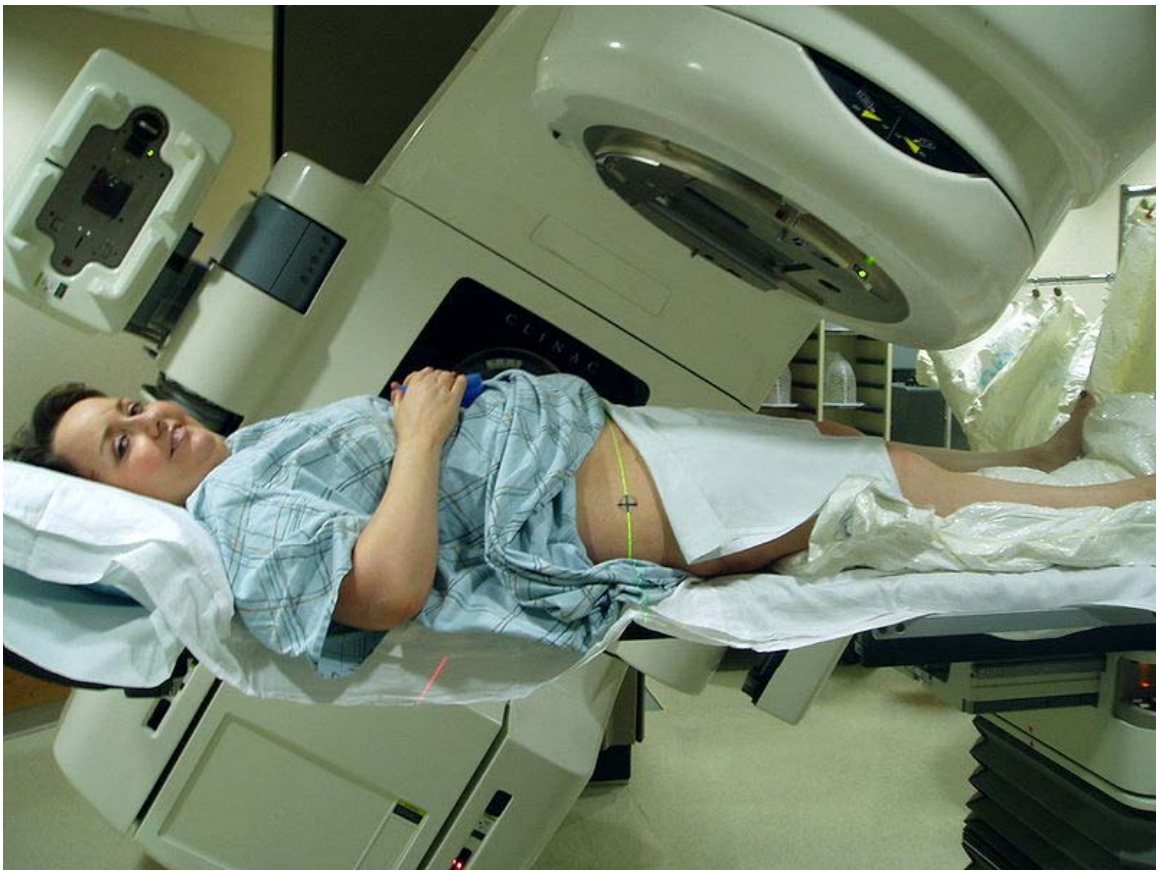
In most countries it is now common that patients are treated by a multidisciplinary team. These teams will meet on regular basis and discuss the patients under their care. These teams consist of the medical oncologist, a clinical oncologist or radiotherapist, a surgeon (sometimes there is a second reconstructive surgeon), a radiologist, a pathologist, an organ specific specialist such as a gynecologist or dermatologist, and sometimes the general practitioner is also involved. These disease oriented teams are sometimes in conflict with the general organisation and operation in hospitals. Historically hospitals are

organised in an organ or technique specific manner. Multidisciplinary teams operate over these borders and it is sometimes difficult to define who is in charge.

In veterinary medicine, veterinary oncology is the sub-specialty that deals with cancer diagnosis and treatment in animals.

Chapter 2

Radiation Therapy



Radiation therapy of the pelvis. Lasers and a mould under the legs are used to determine exact position.

Axesse Radiotherapy

Radiation therapy (in the USA), **radiation oncology**, or **radiotherapy** (in the UK, Canada and Australia), sometimes abbreviated to XRT, is the medical use of ionizing radiation as part of cancer treatment to control malignant cells (not to be confused with radiology, the use of radiation in medical imaging and diagnosis). Radiotherapy may be used for curative or adjuvant treatment. It is used as palliative treatment (where cure is not possible and the aim is for local disease control or symptomatic relief) or as therapeutic treatment (where the therapy has survival benefit and it can be curative). Total body irradiation (TBI) is a radiotherapy technique used to prepare the body to receive a bone marrow transplant. Radiotherapy has several applications in non-malignant conditions, such as the treatment of trigeminal neuralgia, severe thyroid eye disease, pterygium, pigmented villonodular synovitis, prevention of keloid scar growth, and prevention of heterotopic ossification. The use of radiotherapy in non-malignant conditions is limited partly by worries about the risk of radiation-induced cancers.

Radiotherapy is used for the treatment of malignant cancer, and may be used as a primary or adjuvant modality. It is also common to combine radiotherapy with surgery, chemotherapy, hormone therapy, Immunotherapy or some mixture of the four. Most common cancer types can be treated with radiotherapy in some way. The precise treatment intent (curative, adjuvant, neoadjuvant, therapeutic, or palliative) will depend on the tumor type, location, and stage, as well as the general health of the patient.

Radiation therapy is commonly applied to the cancerous tumor. The radiation fields may also include the draining lymph nodes if they are clinically or radiologically involved with tumor, or if there is thought to be a risk of subclinical malignant spread. It is necessary to include a margin of normal tissue around the tumor to allow for uncertainties in daily set-up and internal tumor motion. These uncertainties can be caused by internal movement (for example, respiration and bladder filling) and movement of external skin marks relative to the tumor position.

To spare normal tissues (such as skin or organs which radiation must pass through in order to treat the tumor), shaped radiation beams are aimed from several angles of exposure to intersect at the tumor, providing a much larger absorbed dose there than in the surrounding, healthy tissue.

Brachytherapy, in which a radiation source is placed inside or next to the area requiring treatment, is another form of radiation therapy that minimizes exposure to healthy tissue during procedures to treat cancers of the breast, prostate and other organs.

Mechanism of action

Radiation therapy works by damaging the DNA of cancerous cells. This DNA damage is caused by one of two types of energy, photon or charged particle. This damage is either direct or indirect ionizing the atoms which make up the DNA chain. Indirect ionization happens as a result of the ionization of water, forming free radicals, notably hydroxyl radicals, which then damage the DNA. In the older, most common form of radiation therapy, Intensity-modulated radiotherapy (IMRT) (photons), most of the radiation effect is through free radicals. Because cells have mechanisms for repairing single-strand DNA damage, double-stranded DNA breaks prove to be the most significant technique to cause cell death. Cancer cells generally are undifferentiated and stem cell-like, they reproduce more, and have a diminished ability to repair sub-lethal damage compared to most healthy differentiated cells. This single-strand DNA damage is then passed on through cell division, accumulating damage to the cancer cell's DNA, causing them to die or reproduce more slowly.

One of the major limitations of photon radiotherapy is that the cells of solid tumors become deficient in oxygen. Solid tumors can outgrow their blood supply, causing a low-oxygen state known as hypoxia. Oxygen is a potent radiosensitizer, increasing the effectiveness of a given dose of radiation by forming DNA-damaging free radicals. Tumor cells in a hypoxic environment may be as much as 2 to 3 times more resistant to radiation damage than those in a normal oxygen environment. Much research has been

devoted to overcoming hypoxia including the use of high pressure oxygen tanks, blood substitutes that carry increased oxygen, hypoxic cell radiosensitizer drug's such as misonidazole and metronidazole, and hypoxic cytotoxins (tissue poisons), such as tirapazamine.

Direct damage to cancer cell DNA occurs through high-LET (linear energy transfer) charged particles such as proton, boron, carbon or neon ions which have an antitumor effect which is independent of tumor oxygen supply because these particles act mostly via direct energy transfer usually causing double-stranded DNA breaks. Due to their relatively large mass, protons and other charged particles have little lateral side scatter in the tissue; the beam does not broaden much, stays focused on the tumor shape and delivers small dose side-effects to surrounding tissue. They also more precisely target the tumor using the Bragg peak effect. The cyclotron's, dielectric wall accelerator (DWA), or Still River Systems's super conducting high field magnet (two new compact protron replacements) provide the energy source for charged particle therapy. These particles can be charged to different amounts to provide the desired tissue penetration. This procedure avoids healthy tissue because it releases its energy at the last few milimeters calibrated to be at the target tumor and stops. Because IMRT has little mass it cannot be controlled to as fine a degree as charged particles and is still damaging healthy cells when it exits the body. This is critically important in almost all cases where the close proximity of other organs makes any stray ionization very damaging example: (head and neck cancers). This damage causes secondary inducted cancers. This x-ray exposure is especially bad for children, due to their growing bodies. They have a 30% chance of a second malignancy after 5 years post initial RT.

Dose

The amount of radiation used in photon radiation therapy is measured in gray (Gy), and varies depending on the type and stage of cancer being treated. For curative cases, the typical dose for a solid epithelial tumor ranges from 60 to 80 Gy, while lymphomas are treated with 20 to 40 Gy.

Preventative (adjuvant) doses are typically around 45 - 60 Gy in 1.8 - 2 Gy fractions (for Breast, Head, and Neck cancers.) Many other factors are considered by radiation oncologists when selecting a dose, including whether the patient is receiving chemotherapy, patient comorbidities, whether radiation therapy is being administered before or after surgery, and the degree of success of surgery.

Delivery parameters of a prescribed dose are determined during treatment planning (part of dosimetry). Treatment planning is generally performed on dedicated computers using specialized treatment planning software. Depending on the radiation delivery method, several angles or sources may be used to sum to the total necessary dose. The planner will try to design a plan that delivers a uniform prescription dose to the tumor and minimizes dose to surrounding healthy tissues.

Fractionation

The total dose is fractionated (spread out over time) for several important reasons. Fractionation allows normal cells time to recover, while tumor cells are generally less efficient in repair between fractions. Fractionation also allows tumor cells that were in a relatively radio-resistant phase of the cell cycle during one treatment to cycle into a sensitive phase of the cycle before the next fraction is given. Similarly, tumor cells that were chronically or acutely hypoxic (and therefore more radioresistant) may reoxygenate between fractions, improving the tumor cell kill. Fractionation regimes are individualised between different radiotherapy centres and even between individual doctors. In North America, Australia, and Europe, the typical fractionation schedule for adults is 1.8 to 2 Gy per day, five days a week. In some cancer types, prolongation of the fraction schedule over too long can allow for the tumor to begin repopulating, and for these tumor types, including head-and-neck and cervical squamous cell cancers, radiation treatment is preferably completed within a certain amount of time. For children, a typical fraction size may be 1.5 to 1.8 Gy per day, as smaller fraction sizes are associated with reduced incidence and severity of late-onset side effects in normal tissues.

In some cases, two fractions per day are used near the end of a course of treatment. This schedule, known as a concomitant boost regimen or hyperfractionation, is used on tumors that regenerate more quickly when they are smaller. In particular, tumors in the head-and-neck demonstrate this behavior.

One of the best-known alternative fractionation schedules is Continuous Hyperfractionated Accelerated Radiotherapy (CHART). CHART, used to treat lung cancer, consists of three smaller fractions per day. Although reasonably successful, CHART can be a strain on radiation therapy departments.

Another increasingly well-known alternative fractionation schedule, used to treat breast cancer, is called Accelerated Partial Breast Irradiation (APBI). APBI can be performed with either brachytherapy or with external beam radiation. APBI normally involves two high-dose fractions per day for five days, compared to whole breast irradiation, in which a single, smaller fraction is given five times a week over a six-to-seven-week period.

Implants can be fractionated over minutes or hours, or they can be permanent seeds which slowly deliver radiation until they become inactive.

Effect on different types of cancer

Different cancers respond differently to radiation therapy.

The response of a cancer to radiation is described by its radiosensitivity. Highly radiosensitive cancer cells are rapidly killed by modest doses of radiation. These include leukemias, most lymphomas and germ cell tumors. The majority of epithelial cancers are only moderately radiosensitive, and require a significantly higher dose of radiation (60-70Gy) to achieve a radical cure. Some types of cancer are notably radioresistant, that is,

much higher doses are required to produce a radical cure than may be safe in clinical practice. Renal cell cancer and melanoma are generally considered to be radioresistant.

It is important to distinguish the radiosensitivity of a particular tumor, which to some extent is a laboratory measure, from the radiation "curability" of a cancer in actual clinical practice. For example, leukemias are not generally curable with radiotherapy, because they are disseminated through the body. Lymphoma may be radically curable if it is localised to one area of the body. Similarly, many of the common, moderately radioresponsive tumors are routinely treated with curative doses of radiotherapy if they are at an early stage. For example: non-melanoma skin cancer, head and neck cancer, breast cancer, non-small cell lung cancer, cervical cancer, anal cancer, prostate cancer. Metastatic cancers are generally incurable with radiotherapy because it is not possible to treat the whole body.

Before treatment, a CT scan is often performed to identify the tumor and surrounding normal structures. The patient is then sent for a simulation so that molds can be created to be used during treatment. The patient receives small skin marks to guide the placement of treatment fields.

The response of a tumor to radiotherapy is also related to its size. For complex reasons, very large tumors respond less well to radiation than smaller tumors or microscopic disease. Various strategies are used to overcome this effect. The most common technique is surgical resection prior to radiotherapy. This is most commonly seen in the treatment of breast cancer with wide local excision or mastectomy followed by adjuvant radiotherapy such as brachytherapy. Another method is to shrink the tumor with neoadjuvant chemotherapy prior to radical radiotherapy. A third technique is to enhance the radiosensitivity of the cancer by giving certain drugs during a course of radiotherapy. Examples of radiosensiting drugs include: Cisplatin, Nimorazole, and Cetuximab.

History of radiation therapy

Radiation therapy has been in use as a cancer treatment for more than 100 years, with its earliest roots traced from the discovery of x-rays in 1895 by Wilhelm Röntgen.

The field of radiation therapy began to grow in the early 1900s largely due to the groundbreaking work of Nobel Prize-winning scientist Marie Curie, who discovered the radioactive elements polonium and radium. This began a new era in medical treatment and research. Radium was used in various forms until the mid-1900s when cobalt and caesium units came into use. Medical linear accelerators have been used too as sources of radiation since the late 1940s.

With Godfrey Hounsfield’s invention of computed tomography (CT) in 1971, three-dimensional planning became a possibility and created a shift from 2-D to 3-D radiation delivery; CT-based planning allows physicians to more accurately determine the dose distribution using axial tomographic images of the patient's anatomy. Orthovoltage and

cobalt units have largely been replaced by megavoltage linear accelerators, useful for their penetrating energies and lack of physical radiation source.

The advent of new imaging technologies, including magnetic resonance imaging (MRI) in the 1970s and positron emission tomography (PET) in the 1980s, has moved radiation therapy from 3-D conformal to intensity-modulated radiation therapy (IMRT) and image-guided radiation therapy (IGRT) Tomotherapy. These advances allowed radiation oncologists to better see and target tumors, which have resulted in better treatment outcomes, more organ preservation and fewer side effects.

Types of radiation therapy

Historically, the three main divisions of radiotherapy are external beam radiotherapy (EBRT or XRT) or teletherapy, brachytherapy or sealed source radiotherapy, and systemic radioisotope therapy or unsealed source radiotherapy. The differences relate to the position of the radiation source; external is outside the body, brachytherapy uses sealed radioactive sources placed precisely in the area under treatment, and systemic radioisotopes are given by infusion or oral ingestion. Brachytherapy can use temporary or permanent placement of radioactive sources. The temporary sources are usually placed by a technique called afterloading. In afterloading a hollow tube or applicator is placed surgically in the organ to be treated, and the sources are loaded into the applicator after the applicator is implanted. This minimizes radiation exposure to health care personnel. Particle therapy is a special case of external beam radiotherapy where the particles are protons or heavier ions. Intraoperative radiotherapy or IORT is a special type of radiotherapy that is delivered immediately after surgical removal of the cancer. This method has been employed in breast cancer (TARGeted Introperative radioTherapy or TARGIT), brain tumors and rectal cancers.

External beam radiotherapy

The following three sections refer to treatment using x-rays.

Conventional external beam radiotherapy

Conventional external beam radiotherapy (2DXRT) is delivered via two-dimensional beams using linear accelerator machines. 2DXRT mainly consists of a single beam of radiation delivered to the patient from several directions: often front or back, and both sides. *Conventional* refers to the way the treatment is *planned* or *simulated* on a specially calibrated diagnostic x-ray machine known as a simulator because it recreates the linear accelerator actions (or sometimes by eye), and to the usually well-established arrangements of the radiation beams to achieve a desired *plan*. The aim of simulation is to accurately target or localize the volume which is to be treated. This technique is well established and is generally quick and reliable. The worry is that some high-dose treatments may be limited by the radiation toxicity capacity of healthy tissues which lay close to the target tumor volume. An example of this problem is seen in radiation of the prostate gland, where the sensitivity of the adjacent rectum limited the dose which could

be safely prescribed using 2DXRT planning to such an extent that tumor control may not be easily achievable. Prior to the invention of the CT, physicians and physicists had limited knowledge about the true radiation dosage delivered to both cancerous and healthy tissue. For this reason, 3-dimensional conformal radiotherapy is becoming the standard treatment for a number of tumor sites.

Stereotactic Radiation

Stereotactic radiation is a specialized type of external beam radiation therapy. It uses focused radiation beams targeting a well-defined tumor using extremely detailed imaging scans. Radiation oncologists perform stereotactic treatments, often with the help of a neurosurgeon for tumors in the brain or spine.

There are two types of stereotactic radiation. **Stereotactic radiosurgery** (SRS) is when doctors use a single or several stereotactic radiation treatments of the brain or spine. **Stereotactic body radiation therapy** (SBRT) refers to one or several stereotactic radiation treatments with the body, such as the lungs.

Some doctors say an advantage to stereotactic treatments are they deliver the right amount of radiation to the cancer in a shorter amount of time than traditional treatments, which can often take six to 11 weeks. Plus treatments are given with extreme accuracy, which should limit the effect of the radiation on healthy tissues. One problem with stereotactic treatments is that they are only suitable for certain small tumors.

Stereotactic treatments can be confusing because many hospitals call the treatments by the name of the manufacturer rather than calling it SRS or SBRT. Brand names for these treatments include Axesse, Cyberknife, Gamma Knife, Novalis, Primatom, Synergy, X-Knife, TomoTherapy and Trilogy. This list changes as equipment manufacturers continue to develop new, specialized technologies to treat cancers.

Virtual simulation, 3-dimensional conformal radiotherapy, and intensity-modulated radiotherapy

The planning of radiotherapy treatment has been revolutionized by the ability to delineate tumors and adjacent normal structures in three dimensions using specialized CT and/or MRI scanners and planning software.

Virtual simulation, the most basic form of planning, allows more accurate placement of radiation beams than is possible using conventional X-rays, where soft-tissue structures are often difficult to assess and normal tissues difficult to protect.

An enhancement of virtual simulation is **3-Dimensional Conformal Radiotherapy (3DCRT)**, in which the profile of each radiation beam is shaped to fit the profile of the target from a beam's eye view (BEV) using a multileaf collimator (MLC) and a variable number of beams. When the treatment volume conforms to the shape of the tumor, the relative toxicity of radiation to the surrounding normal tissues is reduced, allowing a

higher dose of radiation to be delivered to the tumor than conventional techniques would allow.

Intensity-Modulated Radiation Therapy (IMRT) is an advanced type of high-precision radiation that is the next generation of 3DCRT. IMRT also improves the ability to conform the treatment volume to concave tumor shapes, for example when the tumor is wrapped around a vulnerable structure such as the spinal cord or a major organ or blood vessel. Computer-controlled x-ray accelerators distribute precise radiation doses to malignant tumors or specific areas within the tumor. The pattern of radiation delivery is determined using highly tailored computing applications to perform optimization and treatment simulation (Treatment Planning). The radiation dose is consistent with the 3-D shape of the tumor by controlling, or modulating, the radiation beam's intensity. The radiation dose intensity is elevated near the gross tumor volume while radiation among the neighboring normal tissue is decreased or avoided completely. The customized radiation dose is intended to maximize tumor dose while simultaneously protecting the surrounding normal tissue. This may result in better tumor targeting, lessened side effects, and improved treatment outcomes than even 3DCRT.

3DCRT is still used extensively for many body sites but the use of IMRT is growing in more complicated body sites such as CNS, head and neck, prostate, breast and lung. Unfortunately, IMRT is limited by its need for additional time from experienced medical personnel. This is because physicians must manually delineate the tumors one CT image at a time through the entire disease site which can take much longer than 3DCRT preparation. Then, medical physicists and dosimetrists must be engaged to create a viable treatment plan. Also, the IMRT technology has only been used commercially since the late 1990s even at the most advanced cancer centers, so radiation oncologists who did not learn it as part of their residency program must find additional sources of education before implementing IMRT.

Proof of improved survival benefit from either of these two techniques over conventional radiotherapy (2DXRT) is growing for many tumor sites, but the ability to reduce toxicity is generally accepted. Both techniques enable dose escalation, potentially increasing usefulness. There has been some concern, particularly with 3DCRT, about increased exposure of normal tissue to radiation and the consequent potential for secondary malignancy. Overconfidence in the accuracy of imaging may increase the chance of missing lesions that are invisible on the planning scans (and therefore not included in the treatment plan) or that move between or during a treatment (for example, due to respiration or inadequate patient immobilization). New techniques are being developed to better control this uncertainty—for example, real-time imaging combined with real-time adjustment of the therapeutic beams. This new technology is called image-guided radiation therapy (IGRT) or four-dimensional radiotherapy.

Particle Therapy

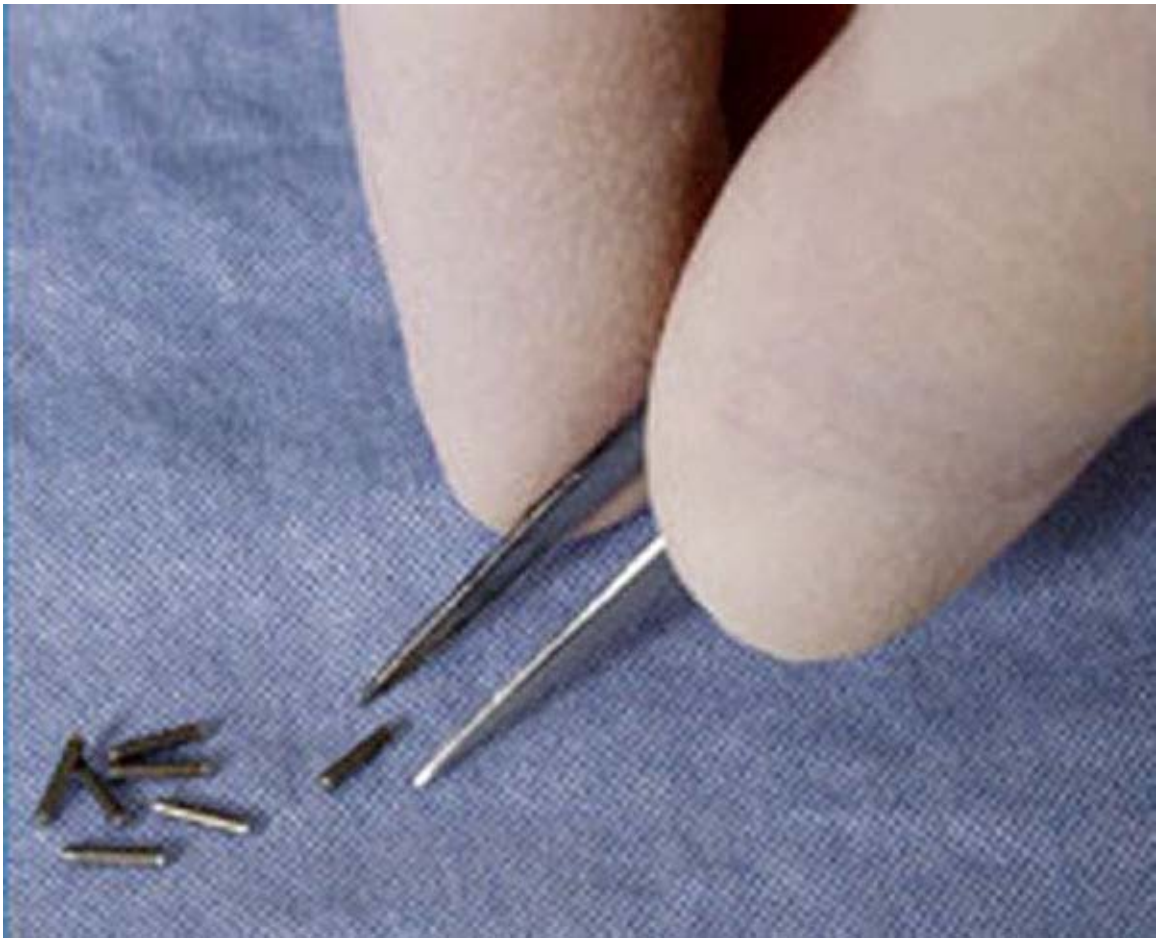
In particle therapy (Proton therapy), energetic ionizing particles (protons or carbon ions) are directed at the target tumor. The dose increases while the particle penetrates the

tissue, up to a maximum (the Bragg peak) that occurs near the end of the particle's range, and it then drops to (almost) zero. The advantage of this energy deposition profile is that less energy is deposited into the healthy tissue surrounding the target tissue.

Brachytherapy

Researcher Cate Yashar, M.D. With SAVI brachytherapy device.

Brachytherapy (internal radiotherapy) is delivered by placing radiation source(s) inside or next to the area requiring treatment. Brachytherapy is commonly used as an effective treatment for cervical, prostate, breast, and skin cancer and can also be used to treat tumours in many other body sites. As with stereotactic radiation, brachytherapy treatments are often known by their brand names. For example, brand names for breast cancer brachytherapy treatments include SAVI, MammoSite, and Contura. Brand names for prostate cancer include Proxcelan, TheraSeed, and I-Seed.



Brachytherapy seeds used to treat prostate cancer

In brachytherapy, radiation sources are precisely placed directly at the site of the cancerous tumour. This means that the irradiation only affects a very localized area – exposure to radiation of healthy tissues further away from the sources is reduced. These characteristics of brachytherapy provide advantages over external beam radiotherapy - the tumour can be treated with very high doses of localized radiation, whilst reducing the probability of unnecessary damage to surrounding healthy tissues. A course of brachytherapy can often be completed in less time than other radiotherapy techniques. This can help reduce the chance of surviving cancer cells dividing and growing in the intervals between each radiotherapy dose.

As one example of the localized nature of breast brachytherapy, the SAVI device delivers the radiation dose through multiple catheters, each of which can be individually controlled. This approach decreases the exposure of healthy tissue and resulting side effects, compared both to external beam radiotherapy and older methods of breast brachytherapy.

Radioisotope Therapy (RIT)

Systemic radioisotope therapy is a form of targeted therapy. Targeting can be due to the chemical properties of the isotope such as radioiodine which is specifically absorbed by the thyroid gland a thousandfold better than other bodily organs. Targeting can also be achieved by attaching the radioisotope to another molecule or antibody to guide it to the target tissue. The radioisotopes are delivered through infusion (into the bloodstream) or ingestion. Examples are the infusion of metaiodobenzylguanidine (MIBG) to treat neuroblastoma, of oral iodine-131 to treat thyroid cancer or thyrotoxicosis, and of hormone-bound lutetium-177 and yttrium-90 to treat neuroendocrine tumors (peptide receptor radionuclide therapy). Another example is the injection of radioactive glass or resin microspheres into the hepatic artery to radioembolize liver tumors or liver metastases.

A major use of systemic radioisotope therapy is in the treatment of bone metastasis from cancer. The radioisotopes travel selectively to areas of damaged bone, and spare normal undamaged bone. Isotopes commonly used in the treatment of bone metastasis are strontium-89 and samarium (^{153}Sm) lexidronam.

In 2002, the United States Food and Drug Administration (FDA) approved ibritumomab tiuxetan (Zevalin), which is an anti-CD20 monoclonal antibody conjugated to yttrium-90. In 2003, the FDA approved the tositumomab/iodine (^{131}I) tositumomab regimen (Bexxar), which is a combination of an iodine-131 labelled and an unlabelled anti-CD20 monoclonal antibody. These medications were the first agents of what is known as radioimmunotherapy, and they were approved for the treatment of refractory non-Hodgkins lymphoma.

Side effects

Radiation therapy is in itself painless. Many low-dose palliative treatments (for example, radiotherapy to bony metastases) cause minimal or no side effects, although short-term pain flare up can be experienced in the days following treatment due to oedema compressing nerves in the treated area. Treatment to higher doses causes varying side effects during treatment (acute side effects), in the months or years following treatment (long-term side effects), or after re-treatment (cumulative side effects). The nature, severity, and longevity of side effects depends on the organs that receive the radiation, the treatment itself (type of radiation, dose, fractionation, concurrent chemotherapy), and the patient.

Most side effects are predictable and expected. Side effects from radiation are usually limited to the area of the patient's body that is under treatment. One of the aims of modern radiotherapy is to reduce side effects to a minimum, and to help the patient to understand and to deal with those side effects which are unavoidable.

The main side effects reported are fatigue and skin irritation, like a mild to moderate sun burn. The fatigue often sets in during the middle of a course of treatment and can last for

weeks after treatment ends. The skin irritation will also go away, but it may not be as elastic as it was before.

Acute side effects

Damage to the epithelial surfaces. Epithelial surfaces may sustain damage from radiation therapy. Depending on the area being treated, this may include the skin, oral mucosa, pharyngeal, bowel mucosa and ureter. The rates of onset of damage and recovery from it depend upon the turnover rate of epithelial cells. Typically the skin starts to become pink and sore several weeks into treatment. The reaction may become more severe during the treatment and for up to about one week following the end of radiotherapy, and the skin may break down. Although this moist desquamation is uncomfortable, recovery is usually quick. Skin reactions tend to be worse in areas where there are natural folds in the skin, such as underneath the female breast, behind the ear, and in the groin.

If the head and neck area is treated, temporary soreness and ulceration commonly occur in the mouth and throat. If severe, this can affect swallowing, and the patient may need painkillers and nutritional support/food supplements. The esophagus can also become sore if it is treated directly, or if, as commonly occurs, it receives a dose of collateral radiation during treatment of lung cancer.

The lower bowel may be treated directly with radiation (treatment of rectal or anal cancer) or be exposed by radiotherapy to other pelvic structures (prostate, bladder, female genital tract). Typical symptoms are soreness, diarrhoea, and nausea.

Swelling (edema or oedema). As part of the general inflammation that occurs, swelling of soft tissues may cause problems during radiotherapy. This is a concern during treatment of brain tumors and brain metastases, especially where there is pre-existing raised intracranial pressure or where the tumor is causing near-total obstruction of a lumen (e.g., trachea or main bronchus). Surgical intervention may be considered prior to treatment with radiation. If surgery is deemed unnecessary or inappropriate, the patient may receive steroids during radiotherapy to reduce swelling.

Infertility. The gonads (ovaries and testicles) are very sensitive to radiation. They may be unable to produce gametes following **direct** exposure to most normal treatment doses of radiation. Treatment planning for all body sites is designed to minimize, if not completely exclude dose to the gonads if they are not the primary area of treatment.

Late side effects

Late side effects occur months to years after treatment and are generally limited to the area that has been treated. They are often due to damage of blood vessels and connective tissue cells. Many late effects are reduced by fractionating treatment into smaller parts.

Fibrosis
: Tissues which have been irradiated tend to become less elastic over time due to a diffuse scarring process.

Epilation (Hair Loss)
: Epilation may occur on any hair bearing skin with doses above 1 Gy. It only occurs within the radiation field/s. Hair loss may be permanent with a single dose of 10 Gy, but if the dose is fractionated permanent hair loss may not occur until dose exceeds 45 Gy.

Dryness
: The salivary glands and tear glands have a radiation tolerance of about 30 Gy in 2 Gy fractions, a dose which is exceeded by most radical head and neck cancer treatments. Dry mouth (xerostomia) and dry eyes (xerophthalmia) can become irritating long-term problems and severely reduce the patient's quality of life. Similarly, sweat glands in treated skin (such as the armpit) tend to stop working, and the naturally moist vaginal mucosa is often dry following pelvic irradiation.

Lymphedema
: Lymphedema, a condition of localized fluid retention and tissue swelling, can result from damage to the lymphatic system sustained during radiotherapy. It is the most commonly reported complication in breast radiotherapy patients.

Cancer
: Radiation is a potential cause of cancer, and secondary malignancies are seen in a very small minority of patients - usually less than 1/1000. It usually occurs 20 - 30 years following treatment, although some haematological malignancies may develop within 5 - 10 years. In the vast majority of cases, this risk is greatly outweighed by the reduction in risk conferred by treating the primary cancer. The cancer occurs within the treated area of the patient.

Heart disease
: Radiation has potentially excess risk of death from heart disease seen after some past breast cancer RT regimens.

Cognitive decline
: In cases of radiation applied to the head radiation therapy may cause cognitive decline.

Radiation Proctitis
: This can involve long-term effects on the rectum including bleeding, diarrhoea and urgency and is associated with radiotherapy to pelvic organs. Pelvic radiotherapy can also cause radiation cystitis when the bladder is affected

Cumulative side effects

Cumulative effects from this process should not be confused with long-term effects—when short-term effects have disappeared and long-term effects are subclinical, reirradiation can still be problematic.

Radiation therapy accidents

There are rigorous procedures in place to minimise the risk of accidental overexposure of radiotherapy to patients. However, mistakes do occasionally occur; for example, the radiation therapy machine Therac-25 was responsible for at least six accidents between 1985 and 1987, where patients were given up to one hundred times the intended dose; two people were killed directly by the radiation overdoses. From 2005 to 2010, a hospital in Missouri overexposed 76 patients (most with brain cancer) during a five-year period because new radiation equipment had been set up incorrectly. Although medical errors are exceptionally rare, radiation oncologists, medical physicists and other members of the radiation therapy treatment team are working diligently to eliminate them. ASTRO has launched a safety initiative called Target Safely that, among other things, aims to record errors nation wide so that doctors can learn from each and every mistake and prevent them from happening. ASTRO also publishes a list of questions for patients to ask their doctors about radiation safety to ensure every treatment is as safe as possible.

Chapter 3

Surgical Oncology and Gynecologic Oncology

Surgical oncology

Surgical oncology is the branch of surgery which focuses on the surgical management of cancer.

The specialty of surgical oncology has evolved in steps similar to medical oncology, which grew out of hematology, and radiation oncology, which grew out of radiology. The Ewing Society known today as the Society of Surgical Oncology was started by surgeons interested in promoting the field of oncology. Though it has not been ratified by a specialty Board certification, the area of expertise is coming to its own by the success of combined treatment with chemotherapy, radiation, and targeted biologic treatments. The proliferation of cancer centers will continue to popularize the field, as will developments in minimally invasive techniques, palliative surgery, and neo-adjuvant treatments.

Debate

Whether surgical oncology constitutes a medical specialty *per se* is the topic of a heated debate. Today, some would agree that it is simply impossible for any one surgeon to be competent in the surgical management of *all* malignant disease. However, there are currently 19 surgical oncology fellowship training programs in the United States that have been approved by the Society of Surgical Oncology. While many general surgeons are actively involved in treating patients with malignant neoplasms, the designation of "surgical oncologist" is generally reserved for those surgeons who have completed one of the approved fellowship programs. However, this is a matter of semantics, as many surgeons who are thoroughly involved in treating cancer patients may consider themselves to be surgical oncologists.

Most often, *surgical oncologist* refers to a general surgical oncologist (cf. General Surgery), but thoracic surgical oncologists, gynecologic oncologists and so forth can all be considered surgeons who specialize in treating cancer patients.

Training

The importance of training surgeons who sub-specialize in cancer surgery lies in evidence, supported by a number of clinical trials, that outcomes in surgical cancer care are positively associated to surgeon volume -- i.e. the more cancer cases a surgeon treats, the more proficient he becomes, and his or her patients experience improved survival rates as a result. This is another controversial point, but it is generally accepted -- even as common sense -- that a surgeon who performs a given operation more often, will achieve superior results when compared with a surgeon who rarely performs the same procedure. This is particularly true of cancer resections such as pancreaticoduodenectomy (Whipple procedure) for pancreatic cancer, and gastrectomy with extended (D2) lymphadenectomy for gastric cancer.

Gynecologic oncology

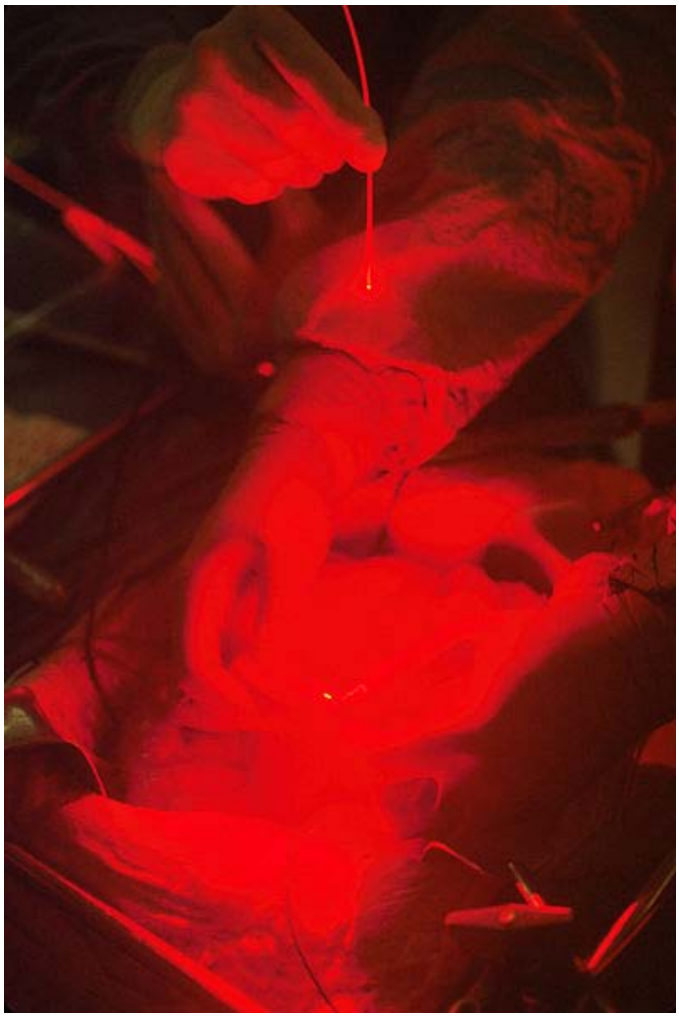
Gynecologic oncology is a specialized field of medicine that focuses on cancers of the female reproductive system, including ovarian cancer, uterine cancer, endometrial cancer, cervical cancer, and vulvar cancer. As specialists, they are generally the most appropriate type of physician to treat these kinds of cancers.

Gynecologic cancer is the fourth most common type of cancer in women, affecting approximately 1 in 20 women. In the United States each year 82,000 women are diagnosed with gynecologic cancer.

The Society of Gynecologic Oncologists and the European Society of Gynaecological Oncology are professional organizations for gynecologic oncologists, and the Gynecologic Oncology Group is a professional organization for gyn-oncs as well as other medical professionals who deal with gynecologic cancers.

Chapter 4

Photodynamic Therapy



Close up of surgeons' hands in an operating room with a "beam of light" traveling along fiber optics for photodynamic therapy. Its source is a laser beam which is split at two different stages to create the proper "therapeutic wavelength". A patient would be given a photo sensitive drug (photofrin) containing cancer killing substances which are absorbed by cancer cells. During the surgery, the light beam is positioned at the tumor site, which then activates the drug that kills the cancer cells, thus photodynamic therapy (PDT).

Photodynamic therapy (PDT), matured as a feasible medical technology in the 1980s at several institutions throughout the world, is used to eradicate premalignant and early-stage cancer and reduce the tumour size in end-stage cancers involving three key components: a photosensitizer, light, and tissue oxygen.

It is an approved treatment for wet macular degeneration, and is also being investigated for treatment of psoriasis.

Treatment of internal organs may be achieved through the use of endoscopes and fiber optic catheters to deliver light, and intravenously-administered photosensitizers.

A great deal of research and clinical study is now underway to determine optimal combinations of photosensitizers, light sources, and treatment parameters for a wide variety of different cancers.

It is currently being tested as a treatment for severe acne.

History

The German physician Friedrich Meyer–Betz performed the first study with what was first called **photoradiation therapy** (PRT) with porphyrins in humans in 1913. Meyer–Betz tested the effects of haematoporphyrin-PRT on his own skin.

Thomas Dougherty of Roswell Park Cancer Center, among others worldwide, became a highly visible advocate and educator. Early patients were treated at Roswell, Los Angeles Children's Hospital, Los Angeles County Hospital, and other clinics and Hospitals in the USA and overseas.

It was John Toth, as product manager for Cooper Medical Devices Corp/Cooper Lasersonics, who acknowledged the "photodynamic chemical effect" of the therapy with early clinical argon dye lasers and wrote the first "white paper" renaming the therapy as "Photodynamic Therapy" (PDT). This was done to support efforts in setting up 10 clinical sites in Japan where the term "radiation" had negative connotations. PDT received even greater interest as result of Thomas Dougherty helping expand clinical trials and forming the International Photodynamic Association, in 1986.

Mechanism of action

A photosensitizer is a chemical compound that can be excited by light of a specific wavelength. This excitation uses visible or near-infrared light. In photodynamic therapy, either a photosensitizer or the metabolic precursor of one is administered to the patient. The tissue to be treated is exposed to light suitable for exciting the photosensitizer. Usually, the photosensitizer is excited from a ground singlet state to an excited singlet state. It then undergoes intersystem crossing to a longer-lived excited triplet state. One of the few chemical species present in tissue with a ground triplet state is molecular oxygen. When the photosensitizer and an oxygen molecule are in proximity, an energy transfer

can take place that allows the photosensitizer to relax to its ground singlet state, and create an excited singlet state oxygen molecule. Singlet oxygen is a very aggressive chemical species and will very rapidly react with any nearby biomolecules. (The specific targets depend heavily on the photosensitizer chosen.) Ultimately, these destructive reactions will kill cells through apoptosis or necrosis.

This mechanism is identical to the mechanism of the disease erythropoietic protoporphyria, which causes blistering in response to sun exposure due to a genetic defect in the same metabolic pathway.

Example treatment of skin cancer

As an example, consider PDT as a treatment for basal cell carcinoma (BCC). BCC is the most common form of skin cancer in humans. Conventional treatment of BCC involves surgical excision, cryogenic treatment with liquid nitrogen, or localized chemotherapy with 5-fluorouracil or other agents.

A PDT treatment would involve the following steps.

- A photosensitizer precursor (aminolevulinic acid (ALA) or methyl aminolevulinate (MAL) or levulinic acid (LA)) is applied.
- A waiting period of a few hours is allowed to elapse, during which time
    - ALA will be taken up by cells, and
    - ALA will be converted by the cells to protoporphyrin IX, a photosensitizer.
- The physician shines a bright red light (from an array of light-emitting diodes or a diode laser) on the area to be treated. The light exposure lasts a few minutes to a few tens of minutes.
    - Protoporphyrin IX absorbs light, exciting it to an excited singlet state;
    - Intersystem crossing occurs, resulting in excited triplet protoporphyrin IX;
    - Energy is transferred from triplet protoporphyrin IX to triplet oxygen, resulting in singlet (ground state) protoporphyrin IX and excited singlet oxygen;
    - Singlet oxygen reacts with biomolecules, fatally damaging some cells in the treatment area.
- Within a few days, the exposed skin and carcinoma will scab over and flake away.
- In a few weeks, the treated area has healed, leaving healthy skin behind. For extensive malignancies, repeat treatments may be required. It is also common to experience pain from the area treated.
- After the treatment the patient will need to avoid excessive exposure to sunlight for a period of time.

Advantages and limitations

Unlike chemotherapy for cancer the effect of PDT can be localised. Specificity of treatment is achieved in three ways.

- First, light is delivered only to tissues that a physician wishes to treat. In the absence of light, there is no activation of the photosensitizer and no cell killing.
- Second, photosensitizers may be administered in ways that restrict their mobility.
- Finally, photosensitizers may be chosen which are selectively absorbed at a greater rate by targeted cells. ALA is taken up much more rapidly by metabolically active cells. Since malignant cells tend to be growing and dividing much more quickly than healthy cells, the ALA targets the unhealthy cells.

PDT can be much cheaper than the alternative radiotherapy or surgical operation and after care. Post operative recovery is typically hours or days rather than weeks.

A major limitation of PDT is that the light needed to activate most photosensitizers can not penetrate through more than one third of an inch (1 cm) of tissue using standard laser technology and low powered LED technology. Laser application of PDT is generally limited to the treatment of tumours on or under the skin, or on the lining of some internal organs. Moreover it is less effective in treatment of large tumours and metastasis for the same reason. However, new high-powered LED technology has been lab-tested to provide a depth of 2 inches from surface in a simulated breast tissue. Also, hollow needles have been used by some units to get the light into deeper tissues.

Photosensitizers

A wide array of photosensitizers for PDT exist. They can be divided into porphyrins, chlorophylls and dyes. Some examples include aminolevulinic acid (ALA), Silicon Phthalocyanine Pc 4, m-tetrahydroxyphenylchlorin (mTHPC), and mono-L-aspartyl chlorin e6 (NPe6).

Several photosensitizers are commercially available for clinical use, such as Photofrin, Visudyne, Levulan, Foscan, Metvix, Hexvix®, Cysview™, and Laserphyrin, with others in development, e.g. Antrin, Photochlor, Photosens, Photrex, Lumacan, Cevira, Visonac, BF-200 ALA. Amphinex.

Although these photosensitizers can be used for wildly different treatments, they all aim to achieve certain characteristics:

- High absorption at long wavelengths
    - Tissue is much more transparent at longer wavelengths (~700-850 nm). Absorbing at longer wavelengths would allow the light to penetrate deeper, and allow the treatment of larger tumors.
- High singlet oxygen quantum yield
- Low photobleaching
- Natural fluorescence
    - Many optical dosimetry techniques, such as fluorescence spectroscopy, depend on the drug being naturally fluorescent
- High chemical stability
- Low dark toxicity

    - The photosensitizer should not be harmful to the target tissue until the treatment beam is applied.
- Preferential uptake in target tissue

The major difference between different types of photosensitizers is in the parts of the cell that they target. Unlike in radiation therapy, where damage is done by targeting cell DNA, most photosensitizers target other cell structures. For example, mTHPC has been shown to localize in the nuclear envelope and do its damage there. In contrast, ALA has been found to localize in the mitochondria and Methylene Blue in the lysosomes.

Targeted PDT

Some photosensitisers naturally accumulate in the endothelial cells of vascular tissue allowing 'vascular targeted' PDT, but there is also research to target the photosensitiser to the tumour (usually by linking it to antibodies or antibody fragments). It is currently only in pre-clinical studies.

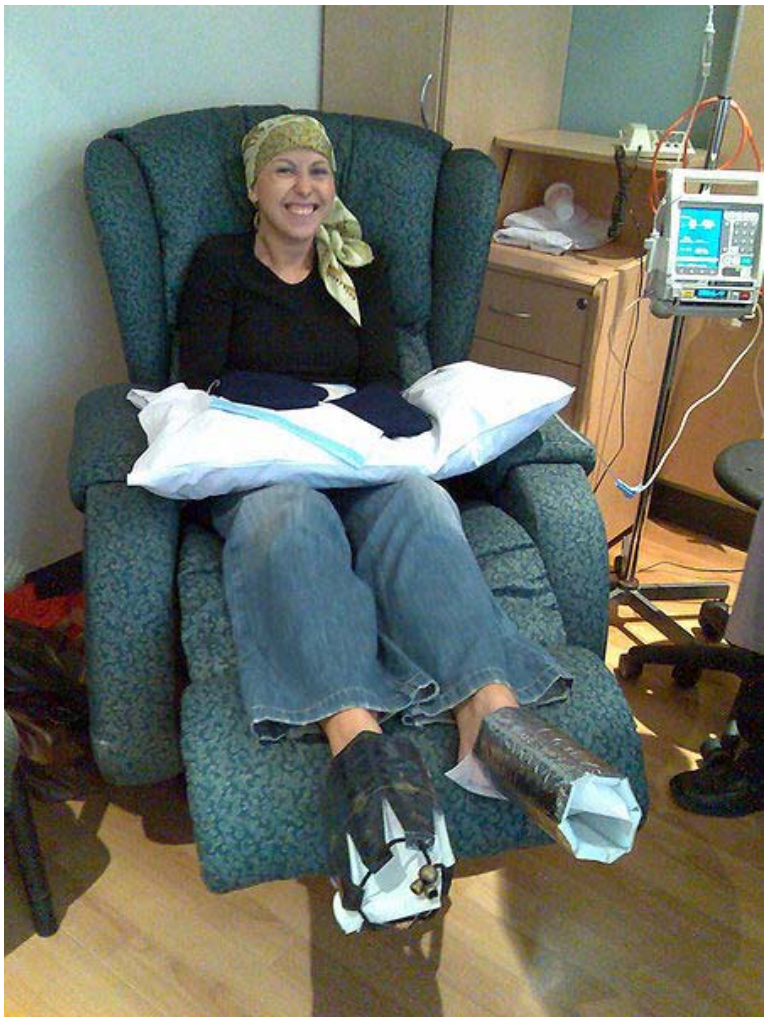
Other research

To allow treatment of deeper tumours some researchers are using internal chemiluminescence to activate the photosensitiser.

PDT is currently in clinical trials to be used as a treatment for severe acne. Initial results show have shown for it to be effective as a treatment only for severe acne, though some question whether it is better than existing acne treatments. The treatment causes severe redness and moderate to severe pain and burning sensation.

Chapter 5

Chemotherapy



A woman being treated with docetaxel chemotherapy for breast cancer. Cold mittens and wine coolers are placed on her hands and feet to reduce harm to her nails.

Chemotherapy, in the most simple sense, is the treatment of an ailment by chemicals especially by killing micro-organisms or cancerous cells. In popular usage, it refers to antineoplastic drugs used to treat cancer or the combination of these drugs into a cytotoxic standardized treatment regimen. In its non-oncological use, the term may also

refer to antibiotics (*antibacterial chemotherapy*). In that sense, the first modern chemotherapeutic agent was arsphenamine, an arsenic compound discovered in 1909 and used to treat syphilis. This was later followed by sulfonamides (sulfa drugs) and penicillin.

Most commonly, chemotherapy acts by killing cells that divide rapidly, one of the main properties of most cancer cells. This means that it also harms cells that divide rapidly under normal circumstances: cells in the bone marrow, digestive tract and hair follicles; this results in the most common side effects of chemotherapy : myelosuppression (decreased production of blood cells, hence also immunosuppression), mucositis (inflammation of the lining of the digestive tract), and alopecia (hair loss).

Other uses of cytostatic chemotherapy agents (including the ones mentioned below) are the treatment of autoimmune diseases such as multiple sclerosis, dermatomyositis, polymyositis, lupus, rheumatoid arthritis and the suppression of transplant rejections.

Newer anticancer drugs act directly against abnormal proteins in cancer cells; this is termed targeted therapy.

History

The use of minerals and plant-based medicines are believed to date back to prehistoric medicine.

The first use of drugs to treat cancer, however, was in the early 20th century, although it was not originally intended for that purpose. Mustard gas was used as a chemical warfare agent during World War I and was studied further during World War II. During a military operation in World War II, a group of people were accidentally exposed to mustard gas and were later found to have very low white blood cell counts. It was reasoned that an agent that damaged the rapidly growing white blood cells might have a similar effect on cancer. Therefore, in the 1940s, several patients with advanced lymphomas (cancers of certain white blood cells) were given the drug by vein, rather than by breathing the irritating gas. Their improvement, although temporary, was remarkable. That experience led researchers to look for other substances that might have similar effects against cancer. As a result, many other drugs have been developed to treat cancer, and drug development since then has exploded into a multibillion-dollar industry, although the principles and limitations of chemotherapy discovered by the early researchers still apply.

Principles

Cancer is the uncontrolled growth of cells coupled with malignant behavior: invasion and metastasis. Cancer is thought to be caused by the interaction between genetic susceptibility and environmental toxins.

In the broad sense, most *chemotherapeutic* drugs work by impairing mitosis (cell division), effectively targeting fast-dividing cells. As these drugs cause damage to cells

they are termed *cytotoxic*. Some drugs cause cells to undergo apoptosis (so-called "self programmed cell death").

Scientists have yet to identify specific features of malignant and immune cells that would make them uniquely targetable (barring some recent examples, such as the Philadelphia chromosome as targeted by imatinib). This means that other fast-dividing cells, such as those responsible for hair growth and for replacement of the intestinal epithelium (lining), are also often affected. However, some drugs have a better side effect profile than others, enabling doctors to adjust treatment regimens to the advantage of patients in certain situations.

As chemotherapy affects cell division, tumors with high *growth fractions* (such as acute myelogenous leukemia and the aggressive lymphomas, including Hodgkin's disease) are more sensitive to chemotherapy, as a larger proportion of the targeted cells are undergoing cell division at any time. Malignancies with slower growth rates, such as indolent lymphomas, tend to respond to chemotherapy much more modestly.

Drugs affect "younger" tumors (i.e., more differentiated) more effectively, because mechanisms regulating cell growth are usually still preserved. With succeeding generations of tumor cells, differentiation is typically lost, growth becomes less regulated, and tumors become less responsive to most chemotherapeutic agents. Near the center of some solid tumors, cell division has effectively ceased, making them insensitive to chemotherapy. Another problem with solid tumors is the fact that the chemotherapeutic agent often does not reach the core of the tumor. Solutions to this problem include radiation therapy (both brachytherapy and teletherapy) and surgery.

Over time, cancer cells become more resistant to chemotherapy treatments. Recently, scientists have identified small pumps on the surface of cancer cells that actively move chemotherapy from inside the cell to the outside. Research on p-glycoprotein and other such chemotherapy efflux pumps, is currently ongoing. Medications to inhibit the function of p-glycoprotein are undergoing testing as of June, 2007 to enhance the efficacy of chemotherapy.

Treatment schemes

There are a number of strategies in the administration of chemotherapeutic drugs used today. Chemotherapy may be given with a curative intent or it may aim to prolong life or to palliate symptoms.

Combined modality chemotherapy is the use of drugs with other cancer treatments, such as radiation therapy or surgery. Most cancers are now treated in this way. *Combination chemotherapy* is a similar practice that involves treating a patient with a number of different drugs simultaneously. The drugs differ in their mechanism and side effects. The biggest advantage is minimising the chances of resistance developing to any one agent.

In *neoadjuvant chemotherapy* (*preoperative* treatment) initial chemotherapy is designed to shrink the primary tumour, thereby rendering local therapy (surgery or radiotherapy) less destructive or more effective.

Adjuvant chemotherapy (*post*operative treatment) can be used when there is little evidence of cancer present, but there is risk of recurrence. This can help reduce chances of developing resistance if the tumour does develop. It is also useful in killing any cancerous cells which have spread to other parts of the body. This is often effective as the newly growing tumours are fast-dividing, and therefore very susceptible.

Palliative chemotherapy is given without curative intent, but simply to decrease tumor load and increase life expectancy. For these regimens, a better toxicity profile is generally expected.

All chemotherapy regimens require that the patient be capable of undergoing the treatment. Performance status is often used as a measure to determine whether a patient can receive chemotherapy, or whether dose reduction is required. Because only a fraction of the cells in a tumor die with each treatment (fractional kill), repeated doses must be administered to continue to reduce the size of the tumor. Current chemotherapy regimens apply drug treatment in cycles, with the frequency and duration of treatments limited by toxicity to the patient.

Types

The majority of chemotherapeutic drugs can be divided in to alkylating agents, antimetabolites, anthracyclines, plant alkaloids, topoisomerase inhibitors, and other antitumour agents. All of these drugs affect cell division or DNA synthesis and function in some way.

Some newer agents do not directly interfere with DNA. These include monoclonal antibodies and the new tyrosine kinase inhibitors e.g. *imatinib mesylate* (*Gleevec* or *Glivec*), which directly targets a molecular abnormality in certain types of cancer (chronic myelogenous leukemia, gastrointestinal stromal tumors). These are examples of targeted therapies.

In addition, some drugs that modulate tumor cell behaviour without directly attacking those cells may be used. Hormone treatments fall into this category.

Where available, Anatomical Therapeutic Chemical Classification System codes are provided for the major categories.

Alkylating agents (L01A)

Alkylating agents are so named because of their ability to alkylate many nucleophilic functional groups under conditions present in cells. Cisplatin and carboplatin, as well as oxaliplatin, are alkylating agents. They impair cell function by forming covalent bonds

with the amino, carboxyl, sulfhydryl, and phosphate groups in biologically important molecules.

Other agents are mechlorethamine, cyclophosphamide, chlorambucil, ifosfamide. They work by chemically modifying a cell's DNA.

Anti-metabolites (L01B)

Anti-metabolites masquerade as purines ((azathioprine, mercaptopurine)) or pyrimidines—which become the building blocks of DNA. They prevent these substances from becoming incorporated in to DNA during the "S" phase (of the cell cycle), stopping normal development and division. They also affect RNA synthesis. Due to their efficiency, these drugs are the most widely used cytostatics.

Plant alkaloids and terpenoids (L01C)

These alkaloids are derived from plants and block cell division by preventing microtubule function. Microtubules are vital for cell division, and, without them, cell division cannot occur. The main examples are vinca alkaloids and taxanes.

Vinca alkaloids (L01CA)

Vinca alkaloids bind to specific sites on tubulin, inhibiting the assembly of tubulin into microtubules (M phase of the cell cycle). They are derived from the Madagascar periwinkle, *Catharanthus roseus* (formerly known as *Vinca rosea*). The vinca alkaloids include:

- Vincristine
- Vinblastine
- Vinorelbine
- Vindesine

Podophyllotoxin (L01CB)

Podophyllotoxin is a plant-derived compound which is said to help with digestion as well as used to produce two other cytostatic drugs, etoposide and teniposide. They prevent the cell from entering the G1 phase (the start of DNA replication) and the replication of DNA (the S phase). The exact mechanism of its action is not yet known.

The substance has been primarily obtained from the American Mayapple (*Podophyllum peltatum*). Recently it has been discovered that a rare Himalayan Mayapple (*Podophyllum hexandrum*) contains it in a much greater quantity, but, as the plant is endangered, its supply is limited. Studies have been conducted to isolate the genes involved in the substance's production, so that it could be obtained recombinantly.

Taxanes (L01CD)

The prototype taxane is the natural product paclitaxel, originally known as Taxol and first derived from the bark of the Pacific Yew tree. Docetaxel is a semi-synthetic analogue of paclitaxel. Taxanes enhance stability of microtubules, preventing the separation of chromosomes during anaphase.

Topoisomerase inhibitors (L01CB and L01XX)

Topoisomerases are essential enzymes that maintain the topology of DNA. Inhibition of type I or type II topoisomerases interferes with both transcription and replication of DNA by upsetting proper DNA supercoiling.

- Some type I topoisomerase inhibitors include *camptothecins*: irinotecan and topotecan.

- Examples of type II inhibitors include amsacrine, etoposide, etoposide phosphate, and teniposide. These are semisynthetic derivatives of epipodophyllotoxins, alkaloids naturally occurring in the root of American Mayapple (*Podophyllum peltatum*).

Antineoplastics (L01D)

These include the immunosuppressant dactinomycin (which is used in kidney transplantations), doxorubicin, epirubicin, bleomycin and others.

Newer and experimental approaches

Hematopoietic stem cell transplant approaches

Stem cell harvesting and autologous or hematopoietic stem cell transplantation has been used to allow for higher doses of chemotherapeutic agents where dosages are primarily limited by hematopoietic damage. Years of research in treating solid tumors, particularly breast cancer, with hematopoeitic stem cell transplants, has yielded little proof of efficacy. Hematological malignancies such as myeloma, lymphoma, and leukemia remain the main indications for stem cell transplants.

Isolated infusion approaches

Isolated limb perfusion (often used in melanoma), or isolated infusion of chemotherapy into the liver or the lung have been used to treat some tumours. The main purpose of these approaches is to deliver a very high dose of chemotherapy to tumor sites without causing overwhelming *systemic* damage. These approaches can help control solitary or limited metastases, but they are by definition *not* systemic, and, therefore, do not treat distributed metastases or micrometastases.

Targeted delivery mechanisms

Specially targeted delivery vehicles aim to increase effective levels of chemotherapy for tumor cells while reducing effective levels for other cells. This should result in an increased tumor kill and/or reduced toxicity.

Specially targeted delivery vehicles have a differentially higher affinity for tumor cells by interacting with tumor-specific or tumour-associated antigens.

In addition to their targeting component, they also carry a payload - whether this is a traditional chemotherapeutic agent, or a radioisotope or an immune stimulating factor. Specially targeted delivery vehicles vary in their stability, selectivity, and choice of target, but, in essence, they all aim to increase the maximum effective dose that can be delivered to the tumor cells. Reduced systemic toxicity means that they can also be used in sicker patients, and that they can carry new chemotherapeutic agents that would have been far too toxic to deliver via traditional systemic approaches.

Light water

Light water or Deuterium-Depleted Water (DDW) is a form of water with lower-than-normal levels of the isotope deuterium. Whereas deuterium-rich heavy water is harmful to many animals, experiments have shown that consumption of light water may be beneficial to humans, particularly those undergoing chemotherapy. A 1999 Romanian study found that water with only 30ppm deuterium produced marked improvement in survival rates of mice bombarded with ionizing radiation. A study of four patients with brain metastases from lung cancer found a three-month regimen of light water "noticeably prolonged" their survival time. A 2010 Hungarian study found significant improvement in the survival times of prostate cancer patients treated with light water.

Nanoparticles

Nanoparticles have emerged as a useful vehicle for poorly soluble agents such as paclitaxel. Protein-bound paclitaxel (e.g., Abraxane) or nab-paclitaxel was approved by the U.S. Food and Drug Administration (FDA) in January 2005 for the treatment of refractory breast cancer. This formulation of paclitaxel uses human albumin as a vehicle and not the Cremophor vehicle used in Taxol. Nanoparticles made of magnetic material can also be used to concentrate agents at tumour sites using an externally applied magnetic field.

Dosage

Dosage of chemotherapy can be difficult: If the dose is too low, it will be ineffective against the tumor, whereas, at excessive doses, the toxicity (side effects, neutropenia) will be intolerable to the patient. This has led to the formation of detailed "dosing schemes" in most hospitals, which give guidance on the correct dose and adjustment in case of

toxicity. In immunotherapy, they are in principle used in smaller dosages than in the treatment of malignant diseases.

In most cases, the dose is adjusted for the patient's body surface area, a measure that correlates with blood volume. The BSA is usually calculated with a mathematical formula or a nomogram, using a patient's weight and height, rather than by direct measurement.

Delivery

Most chemotherapy is delivered intravenously, although a number of agents can be administered orally (e.g., melphalan, busulfan, capecitabine). In some cases, isolated limb perfusion (often used in melanoma), or isolated infusion of chemotherapy into the liver or the lung have been used. The main purpose of these approaches is to deliver a very high dose of chemotherapy to tumour sites without causing overwhelming systemic damage.

Depending on the patient, the cancer, the stage of cancer, the type of chemotherapy, and the dosage, intravenous chemotherapy may be given on either an inpatient or an outpatient basis. For continuous, frequent or prolonged intravenous chemotherapy administration, various systems may be surgically inserted into the vasculature to maintain access. Commonly used systems are the Hickman line, the Port-a-Cath or the PICC line. These have a lower infection risk, are much less prone to phlebitis or extravasation, and abolish the need for repeated insertion of peripheral cannulae.

Harmful and lethal toxicity from chemotherapy limits the dosage of chemotherapy that can be given. Some tumors can be destroyed by sufficiently high doses of chemotherapeutic agents. However, these high doses cannot be given because they would be fatal to the patient.

Adverse effects

Chemotherapeutic techniques have a range of side effects that depend on the type of medications used. The most common medications mainly affect the fast-dividing cells of the body, such as blood cells and the cells lining the mouth, stomach, and intestines. Common side effects include:

- Depression of the immune system, which can result in potentially fatal infections. Although patients are encouraged to wash their hands, avoid sick people, and to take other infection-reducing steps, about 85% of infections are due to naturally occurring microorganisms in the patient's own gut and skin. This may manifest as systemic infections, such as sepsis, or as localized outbreaks, such as shingles. Sometimes, chemotherapy treatments are postponed because the immune system is suppressed to a critically low level.
- Fatigue. The treatment can be physically exhausting for the patient, who might already be very tired from cancer-related fatigue. It may produce mild to severe

anemia. Treatments to mitigate anemia include hormones to boost blood production (erythropoietin), iron supplements, and blood transfusions.
- Tendency to bleed easily. Medications that kill rapidly dividing cells or blood cells are likely to reduce the number of platelets in the blood, which can result in bruises and bleeding. Extremely low platelet counts may be temporarily boosted through platelet transfusions. Sometimes, chemotherapy treatments are postponed to allow platelet counts to recover.
- Gastrointestinal distress. Nausea and vomiting are common side effects of chemotherapeutic medications that kill fast-dividing cells. This can also produce diarrhea or constipation. Malnutrition and dehydration can result when the patient doesn't eat or drink enough, or when the patient vomits frequently, because of gastrointestinal damage. This can result in rapid weight loss, or occasionally in weight gain, if the patient eats too much in an effort to allay nausea or heartburn. Weight gain can also be caused by some steroid medications. These side effects can frequently be reduced or eliminated with antiemetic drugs. Self-care measures, such as eating frequent small meals and drinking clear liquids or ginger tea, are often recommended. This is a temporary effect, and frequently resolves within a week of finishing treatment.
- Hair loss. Some medications that kill rapidly dividing cells cause dramatic hair loss; other medications may cause hair to thin. These are temporary effects: hair usually starts growing back a few weeks after the last treatment, sometimes with a tendency to curl that may be called a "chemo perm".

Damage to specific organs may occur, with resultant symptoms:

- Cardiotoxicity (heart damage)
- Hepatotoxicity (liver damage)
- Nephrotoxicity (kidney damage)
- Ototoxicity (damage to the inner ear), producing vertigo
- Encephalopathy (brain dysfunction)

Immunosuppression and myelosuppression

Virtually all chemotherapeutic regimens can cause depression of the immune system, often by paralysing the bone marrow and leading to a decrease of white blood cells, red blood cells, and platelets. The latter two, when they occur, are improved with blood transfusion. Neutropenia (a decrease of the neutrophil granulocyte count below 0.5 x 10^9/litre) can be improved with synthetic G-CSF (granulocyte-colony stimulating factor, e.g., filgrastim, lenograstim).

In very severe myelosuppression, which occurs in some regimens, almost all the bone marrow stem cells (cells that produce white and red blood cells) are destroyed, meaning *allogenic* or *autologous* bone marrow cell transplants are necessary. (In autologous BMTs, cells are removed from the patient before the treatment, multiplied and then re-injected afterwards; in *allogenic* BMTs the source is a donor.) However, some patients still develop diseases because of this interference with bone marrow.

In Japan the government has approved the use of some medicinal mushrooms like *Trametes versicolor*, to counteract depression of the immune system in patients undergoing chemotherapy. The United States' top-ranked cancer hospital, the MD Anderson, has reported that polysaccharide-K (PSK; an extract from *Trametes versicolor*) is a "promising candidate for chemoprevention due to the multiple effects on the malignant process, limited side effects and safety of daily oral doses for extended periods of time." PSK is already used in pharmaceuticals designed to complement chemotherapy such as MC-S. The MD Anderson has also reported that there are 40 human studies, 55 animal studies, 37 *in vitro* studies, and 11 reviews published concerning *Trametes versicolor* or its extract PSK.

Nausea and vomiting

Chemotherapy-induced nausea and vomiting (CINV) is common with many treatments and some forms of cancer. However, some chemotherapy regimens do not have this side effect, and very effective drugs to stop or noticeably reduce this adverse effect are available.

A class of drugs called 5-HT_3 antagonists are the most effective antiemetics and constitute the single greatest advance in the management of nausea and vomiting in patients with cancer. These drugs block one or more of the nerve signals that cause nausea and vomiting. During the first 24 hours after chemotherapy, the most effective approach appears to be blocking the 5-HT_3 nerve signal. Approved 5-HT_3 inhibitors include dolasetron, granisetron, and ondansetron (Zofran). The newest 5-HT_3 inhibitor, palonosetron, also prevents delayed nausea and vomiting, which occurs during the 2–5 days after treatment. Since some patients have trouble swallowing pills, these drugs are often available by injection, as orally disintegrating tablets, or as transdermal patchs.

The substance P inhibitor aprepitant, which became available in 2005, is also effective in controlling the nausea of cancer chemotherapy.

Some studies and patient groups say that the use of cannabinoids derived from marijuana during chemotherapy greatly reduces the associated nausea and vomiting, and enables the patient to eat. Some synthetic derivatives of the active substance in marijuana (Tetrahydrocannabinol or THC) such as Marinol may be practical for this application. Natural marijuana, known as medical cannabis is also used and recommended by some oncologists, though its use is regulated and not legal everywhere.

Secondary neoplasm

Development of secondary neoplasia after successful chemotherapy and/or radiotherapy treatment can occur. The most common secondary neoplasm is secondary acute myeloid leukemia, which develops primarily after treatment with alkylating agents or topoisomerase inhibitors. Other studies have shown a 13.5 fold increase from the general population in the incidence of secondary neoplasm occurrence after 30 years from treatment.

Infertility

Some types of chemotherapy are gonadotoxic and may cause infertility. Chemotherapies with high risk include procarbazine and alkylating drugs such as cyclophosphamide, ifosfamide, busulfan, melphalan, chlorambucil and chlormethine. Drugs with medium risk include doxorubicin and platinum analogs such as cisplatin and carboplatin. On the other hand, therapies with low risk of gonadotoxicity include plant derivatives such as vincristine and vinblastine, antibiotics such as bleomycinand dactinomycin and antimetabolites such as methotrexate, mercaptopurine and 5-fluoruracil.

Patients may choose between several methods of fertility preservation prior to chemotherapy, including cryopreservation of semen, ovarian tissue, oocytes or embryos.

Other side effects

In particularly large tumors, such as large lymphomas, some patients develop tumor lysis syndrome from the rapid breakdown of malignant cells. Although prophylaxis is available and is often initiated in patients with large tumors, this is a dangerous side effect that can lead to death if left untreated.

Less common side effects include pain, red skin (erythema), dry skin, damaged fingernails, a dry mouth (xerostomia), water retention, and sexual impotence. Some medications can trigger allergic or pseudoallergic reactions.

Some patients report fatigue or non-specific neurocognitive problems, such as an inability to concentrate; this is sometimes called post-chemotherapy cognitive impairment, referred to as "chemo brain" by patients' groups.

Specific chemotherapeutic agents are associated with organ-specific toxicities, including cardiovascular disease (e.g., doxorubicin), interstitial lung disease (e.g., bleomycin) and occasionally secondary neoplasm (e.g., MOPP therapy for Hodgkin's disease).

Chapter 6

Post-Chemotherapy Cognitive Impairment

Post-chemotherapy cognitive impairment (PCCI) (also known as **chemotherapy-induced cognitive dysfunction**, **chemo brain** or **chemo fog**) describes the cognitive impairment that can result from chemotherapy treatment. Approximately 20-30% of people who undergo chemotherapy experience some level of post-chemotherapy cognitive impairment. The phenomenon first came to light because of the large number of breast cancer survivors who complained of changes in memory, fluency, and other cognitive abilities that impeded their ability to function as they had pre-chemotherapy.

Although the causes and existence of post-chemotherapy cognitive impairment have been a subject of debate, recent studies have confirmed that post-chemotherapy cognitive impairment is a real, measurable side effect of chemotherapy that appears in some patients. While any cancer patient may experience temporary cognitive impairment due to stress, fatigue, and depression, the long-term symptoms of PCCI are almost exclusively seen in patients treated for breast cancer, ovarian cancer, prostate cancer, and other cancers of the reproductive system.

PCCI is clinically important due to the large number of women who survive breast cancer, more aggressive dosing of chemotherapeutic agents, and the use of chemotherapy as an adjuvant to other forms of treatment. In some patients, fear of PCCI can impact treatment decisions. The magnitude of chemotherapy-related cognitive changes and their impact on the activities of daily living are uncertain.

Incidence

PCCI affects a subset of cancer survivors, though the overall epidemiology and prevalence is not well known and may depend on many factors.

It generally affects about 10-40% of breast cancer patients, with higher rates among pre-menopausal women and patients who receive high-dose chemotherapy.

Symptoms

The systems of the body most affected by chemotherapy drugs include visual and semantic memory, attention and motor coordination. These effects can impair a chemotherapy patient's ability to understand and make decisions regarding treatment, perform in school or employment and can reduce quality of life. Survivors often report difficulty multitasking, comprehending what they've just read, following the thread of a conversation, and retrieving words

Breast cancer survivors who were treated with chemotherapy have to work harder to perform tasks than survivors whose treatment was surgical. A year after treatment the brains of cancer survivors treated with chemotherapy had physically shrunk while those of people not treated with chemotherapy had not.

Post-chemotherapy cognitive impairment comes as a surprise to many cancer survivors. Often, survivors think their lives will return to normal when the cancer is gone, only to find that the lingering effects of post-chemotherapy cognitive impairment impede their efforts. Working, connecting with loved ones, carrying out day-to-day tasks—all can be very challenging for an impaired brain. Although post-chemotherapy cognitive impairment appears to be temporary, it can be quite long-lived, with some cases lasting 10 years or more.

Cause

The details of PCCI's causes and boundaries are not well known. Two major theories have been advanced: the direct effect of chemotherapy drugs on the brain, and the role of hormones in nervous system health.

Bortezomib is known to cause neuropathy to the sensory and peripheral nervous systems that is reversible. In most cases there is no known way of reducing the effects of chemotherapeutic agents related to taxanes, thalidomide and platinum-based compounds (oxaliplatin is a notable exception to the latter category - though it does cause PCCI its effects can be buffered by infusion of calcium and thought related to PCCI include the ability of the nerves to repair themselves, the ability of cells to excrete compounds, permeability of the blood-brain barrier, damage done to DNA including shortening of telomeres and cellular oxidative stress.

The importance of hormones, particularly estrogen, on cognitive function is underscored by the presence of cognitive impairment in breast cancer patients before chemotherapy is begun, the similarity of the cognitive impairments to several menopausal symptoms, the increased rate of PCCI in pre-menopausal women, and the fact that the symptoms can frequently be reversed by taking estrogen.

Other theories suggest vascular injury, inflammation, autoimmunity, anemia and the presence of the epsilon 4 version of the apolipoprotein E gene.

PCCI is complex and factors other than the chemotherapeutic agents may impact cognitive functioning. Menopause, the biological impact of a surgical procedure with anesthesia, medications prescribed in addition to the chemotherapy, genetic predisposition, hormone therapy, emotional states (including anxiety, depression and fatigue), comorbid conditions and paraneoplastic syndrome may all co-occur and act as confounding factors in the study or experience of PCCI.

Treatment

Hypothesized treatment options include the use of antioxidants, cognitive behavior therapy, erythropoietin and stimulant drugs such as methylphenidate, though as the mechanism of PCCI is not well understood the potential treatment options are equally theoretical.

Modafinil, approved for narcolepsy, has been used off-label in trials with people with symptoms of chemobrain. Modafinil is a wakefulness promoting agent that can improve alertness and concentration. A University of Rochester study of 68 subjects had significant results. "We knew from previous studies that modafinil does alleviate problems with memory and attention, and were hoping it would do the same for breast-cancer patients experiencing chemo-brain, which it did," related the study's lead author Sadhna Kohli, Ph.D, a research assistant professor at the University of Rochester's James P. Wilmot Cancer Center.

While taking estrogen will frequently reverse the symptoms, this would be dangerous because of the many health risks associated with taking this hormone as a drug.

Research

Research on PCCI is limited, and studies on the subject have often been conflicting in results, in part due to differing means of assessing and defining the phenomenon, which makes comparison and synthesis difficult. Most studies have involved small samples, making generalization difficult, and there has been a focus on younger patients which makes conclusions about the largest group of cancer patients, the elderly, difficult to draw.

The drug doxorubicin (adriamycin) has been investigated as a PCCI-causing agent due to its production of reactive oxygen species. It has been investigated in an animal model with mice.

Prognosis

While frustrating, the ultimate outcome is very good: symptoms typically disappear in about four years.

History

The symptoms of chemobrain (a phrase coined by June Mari-Gras in 2004) were recognized by researchers in the 1980s, who typically described it as mild cognitive impairment subsequent to successful cancer treatment. Some authors say that it was identified primarily in breast cancer survivor and support groups as affecting a subset of individuals treated with chemotherapy, who attributed it to the effects of the medication taken to treat their cancers.

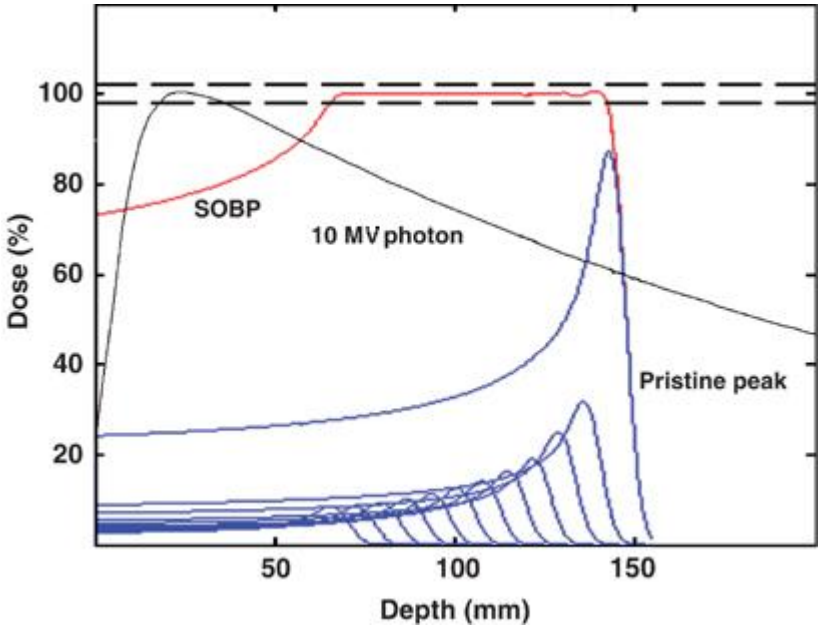
Chapter 7

Proton Therapy

2005 image of the control panel of the synchrocyclotron at the Orsay proton therapy center

Proton therapy is a type of particle therapy which uses a beam of protons to irradiate diseased tissue, most often in the treatment of cancer. The chief advantage of proton therapy is the ability to more precisely localize the radiation dosage when compared with other types of external beam radiotherapy.

Description



In a typical treatment plan for proton therapy the red line representing the spread out Bragg peak (SOBP) is the therapeutic radiation distribution. The SOBP is the sum of several pristine Bragg peaks (blue lines) at staggered depths. The depth-dose plot of a 10 MV x-ray (photon) beam is provided for comparison.

Proton therapy is a type of external beam radiotherapy using ionizing radiation. During treatment, a particle accelerator is used to target the tumor with a beam of protons. These charged particles damage the DNA of cells, ultimately causing their death or interfering with their ability to reproduce. Cancerous cells, because of their high rate of division and their reduced ability to repair damaged DNA, are particularly vulnerable to attack on their DNA.

Due to their relatively large mass, protons have little lateral side scatter in the tissue; the beam does not broaden much, stays focused on the tumor shape and delivers small dose side-effects to surrounding tissue. All protons of a given energy have a certain range; very few protons penetrate beyond that distance. Furthermore, the dose delivered to tissue is maximum just over the last few millimeters of the particle's range; this maximum is called the Bragg peak.

To treat tumors at greater depths, the proton accelerator must produce a beam with higher energy, typically given in eV or electron volts. Tumors closer to the surface of the body are treated using protons with lower energy. The accelerators used for proton therapy typically produce protons with energies in the range of 70 to 250 MeV (Mega electron Volts: million electron Volts). By adjusting the energy of the protons during application of treatment, the cell damage due to the proton beam is maximized within the tumor itself. Tissues closer to the surface of the body than the tumor receive reduced radiation,

and therefore reduced damage. Tissues deeper within the body receive very few protons so that the dosage becomes immeasurably small.

In most treatments, protons of different energies with Bragg peaks at different depths are applied to treat the entire tumor. These Bragg peaks are shown as blue lines in the figure to the left. The total radiation dosage of the protons is called the *Spread-Out Bragg Peak* (SOBP), shown as a red line in figure to the left. It is important to understand that, while tissues *behind* or *deeper than* the tumor receive no radiation from proton therapy, the tissue *in front of* or *shallower than* the tumor receive radiation dosage based on the SOBP.

History

The first suggestion that energetic protons could be an effective treatment method was made by Robert R. Wilson in a paper published in 1946 while he was involved in the design of the Harvard Cyclotron Laboratory (HCL). The first treatments were performed with particle accelerators built for physics research, notably Berkeley Radiation Laboratory in 1954 and at Uppsala in Sweden in 1957. In 1961, a collaboration began between HCL and the Massachusetts General Hospital (MGH) to pursue proton therapy. Over the next 41 years, this program refined and expanded these techniques while treating 9,116 patients before the Cyclotron was shut down in 2002. The world's first hospital-based proton therapy center was built in 1990 at the Loma Linda University Medical Center (LLUMC) in Loma Linda, California. Later, The Northeast Proton Therapy Center at Massachusetts General Hospital was brought online, and the HCL treatment program was transferred to it during 2001 and 2002.

Application

The types of treatments for which protons are used can be separated into two broad categories. The first are those for disease sites that favor the delivery of higher doses of radiation, i.e. dose escalation. In some instances dose escalation has been shown to achieve a higher probability of "cure" (i.e. local control) than conventional radiotherapy. These include (but are not limited to) uveal melanoma (ocular tumors), skull base and paraspinal tumors (chondrosarcoma and chordoma), and unresectable sarcomas. In all these cases proton therapy achieves significant improvements in the probability of local control over conventional radiotherapy.

The second broad class are those treatments where the increased precision of proton therapy is used to reduce unwanted side effects, by limiting the dose to normal tissue. In these cases the tumor dose is the same as that used in conventional therapy, and thus there is no expectation of an increased probability of curing the disease. Instead, the emphasis is on the reduction of the integral dose to normal tissue, and thus a reduction of unwanted effects. Two prominent examples are pediatric neoplasms (such as medulloblastoma) and prostate cancer. In the case of pediatric treatments there is convincing clinical data showing the advantage of sparing developing organs by using protons, and the resulting reduction of long term damage to the surviving child.

In the case of prostate cancer the issue is not so clear. Some published studies found a reduction in long term rectal and genitio-urinary damage when treating with proton rather than photon also known as X-ray or gamma ray therapy. Others showed the difference is small, and limited to cases where the prostate is particularly close to certain anatomical structures. The relatively small improvement found may be the result of inconsistent patient set-up and internal organ movement during treatment, which offsets most of the advantage due to increased precision. One source suggests that dose errors around 20% can result from motion errors of just 2.5 mm, and another that prostate motion is between 5–10 mm.

However, the number of cases of prostate cancer diagnosed each year far exceeds those of the other diseases referred to above, and this has led some, but not all, facilities to devote a majority of their treatments slots to prostate treatments. For example two hospital facilities devote roughly 65% and 50% of their proton treatment capacity to prostate cancer, while a third devotes only 7.1%

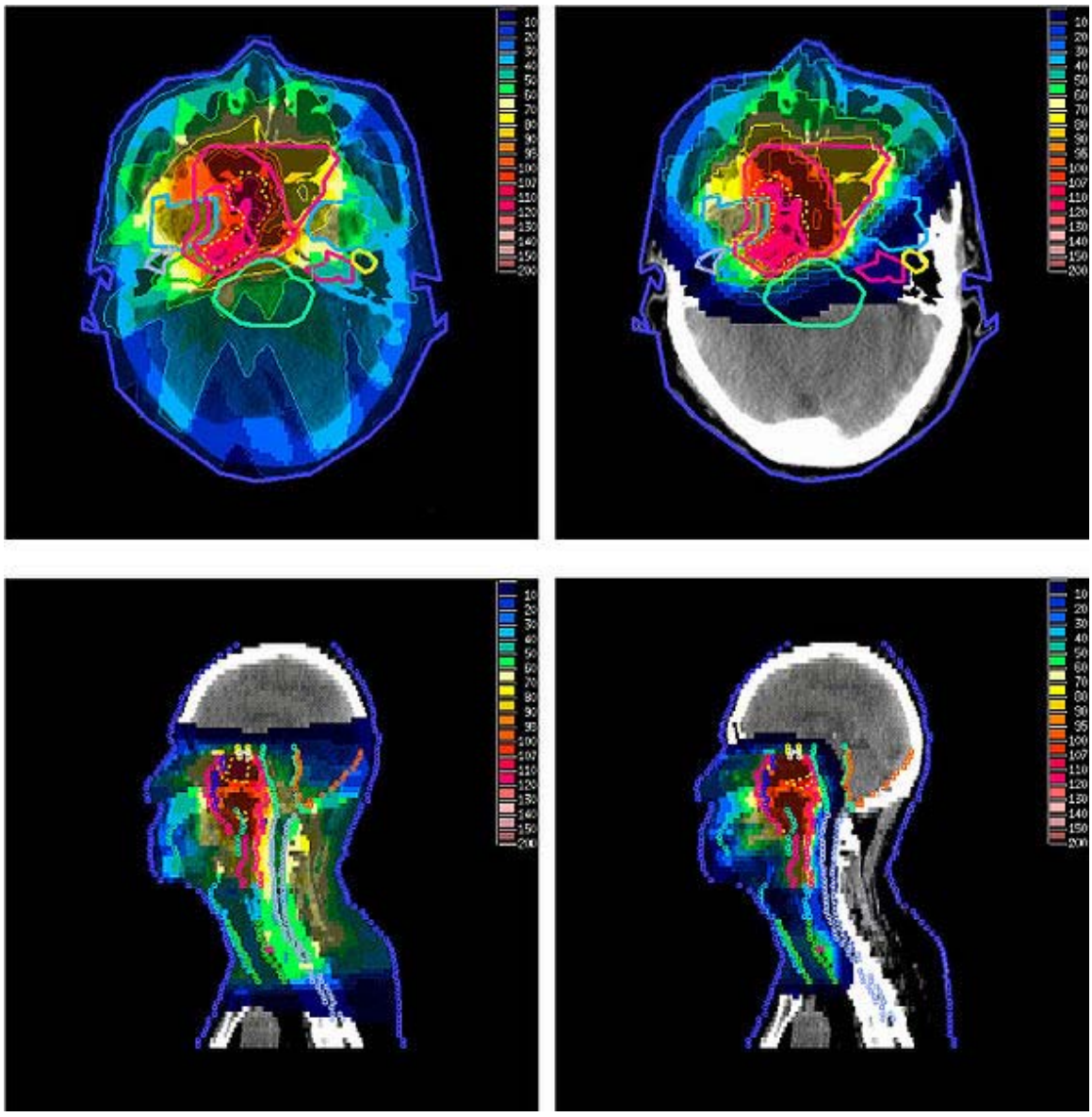
Current overall world wide numbers are hard to compile, but one example in the literature shows that in 2003 roughly 26% of proton therapy treatments world wide were for prostate cancer. Proton therapy for ocular (eye) tumors is a special case since this treatment requires only a comparably low energy (about 70 MeV). Owing to this low energy requirement, some particle therapy centers only treat ocular tumors. Proton, or more generally, Hadron therapy of tissue close to the eye affords sophisticated methods to assess the alignment of the eye that can vary significantly from other patient position verification approaches in image guided particle therapy. Position verification and correction have to ensure that sensitive tissue like the optic nerve is spared from the radiation in order to preserve the patient's vision.

Comparison with other treatment options

The issue of when, whether, and how best to apply this technology is controversial. As of 2009 it is not yet known whether proton therapy yields better clinical outcomes than other types of radiation therapy for people with many common cancers. Proton therapy is far more expensive than conventional therapy. It requires a large capital investment (roughly $100M to $150M) for 2009 technology.

Preliminary results from a three-year 2009 study, including high dose treatments, show very few side effects.

X-ray radiotherapy



Irradiation of nasopharyngeal carcinoma by photon(X-ray) therapy (left) and proton therapy (right)

The figure at the right of the page shows how beams of x-rays (IMRT) left frame and beams of protons right frame, of different energies, penetrate human tissue. A tumor with a sizable thickness is covered by the IMPT spread out Bragg peak (SOBP) shown as the red lined distribution in the figure. The SOBP is an overlap of several pristine Bragg peaks (blue lines) at staggered depths.

X-ray therapy may be described as having more "skin sparing potential" than proton therapy: x-ray radiation at the skin and at very small depths is lower than for proton therapy. One study estimates that passively scattered proton fields have a slightly higher

entrance dose at the skin (~75%) compared to therapeutic megavoltage (MeV) photon beams (~60%). X-ray radiation dose falls off gradually, while tissues deeper in the body than the tumor receive essentially no radiation during proton therapy. Thus, x-ray therapy causes less damage to the skin and surface tissues, and proton therapy causes less damage to tissues beyond the target.

Surgery

The decision to use surgery or proton therapy (or in fact any radiation therapy) is based on the tumor type, stage, and location. In some instances surgery is superior (e.g. cutaneous melanoma), in some instances radiation is superior (e.g. skull base chondrosarcoma), and in some instances they are comparable (e.g. prostate cancer). In some instances, they are used together (e.g. rectal cancer or early stage breast cancer). The benefit of external beam proton radiation lies in the dosimetric difference from external beam x-ray radiation and brachytherapy in cases, where the use of radiation therapy is already indicated, rather than as a direct competition with surgery.

Side effects and risks

Proton therapy is a type of external beam radiotherapy, and shares risks and side effects of other forms of radiation therapy. Proton therapy has been in use for over 40 years, and is a mature treatment technology. However, as with all medical knowledge, understanding of the interaction of radiation (proton, X-ray, etc.) with tumor and normal tissue is still imperfect.

Treatment centers

As of April 2010, there were a total of 29 proton therapy centers in Canada, China, England, France, Germany, Italy, Japan, Korea, Russia, South Africa, Sweden, Switzerland, and USA; and more than 67000 patients had been treated. One hindrance to universal use of the proton in cancer treatment is the size and cost of the cyclotron or synchrotron equipment necessary. Several industrial teams are working on development of comparatively small cyclotron or synchrotron systems to deliver the proton therapy to patients.

Chapter 8

Cancer Immunotherapy

Cancer immunotherapy is the use of the immune system to reject cancer. The main premise is stimulating the patient's immune system to attack the malignant tumor cells that are responsible for the disease. This can be either through immunization of the patient (eg. by administering a cancer vaccine, such as Dendreon's Provenge), in which case the patient's own immune system is trained to recognize tumor cells as targets to be destroyed, or through the administration of therapeutic antibodies as drugs, in which case the patient's immune system is recruited to destroy tumor cells by the therapeutic antibodies.

Since the immune system responds to the environmental factors it encounters on the basis of discrimination between self and non-self, many kinds of tumor cells that arise as a result of the onset of cancer are more or less tolerated by the patient's own immune system since the tumor cells are essentially the patient's own cells that are growing, dividing and spreading without proper regulatory control.

In spite of this fact, however, many kinds of tumor cells display unusual antigens that are either inappropriate for the cell type and/or its environment, or are only normally present during the organisms' development (e.g. fetal antigens). Examples of such antigens include the glycosphingolipid GD2, a disialoganglioside that is normally only expressed at a significant level on the outer surface membranes of neuronal cells, where its exposure to the immune system is limited by the blood-brain barrier. GD2 is expressed on the surfaces of a wide range of tumor cells including neuroblastoma, medulloblastomas, astrocytomas, melanomas, small-cell lung cancer, osteosarcomas and other soft tissue sarcomas. GD2 is thus a convenient tumor-specific target for immunotherapies.

Other kinds of tumor cells display cell surface receptors that are rare or absent on the surfaces of healthy cells, and which are responsible for activating cellular signal transduction pathways that cause the unregulated growth and division of the tumor cell. Examples include ErbB2, a constitutively active cell surface receptor that is produced at abnormally high levels on the surface of breast cancer tumor cells.

Monoclonal antibody therapy

Antibodies are a key component of the adaptive immune response, playing a central role in both in the recognition of foreign antigens and the stimulation of an immune response to them. It is not surprising therefore, that many immunotherapeutic approaches involve the use of antibodies. The advent of monoclonal antibody technology has made it possible to raise antibodies against specific antigens such as the unusual antigens that are presented on the surfaces of tumors.

A number of therapeutic monoclonal antibodies have been approved for use in humans; approvals mentioned here are by the U.S. Food and Drug Administration (FDA).

Cancer immunotherapy:Monoclonal antibodies

Antibody	Brand name	Approval date	Type	Target	Approved treatment(s)
Alemtuzumab	Campath	2001	humanized	CD52	Chronic lymphocytic leukemia
Bevacizumab	Avastin	2004	humanized	vascular endothelial growth factor	colorectal cancer
Cetuximab	Erbitux	2004	chimeric	epidermal growth factor receptor	colorectal cancer
Gemtuzumab ozogamicin	Mylotarg	2000	humanized	CD33	acute myelogenous leukemia (with calicheamicin)
Ibritumomab tiuxetan	Zevalin	2002	murine	CD20	non-Hodgkin lymphoma (with yttrium-90 or indium-111)
Panitumumab	Vectibix	2006	human	epidermal	colorectal cancer

				growth factor receptor	
Rituximab	Rituxan, Mabthera	1997	chimeric	CD20	non-Hodgkin lymphoma
Trastuzumab	Herceptin	1998	humanized	ErbB2	breast cancer

Alemtuzumab

Alemtuzumab is an anti-CD52 humanized IgG1 monoclonal antibody indicated for the treatment of Chronic lymphocytic leukemia(CLL), the most frequent form of leukaemia in Western countries. The function of CD52 is unknown, but it is found on >95% of peripheral blood lymphocytes and monocytes. Upon binding to CD52, alemtuzumab initiates its cytotoxic effect by complement fixation and antibody-dependent cell-mediated cytotoxicity mechanisms. Alemtuzumab therapy is also indicated for T-prolymphocytic leukaemia (TPPL), for which no standard treatment exists. This is a highly aggressive tumour, with a median survival of 7.5 months.

Bevacizumab

Bevacizumab is a humanized IgG1 monoclonal antibody which binds to and sterically interferes with the vascular endothelial growth factor-A (VEGF-A), preventing receptor activation. A marked increase in VEGF expression is thought to play a role in tumor angiogenesis. Bevacizumab is indicated for colon cancer; but has been applied to numerous other cancers in small scale studies, especially renal cell carcinoma. Results obtained showed that bevacizumab increased the duration of survival, progression-free survival, the rate of response and the duration of response in a statistically relevant manner.

Cetuximab

Cetuximab is a chimeric IgG1 monoclonal antibody which targets the extracellular domain of the epidermal growth factor receptor (EGFR). It functions by competitively inhibiting ligand binding, thereby preventing EGFR activation, and is indicated for the treatment of colorectal cancer. Studies have also been carried out on numerous other malignancies, especially non-small cell lung cancer and head and neck cancer. As a single agent, cetuximab showed a response rate of 10.8% in patients with EGFR overexpressed metastatic colon cancer. Other anti-EGFR monoclonal antibodies in development include: ABX-EGF, hR3, and EMD 72000. Although they hold significant promise for the future, as of yet none of the agents are currently beyond phase I clinical trials.

Gemtuzumab ozogamicin

Gemtuzumab ozogamicin is an "immuno-conjugate" of an anti-CD33 antibody chemically linked to calicheamicin, a cytotoxic agent. It is indicated for the treatment of acute myeloid leukaemia (AML). The patient group most likely to benefit from gemtuzumab is young adults, and trials have reported high complete responses (85%), when combined with intensive chemotherapy. There are minimal side-effects associated with Gemtuzumab therapy.

Rituximab

Rituximab is a chimeric monoclonal antibody specific for CD20. CD20 is widely expressed on B-cells. Although the function of CD20 is relatively unknown it has been suggested that CD20 could play a role in calcium influx across plasma membrane, maintaining intracellular calcium concentration and allowing for the activation of B cells. The exact mode of action of rituximab is also unclear, but it has been found to have a general regulatory effect on the cell cycle and on immune-receptor expression. Experiments involving primates showed that treatment with anti-CD20 reduced peripheral B-cells by 98%, and peripheral lymph node and bone marrow B-cells by up to 95%.

Trastuzumab

Trastuzumab is a monoclonal IgG1 humanized antibody specific for the epidermal growth factor receptor 2 protein (HER2). It received FDA-approval in 1998, and is clinically used for the treatment of breast cancer. The use of Trastuzumab is restricted to patients whose tumours over-express HER-2, as assessed by immunohistochemistry (IHC) and either chromogenic or Fluorescent in situ hybridisation (FISH), as well as numerous PCR-based methodologies.

HER-2 is a member of the epidermal growth factor receptor (EGFR) family of transmembrane tyrosine kinases, and is normally involved in regulation of cell proliferation and differentiation. Amplification or overexpression of HER-2 is present in 25-30% of breast carcinomas and has been associated with aggressive tumour phenotype, poor prognosis, non-responsiveness to hormonal therapy and reduced sensitivity to conventional chemotherapeutic agents.

Radioimmunotherapy

Radioimmunotherapy involves the use of radioactively conjugated murine antibodies against cellular antigens. Most research currently involved their application to lymphomas, as these are highly radio-sensitive malignancies. To limit radiation exposure, murine antibodies were especially chosen, as their high immunogenicity promotes rapid clearance from the body.

Ibritumomab tiuxetan

Ibritumomab tiuxetan is a murine antibody chemically linked to a chelating agent which binds yttrium-90. ^{90}Y is a beta radiator, has a half-life of 64 h and a tissue penetration of 1-5 millimetres. Its use has been investigated, primarily in the treatment of follicular lymphoma.

Tositumomab/iodine (^{131}I) tositumomab regimen

Tositumomab is a murine IgG2a anti-CD20 antibody. Iodine (^{131}I) tositumomab is covalently bound to Iodine 131. ^{131}I emits both beta and gamma radiation, and is broken down rapidly in the body. Clinical trials have established the efficacy of a sequential application of tositumomab and iodine (^{131}I) tositumomab in patients with relapsed follicular lymphoma.

Advances in immunotherapy

The development and testing of second generation immunotherapies are already under way. While antibodies targeted to disease-causing antigens can be effective under certain circumstances, in many cases, their efficacy may be limited by other factors. In the case of cancer tumors, the microenvironment is immunosuppressive, allowing even those tumors that present unusual antigens to survive and flourish in spite of the immune response generated by the cancer patient, against his or her own tumor tissue. Certain members of a group of molecules known as cytokines, such as Interleukin-2 also play a key role in modulating the immune response, and have been tried in conjunction with antibodies in order to generate an even more devastating immune response against the tumor. While the therapeutic administration of such cytokines may cause systemic inflammation, resulting in serious side effects and toxicity, a new generation of chimeric molecules consisting of an immune-stimulatory cytokine attached to an antibody that targets the cytokine's activity to a specific environment such as a tumor, are able to generate a very effective yet localized immune response against the tumor tissue, destroying the cancer-causing cells without the unwanted side-effects. A different type of chimeric molecule is an artificial T cell receptor.

The targeted delivery of cytokines by anti-tumor antibodies is one example of using antibodies to delivery payloads rather than simply relying on the antibody to trigger an immune response against the target cell. Another strategy is to deliver a lethal radioactive dose directly to the target cell, which has been utilized in the case of the Zevalin therapeutic. A third strategy is to deliver a lethal chemical dose to the target, as used in the Mylotarg therapeutic. Engineering the antibody-payload pair in such a way that they separate after entry into a cell by endocytosis can potentially increase the efficacy of the payload. One strategy to accomplish this is the use of a disulfide linkage which could be severed by the reducing conditions in the cellular interior. However, recent evidence suggests that the actual intracellular trafficking of the antibody-payload after endocytosis is such to make this strategy not generally applicable. Other potentially useful linkage types include hydrazone and peptide linkages.

Latest research

In 2001, two U.S. based non-profit organizations, the Cancer Research Institute and the Ludwig Institute for Cancer Research, formed the Cancer Vaccine Collaborative, a unique global network of clinical trial sites with special expertise in immunology, built to centrally design and coordinate early-stage clinical trials to be run in parallel in order to identify more quickly the optimal combination of reagents, or vaccine components, necessary for a successful therapeutic cancer vaccine. The Cancer Vaccine Collaborative has to-date (June 2009) completed or is currently running more than 40 clinical trials of different therapeutic cancer vaccines, including 37 phase I, 6 phase II, and 1 fully-randomized phase II clinical trials, and has published more than 130 scientific papers in peer-reviewed journals. Nearly all of these trials featured vaccines targeting various forms of the cancer-testes antigen, NY-ESO-1, a highly-immunogenic, prototypical protein marker limited in expression to a wide variety of cancer types but not in normal tissue, with the exception of the immune-privileged testes. Vaccines tested in Cancer Vaccine Collaborative trials have induced integrated immune responses composed of target-specific antibodies and CD4+ and CD8+ T lymphocytes, all of which are held to be essential for effective long-term control of cancer. Insights from these trials have generated a strong framework for the selection of components that will likely comprise an ideal therapeutic cancer vaccine, including: multiple cancer-antigens in various forms delivered with potent adjuvants and all administered in a prime-boost setting in conjunction with a modulator of cancer immunosuppression.

In June 2008, it was announced that US doctors from the Clinical Research Division led by Cassian Yee at Fred Hutchinson Cancer Research Center in Seattle have for the first time successfully treated a skin cancer patient by using immune cells cloned from his own immune system which were then re-injected into him. The patient, who was suffering from advanced skin cancer, was free from tumours within eight weeks of being injected with billions of his own immune cells in the first case of its kind. Experts say that this case could be a landmark in the treatment of cancer in general. Larger trials are now under way.

More new research is being conducted by Drs. Richard O'Reilly and Michel Sadelain. Drs. O'Reilly and Sadelain have done extensive research at Memorial Sloan-Kettering Cancer Center hospital and are among leaders of the cancer adoptive Immunotherapy field

Topical immunotherapy

Dermatologists use new creams and injections in the management of benign and malignant skin tumors. Topical immunotherapy utilizes an immune enhancement cream (imiquimod) which is an interferon producer causing the patient's own killer T cells to destroy warts, actinic keratoses, basal cell carcinoma, squamous cell carcinoma, cutaneous T cell lymphoma, and Superficial spreading melanoma. Injection immunotherapy uses mumps, candida or trichophytin antigen injections to treat warts (HPV induced tumors).

Natural products

Some types of natural products have shown promise to stimulate the immune system. Research suggests that mushrooms like Reishi and *Agaricus blazei* may be able to stimulate the immune system. Research has shown that *Agaricus blazei* may be a potent stimulator of natural killer cells. *Agaricus blazei* is rich in proteoglucans and beta-glucans, which are potent stimulators of macrophages.

Research ss the compounds in medicinal mushrooms most responsible for up-regulating the immune system and providing an anti-cancer effect, are a diverse collection of polysaccharide compounds, particularly beta-glucans. Beta-glucans are known as "biological response modifiers", and their ability to activate the immune system is well documented. Specifically, beta-glucans stimulate the innate branch of the immune system. Research has shown beta-glucans have the ability to stimulate macrophage, NK cells, T cells, and immune system cytokines. The mechanisms in which beta-glucans stimulate the immune system is only partially understood. One mechanism in which beta-glucans are able to activate the immune system, is by interacting with the Macrophage-1 antigen (CD18) receptor on immune cells.

Highly purified compounds isolated from medicinal mushrooms such as lentinan (isolated from Shiitake), and Polysaccharide-K, (isolated from *Trametes versicolor*), have become incorporated into the health care system of a few countries, such as Japan. Japan's Ministry of Health, Labour and Welfare approved the use of Polysaccharide-K in the 1980s, to stimulate the immune systems of patients undergoing chemotherapy. In Australia, a pharmaceutical based on a mixture of several mycological extracts including lentinan and Polysaccharide-K is sold commercially as MC-S.

Chapter 9

Hyperthermia Therapy

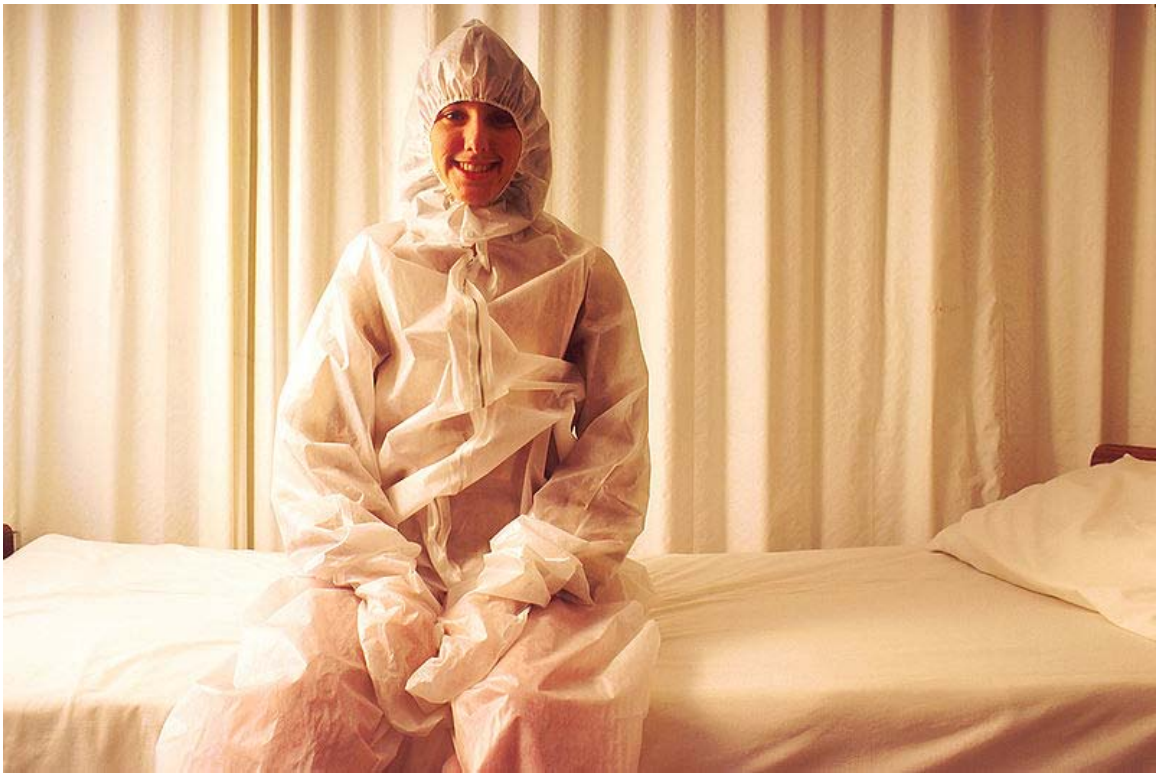
Hyperthermia therapy is a type of medical treatment in which body tissue is exposed to high temperatures to damage and kill cancer cells or to make cancer cells more sensitive to the effects of radiation and certain anti-cancer drugs. When combined with radiation therapy, it is called **thermoradiography**.

Local hyperthermia for certain small tumors is generally accepted, similar to surgically removing a tumor. Whole-body hyperthermia is generally considered to be a promising but experimental cancer treatment.

Hyperthermia is only useful for certain kinds of cancer, and is not in widespread use. Hyperthermia is most effective when used alongside conventional therapies, so it is normally used as an adjuvant therapy. The most effective uses are currently being studied.

Mechanism

Hyperthermia may kill or weaken tumor cells, and is controlled to limit effects on healthy cells. Tumor cells, with a disorganized and compact vascular structure, have difficulty dissipating heat. Hyperthermia may therefore cause cancerous cells to undergo apoptosis in direct response to applied heat, while healthy tissues can more easily maintain a normal temperature.



Whole-body suit used in hyperthermia therapy

Even if the cancerous cells do not die outright, they may become more susceptible to ionizing radiation therapy or to certain chemotherapy drugs, which may allow such therapy to be given in smaller doses.

Intense heating will cause denaturation and coagulation of cellular proteins, rapidly killing cells within a tumour. More prolonged moderate heating to temperatures just a few degrees above normal can cause more subtle changes. A mild heat treatment combined with other stresses can cause cell death by apoptosis. There are many biochemical consequences to the heat shock response within in cell, including slowed cell division and increased sensitivity to ionizing radiation therapy.

Hyperthermia can kill cells directly, but its more important use is in combination with other treatments for cancer. Hyperthermia increases blood flow to the warmed area, perhaps doubling perfusion in tumors, while increasing perfusion in normal tissue by ten times or even more. This enhances the delivery of medications. Hyperthermia also increases oxygen delivery to the area, which may make radiation more likely to damage and kill cells, as well as preventing cells from repairing the damage induced during the radiation session.

Cancerous cells are not inherently more susceptible to the effects of heat. When compared in *in vitro* studies, normal cells and cancer cells show the same responses to heat. However, the vascular disorganization of a solid tumor results in an unfavorable microenvironment inside tumors. Consequently, the tumor cells are already stressed by

low oxygen, higher than normal acid concentrations, and insufficient nutrients, and are thus significantly less able to tolerate the added stress of heat than a healthy cell in normal tissue.

Mild hyperthermia, which provides temperatures equal to that of a naturally high fever, may stimulate natural immunological attacks against the tumor, as part of a natural physiological response called thermotolerance.

Moderate hyperthermia, which heats cells in the range of 40 to 42 °C, damages cells directly, in addition to making the cells radiosensitive and increasing the pore size to improve delivery of large-molecule chemotherapeutic and immunotherapeutic agents (molecular weight greater than 1,000 Daltons), such as monoclonal antibodies and liposome-encapsulated drugs. Cellular uptake of certain small molecule drugs is also increased. Most local and regional cancer treatments are in this temperature range.

Very high temperatures, above 50 °C (122 °F), are used for ablation (direct destruction) of some tumors. This generally involves inserting a metal tube directly into the tumor, and heating the tip until the tissue next to the tube has been killed.

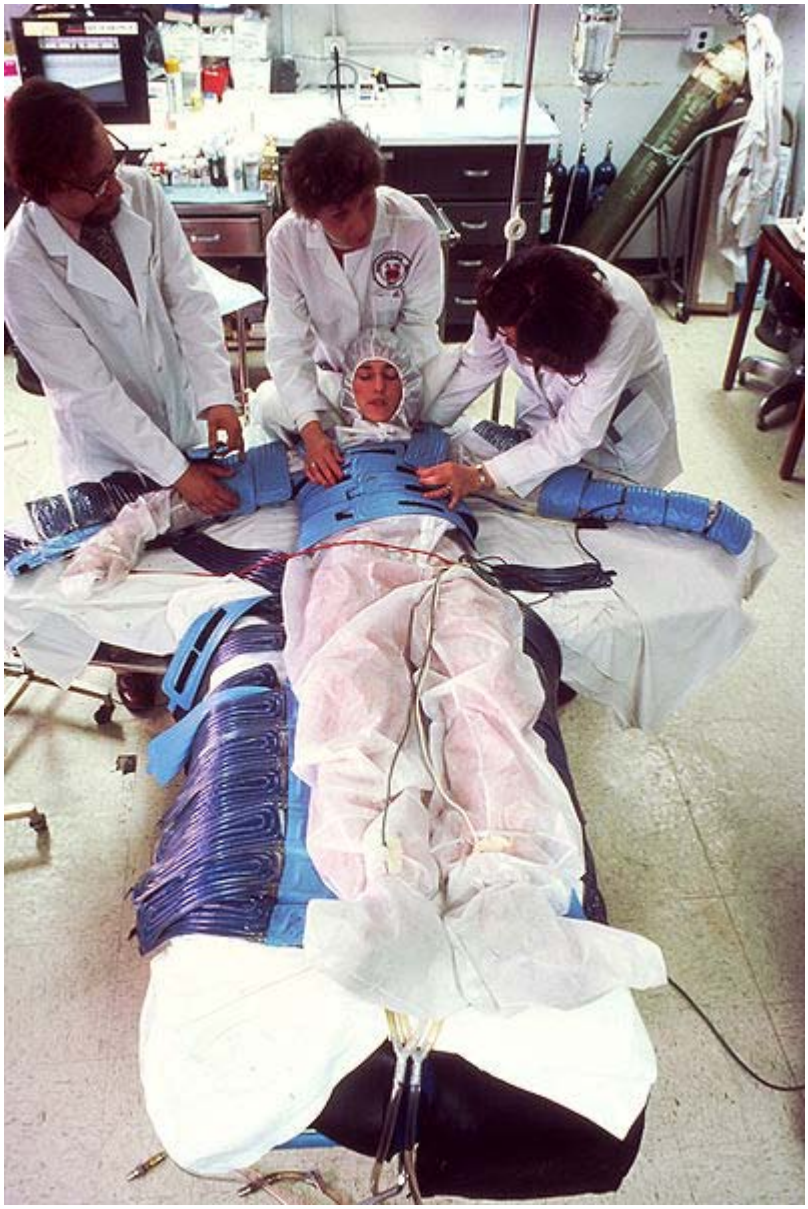
Heat sources

There are many techniques by which heat may be delivered. Some of the most common involve the use of focused ultrasound (FUS or HIFU), infrared sauna, microwave heating, induction heating, magnetic hyperthermia, infusion of warmed liquids, or direct application of heat such as through sitting in a hot room or wrapping a patient in hot blankets.

Types

- *Local hyperthermia* heats a very small area, usually the tumor itself. In some instances, the goal is to kill the tumor by "cooking" it, without damaging anything else. The heat may be created with microwave, radiofrequency, ultrasound energy or using magnetic hyperthermia. Depending on the location of the tumor, the heat may be applied to the surface of the body, inside normal body cavities, or deep in tissue through the use of needles or probes. One relatively common type is radiofrequency ablation of small tumors. This is easiest to achieve when the tumor is on a superficial part of the body, which is called *superficial hyperthermia*, or when needles or probes are inserted directly into the tumor, which is called *interstitial hyperthermia*.
- *Regional hyperthermia* heats a larger part of the body, such as an entire organ or limb. Usually, the goal is to weaken cancer cells so that they are more likely to be killed by radiation and chemotherapeutic medications. This may use the same techniques as local hyperthermia treatment, or it may rely on blood perfusion. In blood perfusion, the patient's blood is removed from the body, heated up, and returned to blood vessels that lead directly through the desired body part. Normally, chemotherapy drugs are infused at the same time. One specialized type

of this approach is continuous hyperthermic peritoneal perfusion (CHPP), which is used to treat difficult cancers within the peritoneal cavity (the abdomen), including primary peritoneal mesothelioma and stomach cancer. Hot chemotherapy drugs are pumped directly into the peritoneal cavity to kill the cancer cells.



Patient undergoing whole-body hyperthermia with doctors looking on

- *Whole-body hyperthermia* heats the entire body to temperatures of about 39 to 41 °C. It is typically used to treat metastatic cancer (cancer that spread to many parts of the body). Techniques include infrared hyperthermia domes which include the whole body apart from the head, putting the patient in a very hot room, or wrapping the patient in hot, wet blankets.

Treatment

Moderate hyperthermia treatments usually maintain the temperature for about an hour or so.

The schedule for treatments depends on the effect desired. After being heated, cells develop resistance to heat, which persists for about three days and reduces the likelihood that they will die from direct cytotoxic effects of the heat. This suggests a maximum treatment schedule of about twice a week. However, if the desired goal is increased radiosensitivity in a poorly oxygenated tumor, rather than directly killing the cells, then application of heat with every radiation treatment is acceptable.

Controlling temperatures

One of the challenges in thermal therapy is delivering the appropriate amount of heat to the correct part of the patient's body. For this technique to be effective, the temperatures must be high enough, and the temperatures must by sustained long enough, to damage or kill the cancer cells. However, if the temperatures are too high, or if they are kept elevated for too long, then serious side effects, including death, can result. The smaller the place that is heated, and the shorter the treatment time, the lower the side effects.

To minimize damage to healthy tissue and other adverse effects, physicians carefully monitor the temperature of the affected area. The goal is to keep local temperatures under 44 °C (111 °F) to avoid damage to surrounding tissues, and the whole body temperatures under 42 °C (108 °F), which is the upper limit compatible with life. These temperatures compare to the normal human body temperature, taken internally, of about 37.6 °C (99.6 °F).

A great deal of current research focuses on precisely positioning heat-delivery devices (catheters, microwave and ultrasound applicators, etc.) using ultrasound or magnetic resonance imaging, as well as developing new types of nanoparticles that make them particularly efficient absorbers while offering little or no concerns about toxicity to other tissues. Clinicians also hope to use advanced imaging techniques to monitor heat treatments in real time; heat-induced changes in tissue are sometimes perceptible using these imaging instruments.

The thermoacoustic (TA) effect refers to the generation of acoustic waves by electromagnetic (EM) irradiation, such as optical or microwave/radio frequency waves. In the past ten years, thermoacoustic tomography (TAT) using pulsed EM excitation has undergone tremendous growth. Energy deposition inside biological tissue through the absorption of incident EM pulses will create a transient temperature rise on the order of 10 mK. In the thermoelastic mechanism of acoustic generation, a sound or stress wave is produced as a consequence of the expansion induced by the temperature variation. Thermoacoustic signals are temperature dependent, which is an ideal characteristic for use in monitoring biological tissue temperature. The thermoacoustic pressure has the following expression

$$P=\mu_a H ß c^2/c_p,$$

where μ_a is the microwave absorption coefficient, H is the heating function can be written as the product of a spatial absorption function and a temporal illumination function, ß is the isobaric volume expansion coefficient, c_0 is the speed of sound, c_p is the heat capacity. The thermal expansion coefficient defines the fractional changes in the volume of a material with temperature-normally its value increases almost linearly with temperature except for the lowest temperatures. Thus, the thermoacoustic pressure can be written in the following forms:

$$P=(A+BT)*P_0$$

where A and B is a constant,whichi can be gotten by the linearship between temperature and thermal expansion coefficient. T is the temperature, P_0 is the thermoacoustic pressure at baseline temperature. The equation demonstrates that the thermoacoustic pressure is directly proportional to temperature where its variation is the reaction of sample thermodynamic parameter changes with heat.

This character of thermoacoustic signals give us a new method to monitor thermotherapy temperature, has the potential to be developed into a viable alternative to current clinical temperature monitoring device for microwave thermotherapy.

Adverse effects

External application of heat may cause blisters, which generally heal quickly, and burns, which do not. All techniques may result in pain or fatigue. Perfusion and moderate or high levels of hyperthermia can cause swelling, blood clots, and bleeding. Whole-body hyperthermia, which is the riskiest treatment, usually results in diarrhea, nausea, vomiting, fatigue, and other symptoms of sunstroke; it may also cause cardiovascular problems.

Effectiveness

By itself, hyperthermia is generally ineffective, with only small numbers of patients receiving lasting benefit. However, it may significantly increase the effectiveness of other treatments.

When combined with radiation, hyperthermia is particularly effective at increasing the damage to acidic, poorly oxygenated parts of a tumor, and cells that are preparing to divide. Hyperthermia treatment is most effective when provided at the same time, or within an hour, of the radiation. Irradiation alone produces a complete response in about 30% of patients. Combining irradiation and hyperthermia increases the complete response rate to about 70% of patients. In the past decade hyperthermia treatments in conjunction with radiation have been used with curative intent in patients with early stage cancers of the breast, head and neck, and prostate. In his observations, James Bicher recorded complete response rates were 82% for breast patients, 88% for head and neck,

and 93% for prostate patients. Projected 5 year survival rates were 80% for breast patients, 88% for head and neck, 87% for prostate patients.

Whole body hyperthermia cannot safely reach the temperatures necessary to improve the effectiveness of radiation, and thus is not used with radiation, but it may be useful for chemotherapy and immunotherapy.

History

The application of heat to treat certain conditions, including possible tumors, has a long history. Ancient Greeks, Romans, and Egyptians used heat to treat breast masses; this is still a recommended self-care treatment for breast engorgement. Medical practitioners in ancient India used regional and whole-body hyperthermia as treatments.

During the 19th century, tumor shrinkage after a high fever due to infection had been reported in a small number of cases. Typically, the reports documented the rare regression of a soft tissue sarcoma after erysipelas (an acute streptococcus bacterial infection of the skin; a different presentation of an infection by "flesh-eating bacteria") was noted. Efforts to deliberately recreate this effect led to the development of Coley's toxin. A sustained high fever after induction of illness was considered critical to treatment success. This treatment is generally considered both less effective than modern treatments and, when it includes live bacteria, inappropriately dangerous.

Around the same period Westermark used localized hyperthermia to produce tumor regression in patients. Encouraging results were also reported by Warren when he treated patients with advanced cancer of various types with a combination of heat, induced with pyrogenic substance, and x-ray therapy. Out of 32 patients, 29 improved for 1 to 6 months.

Properly controlled clinical trials on deliberately induced hyperthermia began in the 1970s.

Future directions

Hyperthermia may be combined with gene therapy, particularly using the heat shock protein 70 promoter.

Two major technological challenges make hyperthermia therapy complicated: the ability to achieve a uniform temperature in a tumor, and the ability to precisely monitor the temperatures of both the tumor and the surrounding tissue. Advances in devices to deliver uniform levels of the precise amount of heat desired, and devices to measure the total dose of heat received, are hoped for.

In locally advanced adenocarcinoma of middle and lower rectum, regional hyperthermia added to chemoradiotherapy achieved good results in terms of rate of sphincter sparing surgery.

Chapter 10

Adjuvant Cancer Therapy

Adjuvant therapy is a term used to describe the role of therapy relative to other cancer treatments.

The terms *adjuvant* and *neoadjuvant* have special meanings in oncology. Adjuvant therapy refers to additional treatment, usually given after surgery where all detectable disease has been removed, but where there remains a statistical risk of relapse due to occult disease. If known disease is left behind following surgery, then further treatment is not technically "adjuvant".

For example, radiotherapy or systemic therapy is commonly given as adjuvant treatment after surgery for a breast cancer. Systemic therapy consists of chemotherapy, immunotherapy or biological response modifiers or hormone therapy. Oncologists use statistical evidence to assess the risk of disease relapse before deciding on the specific adjuvant therapy. The aim of adjuvant treatment is to improve disease-specific and overall survival. Because the treatment is essentially for a risk, rather than for provable disease, it is accepted that a proportion of patients who receive adjuvant therapy will already have been cured by their primary surgery.

Adjuvant systemic therapy and radiotherapy are often given following surgery for many types of cancer, including colon cancer, lung cancer, pancreatic cancer, breast cancer, prostate cancer, and some gynaecological cancers.Some forms of cancer fail to benefit from adjuvant therapy, however. Such cancers include renal cell carcinoma,and certain forms of brain cancer.

Neoadjuvant therapy

Neoadjuvant therapy, in contrast to adjuvant therapy, is given *before* the main treatment. For example, systemic therapy that is given before removal of a breast is considered neoadjuvant chemotherapy. The most common reason for neoadjuvant therapy is to reduce the size of the tumor so as to facilitate more effective surgery.

Concomitant or concurrent systemic therapy

Finally, concomitant or concurrent systemic therapy refers to administering medical treatments at the same time as other therapies, such as radiation. Adjuvant hormonal

therapy is given after prostate removal in prostate cancer, but there are concerns that the side effects, in particular the cardiovascular ones, may outweigh the risk of reoccurrence. In breast cancer, adjuvant therapy may consist of chemotherapy (doxorubicin, herceptin, paclitaxel, docetaxel, cyclophosphamide, 5-fu, and methotrexate) and radiotherapy, especially after lumpectomy, and hormonal therapy (tamoxifen, femra). Adjuvant therapy in breast cancer is used in stage one and two breast cancer following lumpectomy, and in stage three breast cancer due to lymph node involvement. In glioblastoma multiforme, adjuvant chemoradiotherapy is critical in the case of a completely removed tumor, as with no other therapy, reoccurrence occurs in 1–3 months. Adjuvant therapy does not improve prognsis in stage I, II, and III renal cell carcinoma, with the possible exception of radiotherapy, which lowered the risk of local reoccurrence from 41% to 22% in one study.as a result of this resistance to chemotherapy,targeted therapies,including nexavar, sutent, rapamycin and interleukin 2 that are known to be effective in stage IV renal cell carcinoma have been studied in the adjuvant setting,without good results. In early stage one small cell lung carcinoma, adjuvant chemotherapy with gemzar, cisplatin, paclitaxel, docetaxel, and other chemotheraputic agents, and adjuvant radiotherapy is administered to either the lung (to prevent a local reoccurrence) or the brain (to prevent metastases). In testicular cancer, adjuvant either radiotherapy or chemotherapy may be used following orchidectomy. Previously, mainly radiotherapy was used, as a full course of cytotoxic chemotherapy produced far more side effects then a course of external-beam radiotherapy (EBRT) However, it has been found a single dose of carboplatin is as effective as EBRT in stage 11 testicular cancer, with only mild side effects (transeint myelosuppressive action vs severe and prolonged myelosuppresive neutropenic illness in normal chemotherapy, and much less vomiting, diarrhea, mucositis, and no alopecia in 90% of cases)Adjuvant therapy is particularly in certain types of cancer,including colorectal carcinoma, lung cancer,and medulloblastoma. In completely resected medulloblastoma, 5 year survival rate is 85% if adjuvant chemotherapy and/or craniospinal irradiation,and just 10% if no adjuvant chemotherapy or craniospinal irradiation is used.prophylatic cranial irradation for ALL is technically adjuvant, and most experts agree that cranial irradation decreases risk of CNS relapse in ALL and possibly AML,but it can cause severe side effects,and adjuvant intrathecal methotrexate and hydrocortisone may be just as effective as cranial irraditon,without severe late effects,such as developmental disability,dementia,and increased risk for second malignancy.

Side effects of adjuvant therapy

Depending on what form of treatment is used, adjuvant therapy can have side effects, like all therapy for neoplasms. Chemotherapy frequently causes vomiting, nausea, alopecia, mucositis, myelosuppresion particularly neutropenia,sometimes resaulting in septicaemia.some chemotheraputic agents can cause acute myeloid leukaemia, in particular the alkylating agents. Rarely, this risk may outweigh the risk of reoccurrence of the primary tumor. Depending on the agent(s)used, side effects such as neuropathy, leukoencephalopathy, bladder damage, constipation or diarrhea, hemorrhage, or neurocognitive changes, often colloquially called chemobrain. Radiotherapy causes radiation dermatitis and fatigue, and depending on the area being irradiated, may have other side effects. For instance, radiotherapy to the brain can causes memory loss,

headache, alopecia, and radiation necrosis of the brain. If the abdomen or spine is irradiated, nausea, vomiting, diarrhea, and dysphasgia can occur. If the pelvis is irradiated, prostitis, procitis, dysuria, metritis, diarrhea, and abdominal pain can occur. Adjuvant hormonal therapy for prostate cancer may cause cardiovascular disease, and other, possibly severe, side effects.

Specific cancers

Adjuvant therapy in malignant melanoma

The role of adjuvant therapy in malignant melanoma is and has been hotly debated by oncologists. In 1995 a multicenter study reported improved long-term and disease-free survival in melanoma patients using interferon alpha 2b as an adjuvant therapy. Thus, later that year the U.S. Food and Drug Administration (FDA) approved interferon alpha 2b for melanoma patients who are currently free of disease, to reduce the risk of reoccurrence. Since then, however, some doctors have argued that interferon treatment does not prolong survival or decrease the rate of relapse, but only causes harmful side effects those claims have not been validated by scientific research. Adjuvant chemotherapy has been used in malignant melanoma, but there is little hard evidence to use chemotherapy in the adjuvant setting. However, melanoma is not, contrary to some people's thoughts, a chemotherapy-resistant malignancy. Dacarbizine, temozolomide, and cisplatin all have a reproducible 10-20% response rate in metastatic melanoma.; however, these responses are often short lived and almost never complete. multiple studies have shown that adjuvant radiotherapy improves local reoccurrence rates in high-risk melanoma patients. The studies include at least two M.D. Anderson cancer center studies. however, none of the studies showed that adjuvant radiotherapy had a statistically significant survival benefit.

Adjuvant chemotherapy in colorectal cancer

Adjuvant chemotherapy is effective in preventing the outgrowth of micrometastatic disease from colorectal cancer that has been removed surgically. Fluorouracil is one effective agent.

Adjuvant chemotherapy in breast cancer

it has been known for at least 30 years the adjuvant chemotherapy decreases the risk of metastatic recurrence of breast cancer in woman with localized node-positive breast carcinoma.agents used include:

- doxorubicin
- docetaxel
- paclitaxel
- cyclophosphamide
- fluorouracil
- methotrexate

- epirubicin

Combination adjuvant chemotherapy for breast cancer

giving 2 or more chemotheraputic agents at once may decrease the chances of reoccurrence of the cancer,and increase overall survival in patients with breast cancer.commonly used combination chemotherapy regimines used include:

- doxorubicin and cyclophosphamide
- doxorubicin and cyclophosphamide followed by docetaxel
- doxorubicin and cyclophosphamide followed by cyclophosphamide,methotrexate,and fluorouracil
- cyclophosphamide,methotrexate,and fluorouracil.
- docetaxel and cyclophosphamide.
- docetaxel, doxorubicin,and cyclophosphamide
- cyclophosphamide,epirubicin,and fluorouracil.

Chapter 11

Alternative Cancer Treatments

Alternative cancer treatments describes alternative and complementary treatments for cancer that have not been approved by the government agencies responsible for the regulation of therapeutic goods. They include diet and exercise, chemicals, herbs, devices, and manual procedures. The treatments may be untested or unsupported by evidence, either because no proper testing has been conducted, or because testing did not demonstrate statistically significant efficacy. Concerns have been raised about the safety of some of them.

Alternative cancer treatments are typically contrasted with experimental cancer treatments, which are treatments for which experimental testing is currently underway. All currently approved chemotherapeutic cancer treatments were considered experimental cancer treatments before their safety and efficacy testing was completed.

Such therapies can be categorized broadly into three groups: alternative treatments offered as a substitute to standard medical treatment; alternative treatments as an addition to standard treatment; and treatments proposed in the past that have been found in clinical trials to be useless and/or unsafe. Some of these obsolete or disproven treatments continue to be promoted, sold, and used.

Background

Since the 1940s, medical science has developed chemotherapy, radiation therapy, adjuvant therapy and the newer targeted therapies, as well as refining surgical techniques for removing cancer. Before the development of these modern, evidence-based treatments, 90% of cancer patients died within five years. With mainstream treatments, only 34% of cancer patients die within five years. However, while generally prolonging life or permanently curing cancer, most effective, mainstream forms of cancer treatment have side effects ranging from unpleasant to fatal, and permanent cures are not guaranteed. These side effects create appeal for alternative treatments for cancer, which purport to cause fewer side effects or to increase survival rates.

Alternative cancer treatments have typically not undergone properly conducted, well-designed clinical trials, or the results have not been published due to publication bias (a refusal to publish results showing a treatment does not work). Among those that have been published, the methodology is often poor. A 2006 systematic review of 214 articles

covering 198 clinical trials of alternative cancer treatments concluded that almost none conducted dose-ranging studies, which are necessary to ensure that the patients are being given a useful amount of the treatment. These kinds of treatments appear and vanish frequently, and have throughout history.

Complementary versus alternative treatments

Complementary and alternative cancer treatments are often grouped together, but this grouping is controversial. Definitions vary, but generally speaking they are the same methods that are called "complementary" when given alongside mainstream treatments and "alternative" when they are not. Complementary therapies receive more support within the mainstream medical community than alternative treatments.

Complementary treatments are used in conjunction with proven mainstream treatments. They tend to be pleasant for the patient, not involve substances with any pharmacological effects, inexpensive, and intended to treat side effects rather than to kill cancer cells. Medical massage and self-hypnosis to treat pain are examples of complementary treatments. A 2006 systematic review of the effectiveness of complementary techniques in reducing pain concluded that although several seemed promising, conclusive evidence was lacking.

Alternative treatments, by contrast, are used in place of mainstream treatments. The most popular alternative cancer therapies are various, generally strict diets, including the macrobiotic diet. Other therapies include mind-body interventions, bioelectromagnetics, nutritional supplements, and herbs. The popularity and prevalence of different treatments varies widely by region.

People who choose alternative treatments

People who choose alternative treatments tend to believe that evidence-based medicine is ineffective, while still believing that their own health could be improved. They are impressed by physiological and other scientific-sounding information, prefer a healthcare model that treats the patient as an integrated, whole person, and are loyal to their alternative healthcare providers.

Cancer patients who choose complementary or alternative treatments in addition to conventional treatments believe themselves less likely to die than patients who choose only conventional treatments. They feel a greater sense of control over their destinies, and report less anxiety and depression.

However, patients who use alternative treatments have a poorer survival time, even after controlling for type and stage of disease. This may be because patients who accurately perceive that they are likely to survive do not attempt unproven remedies, and patients who accurately perceive that they are unlikely to survive are attracted to unproven remedies. Among patients who believe their condition to be untreatable by evidence-based medicine, "desperation drives them into the hands of anyone with a promise and a

smile." Con artists have long exploited fear, ignorance, and desperation to strip dying people of their money, comfort, and dignity.

About half the practitioners who dispense complementary or alternative treatments are physicians, although they tend to be generalists rather than oncologists. As many as 60% of physicians have referred their patients to a complementary or alternative practitioner for some purpose.

Examples of alternative treatment

None of the cancer treatments on this list have substantial evidence for their effectiveness in treating cancer. Some have shown some benefits as complementary therapy, to reduce pain. Vitamin C, perhaps the most well-known and controversial, is undergoing a clinical trial based on *in vitro* findings and theoretical speculation as to its *in vivo* effectiveness. Very few suppliers of alternative medicines have undertaken scientifically controlled clinical trials for their products, although occasional preliminary testing, or testing as adjuvant therapy, has been performed. For this reason, alternative therapies generally rely on testimonial or anecdotal evidence. In the United States, FDA regulations forbid the makers of unproven products from claiming efficacy against cancer.

Under consideration

- Quercetin
- Vitamin C megadosage by intravenous infusion Oral vitamin C, regardless of dose, is disproven. This is a type of redox therapy.
- Medicinal mushrooms
- Selenium (Selenomethionine and Se-methylselenocysteine)
- Nicholas Gonzalez's metabolic program

Mixed results

- Coley's Toxins

Unknown

- Essiac tea
- Budwig diet, a diet emphasizing flaxseed oil, milk, fruits, vegetables, and fiber. More likely to be useful for preventing cancer rather than treating it.
- Sodium Bicarbonate

Disproven or scientifically implausible

Chemical substances

- Homeopathy (disproven), tiny amounts of substances, ritually diluted according to 18th century standards

- Laetrile (disproven), also known as B-17, is a cyanide-containing extract of crushed apricot pits
- Di Bella Multitherapy (disproven), a mixture of vitamins, melatonin, and other chemicals.
- Emanuel Revici's catabolic/anabolic approach (disproven)
- Hoxsey method (disproven), a caustic, escharotic paste of herbs and arsenic that is banned in the US as "worthless", "discredited" and "quackery".
- 714X, a water-based solution purported to kill "somatids", which the inventor claims cause disease
- Escharotics such as Cansema or "black salve", usually a paste that kills any skin or tissue it is applied to
- Protocel and Cancell (disproven)
- Krebiozen (disproven), diluted blood from horses
- Hydrazine sulfate, a toxic, synthetic drug
- Proteolytic enzyme therapy
- William Koch's "glyoxide antitoxin" (fraud), also called "recrystallized synthetic toxin", which proved to be distilled water
- Radio-Sulfo Brew, a poultice made of Limburger cheese (fraud)
- Livingston-Wheeler, or Virginia Livingston's *Progenitor cryptocides* treatments, made from the patient's urine, to kill a non-existent bacteria claimed to be the cause of cancer
- Lawrence Burton's Immuno-Augmentative Therapy, claimed to energize the immune system
- Antineoplastons (disproven)
- Chaparral
- Shark cartilage

Diets

- Gerson therapy (dangerous and disproven), a combination of diet and enemas
- Johanna Brandt's "Grape Cure" (scientifically implausible), a diet of water and grapes
- Beverly Hills diet (scientifically implausible), a largely fruitarian diet
- Macrobiotic diet (scientifically implausible), a primarily vegetarian diet with no refined or processed foods (may be useful for cancer prevention, but not treatment)
- Edgar Cayce's diet (scientifically implausible), which prefers alkaline foods to acidic ones
- Juice fasting (scientifically implausible)

Electrical or physical treatments

- Orgone accumulators (fraud), a metal and cardboard box that the client sat in
- Magnet therapy (disproven), applying magnets to the body
- The Rife Machine (scientifically implausible), a radio frequency energy 'beam ray' tube machine

Energy and psychological treatments

- Anti-cancer psychotherapy (disproven), claiming that a "cancer personality" caused cancer, which could be cured through talk therapy, e.g., that of the Simonton Cancer Center, Bernie Siegel's "Exceptional Cancer Patients" (ECaP) or Deepak Chopra
- Therapeutic touch (scientifically implausible), a type of energy therapy
- Imagining successful outcomes (scientifically implausible), such as visualizing cancer cells dying, in cancer guided imagery

Examples of complementary therapy

- Acupuncture Useful to control certain symptoms, but does not kill cancer cells.
- Psychotherapy may reduce anxiety and improve quality of life
- Massage therapy may reduce pain

Alternative theories of cancer

Some alternative cancer treatments are based on unproven or disproven theories of how cancer begins or is sustained in the body. Some common concepts are:

- **Mind-body connection**: This idea says that cancer forms because of, or can be controlled through, the person's mental and emotional state. Treatments based on this idea are mind–body interventions. Proponents say that cancer forms because the person is unhappy or stressed, or that a positive attitude can cure cancer after it has formed. A typical claim is that stress, anger, fear, or sadness depresses the immune system, whereas that love, forgiveness, confidence, and happiness cause the immune system to improve, and that this improved immune system will destroy the cancer. This belief is not supported by any scientific research: people with weak immune systems have about the same rate of cancer as people with healthy immune systems. In fact, many cancers require the support of an active immune system to establish the tumor microenvironment necessary for a tumor to grow.
- **Toxin theory of cancer**: In this idea, the body's metabolic processes are overwhelmed by normal, everyday byproducts. These byproducts, called "toxins", are said to build up in the cells and cause cancer and other diseases. Treatments following this approach are usually aimed at detoxification or body cleansing, such as enemas.

Regulatory action

Government agencies around the world routinely investigate purported alternative cancer treatments in an effort to protect their citizens from fraud and abuse.

In 2008, the United States Federal Trade Commission recently acted against companies that made unsupported claims that their products, some of which included highly toxic

chemicals, could cure cancer. Targets included Omega Supply, Native Essence Herb Company, Daniel Chapter One, Gemtronics, Inc., Herbs for Cancer, Nu-Gen Nutrition, Inc., Westberry Enterprises, Inc., Jim Clark's All Natural Cancer Therapy, Bioque Technologies, Inc., Cleansing Time Pro, and Premium-essiac-tea-4less.

Chapter 12

Experimental Cancer Treatment

Experimental cancer treatments are medical therapies intended or claimed to treat cancer by improving on, supplementing or replacing conventional methods (surgery, chemotherapy, radiation, and immunotherapy).

The entries listed below vary between theoretical therapies to unproven controversial therapies. Many of these treatments are alleged to only help against specific forms of cancer. It is not a list of treatments widely available at hospitals.

Bacterial treatments

Chemotherapeutic drugs have a hard time penetrating tumors to kill them at their core because these cells may lack a good blood supply. Researchers have been using anaerobic bacteria, such as *Clostridium novyi*, to consume the interior of oxygen-poor tumours. These should then die when they come in contact with the tumour's oxygenated sides, meaning they would be harmless to the rest of the body. A major problem has been that bacteria don't consume all parts of the malignant tissue. However combining the therapy with chemotheraputic treatments can help to solve this problem.

Another strategy is to use anaerobic bacteria that have been transformed with an enzyme that can convert a non-toxic prodrug into a toxic drug. With the proliferation of the bacteria in the necrotic and hypoxic areas of the tumour the enzyme is expressed solely in the tumour. Thus a systemically applied prodrug is metabolised to the toxic drug only in the tumour. This has been demonstrated to be effective with the non pathogenic anaerobe *Clostridium sporogenes*.

Drug therapies

HAMLET (human alpha-lactalbumin made lethal to tumor cells)

HAMLET (human alpha-lactalbumin made lethal to tumor cells) is a molecular complex derived from human breast milk that kills tumor cells by a process resembling programmed cell death (apoptosis). It has been tested in humans with skin papillomas and bladder cancer.

Dichloroacetate

Dichloroacetate (DCA) has been found to shrink tumors *in vitro* in rats, and has a plausible scientific mechanism: DCA appears to reactivate suppressed mitochondria in some types of oxygen-starved tumor cells, and thus promotes apoptosis. Because it was tested for other conditions, DCA is known to be relatively safe, available, and inexpensive, and it can be taken by mouth as a pill, which is convenient. Early animal studies received attention in the media, and some doctors began controversially using the chemical off-label. As of 2008, no patients had been treated with DCA in a clinical trial, and so its actual effectiveness is unknown.

Quercetin

In vitro, quercetin shows some antitumor activity. Cultured skin and prostate cancer cells showed significant mortality (compared to nonmalignant cells) when treated with a combination of quercetin and ultrasound Note that ultrasound also promotes topical absorption by up to 1,000 times, making the use of topical quercetin and ultrasound wands an interesting proposition.

High dietary intake of fruits and vegetables is associated with reduction in cancer, and some scientists suspect quercetin may be partly responsible. Research shows that quercetin influences cellular mechanisms in vitro and in animal studies, and there is limited evidence from human population studies that quercetin may reduce the risk of lung cancer.

Insulin potentiation therapy

In insulin potentiation therapy (IPT), insulin is a controversial supplement to low-dose chemotherapy. Its proponents claim insulin therapy increases the uptake of chemotherapeutic drugs by malignant cells, permitting the use of lower total drug doses and reducing side effects.

The first clinical trial, involving 30 women with breast cancer, combined insulin with low-dose methotrexate (a common chemotherapy drug) resulted in greatly increased stable disease, and much reduced progressive disease, compared with insulin or low-dose methotrexate alone.

Drugs that restore p53 activity

Several drug therapies are being developed based on p53, the tumour suppressor gene that protects the cell in response to damage and stress. It is like you deciding what to do with a damaged car: p53 brings everything to a halt, and then decides whether to fix the cell or whether the cell is beyond repair and should be destroyed. This protective function of p53 is disabled in most cancer cells, allowing them to multiply without check. Restoration of p53 activity in tumours (where possible) has been shown to inhibit tumour growth and can even shrink the tumour.

As p53 protein levels are usually kept low, one could block its degradation and allow large amounts of p53 to accumulate, thus stimulating p53 activity and its antitumour effects. Drugs that utilize this mechanism include nutlin and MI-219, which are both in phase I clinical trials. There are also other drugs that are still in the preclinical stage of testing, such as RITA and MITA.

Gene therapy

Introduction of tumor suppressor genes into rapidly dividing cells has been thought to slow down or arrest tumor growth. Adenoviruses are a commonly utilized vector for this purpose. Much research has focused on the use of adenoviruses which cannot reproduce, or reproduce only to a limited extent, within the patient to ensure safety via the avoidance of cytolytic destruction of noncancerous cells infected with the vector. However, new studies focus on adenoviruses which can be permitted to reproduce, and destroy cancerous cells in the process, since the adenoviruses' ability to infect normal cells is substantially impaired, potentially resulting in a far more effective treatment. Another use of gene therapy is the introduction of enzymes into these cells that make them susceptible to particular chemotherapy agents; studies with introducing thymidine kinase in gliomas, making them susceptible to aciclovir, are in their experimental stage.

Telomerase therapy

Because most malignant cells rely on the activity of the protein telomerase for their immortality, it has been proposed that a drug which inactivates telomerase might be effective against a broad spectrum of malignancies. At the same time, most healthy tissues in the body express little if any telomerase, and would function normally in its absence. Currently, Inositol hexaphosphate, which is available over-the-counter, is undergoing testing in cancer research due to its telomerase-inhibiting abilities.

A number of research groups have experimented with the use of telomerase inhibitors in animal models, and as of 2005 and 2006 phase I and II human clinical trials are underway. Geron Corporation, is currently conducting two clinical trials involving telomerase inhibitors. One uses a vaccine (GRNVAC1) and the other uses a lipidated drug (GRN163L).

Radiation therapies

Photodynamic therapy

Photodynamic therapy (PDT) is generally a non-invasive treatment using a combination of light and a photosensitive drug, such as 5-ALA, Foscan, Metvix, Tookad, WST09, WST11, Photofrin or Visudyne. The drug is triggered by light of a specific wavelength.

Hyperthermia therapy

Localized and whole-body application of heat has been proposed as a technique for the treatment of malignant tumours. Intense heating will cause denaturation and coagulation of cellular proteins, rapidly killing cells within a tumour.

More prolonged moderate heating to temperatures just a few degrees above normal can cause more subtle changes. A mild heat treatment combined with other stresses can cause cell death by apoptosis. There are many biochemical consequences to the heat shock response within in cell, including slowed cell division and increased sensitivity to ionizing radiation therapy.

There are many techniques by which heat may be delivered. Some of the most common involve the use of focused ultrasound (FUS or HIFU), microwave heating, induction heating, magnetic hyperthermia or direct application of heat through the use of heated saline pumped through catheters. Experiments have been done with carbon nanotubes that selectively bind to cancer cells. Lasers are then used that pass harmlessly through the body, but heat the nanotubes, causing the death of the cancer cells. Similar results have also been achieved with other types of nanoparticles including gold-coated nanoshells and nanorods which exhibit certain degrees of 'tunability' of the absorption properties of the nanoparticles to the wavelength of light for irradiation. The success of this approach to cancer treatment rests on the existence of an 'optical window' in which biological tissue (i.e. healthy cells) are completely transparent at the wavelength of the laser light while nanoparticles are highly absorbing at the same wavelength. Such a 'window' exists in the so-called near infrared region of the electromagnetic spectrum. In this way, the laser light can pass through the system without harming healthy tissue and only diseased cells, where the nanoparticles reside, get hot and are killed.

Magnetic hyperthermia makes use of magnetic nanoparticles, which can be injected into tumours and then generate heat when subjected to an alternating magnetic field.

One of the challenges in thermal therapy is delivering the appropriate amount of heat to the correct part of the patient's body. A great deal of current research focuses on precisely positioning heat delivery devices (catheters, microwave and ultrasound applicators, etc.) using ultrasound or magnetic resonance imaging, as well as of developing new types of nanoparticles that make them particularly efficient absorbers while offering little or no concerns about toxicity to the circulation system. Clinicians also hope to use advanced imaging techniques to monitor heat treatments in real time—heat-induced changes in tissue are sometimes perceptible using these imaging instruments.

Non-invasive RF cancer treatment

This preclinical treatment involves using radio waves to heat up tiny metals which are implanted in cancerous tissue. Gold nanoparticles or carbon nanotubes are the most likely candidate. Promising preclinical trials have been conducted, although clinical trials may not be held for another few years.

Complementary and alternative treatments

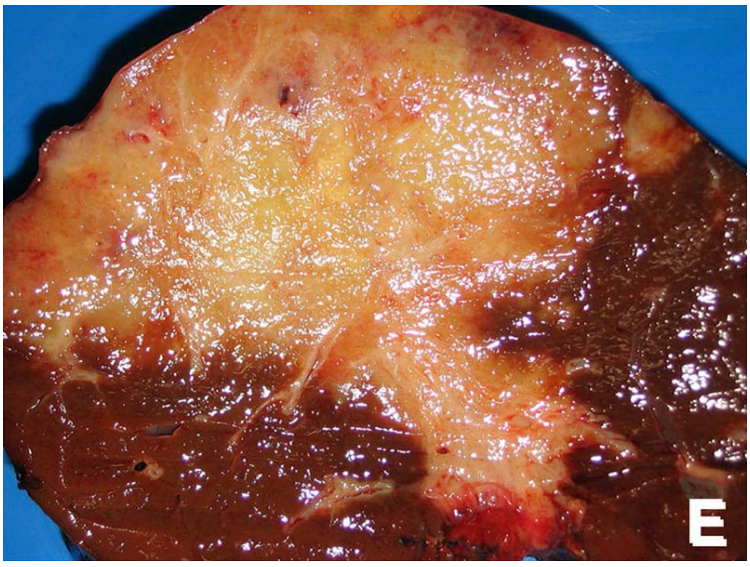
Complementary and alternative medicine (CAM) treatments are the diverse group of medical and health care systems, practices, and products that are not part of conventional medicine and have not been shown to be effective. *Complementary medicine* usually refers to methods and substances used along with conventional medicine, while *alternative medicine* refers to compounds used instead of conventional medicine. CAM use is common among people with cancer.

Most complementary and alternative medicines for cancer have not been rigorously studied or tested. Some alternative treatments that have been proven ineffective continue to be marketed and promoted.

Chapter 13

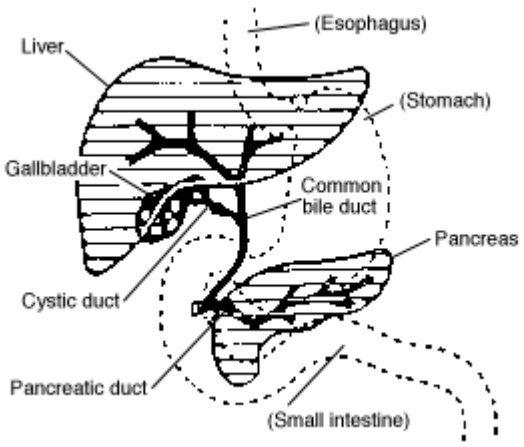
Cholangiocarcinoma

Cholangiocarcinoma



Photograph of cholangiocarcinoma in human liver.

ICD-10	C22.1
ICD-9	155.1, 156.1
ICD-O:	M8160/3
DiseasesDB	2505
MedlinePlus	000291
eMedicine	med/343 radio/153
MeSH	D018281



Digestive system diagram showing bile duct location

Cholangiocarcinoma is a cancer of the bile ducts which drain bile from the liver into the small intestine. Other biliary tract cancers include pancreatic cancer, gall bladder cancer, and cancer of the ampulla of Vater. Cholangiocarcinoma is a relatively rare adenocarcinoma (glandular cancer), with an annual incidence of 1–2 cases per 100,000 in the Western world, but rates of cholangiocarcinoma have been rising worldwide over the past several decades.

Prominent symptoms of cholangiocarcinoma include abnormal liver function tests, abdominal pain, jaundice, weight loss, and sometimes generalized itching, fever, or changes in stool or urine color. The disease is diagnosed through a combination of blood tests, imaging, endoscopy, and sometimes surgical exploration. Cholangiocarcinoma is often in an advanced stage by the time symptoms develop, which may limit treatment options. Known risk factors for cholangiocarcinoma include primary sclerosing cholangitis (an inflammatory disease of the bile ducts), congenital liver malformations, infection with the parasitic liver flukes *Opisthorchis viverrini* or *Clonorchis sinensis*, and exposure to Thorotrast (thorium dioxide), a chemical formerly used in medical imaging. However, most patients with cholangiocarcinoma have no specific risk factors.

Cholangiocarcinoma is considered to be an incurable and rapidly lethal disease unless all of its tumors can be fully resected (cut out surgically). There is no potentially curative treatment except surgery, but unfortunately most patients have advanced and inoperable disease at the time of diagnosis. Patients with cholangiocarcinoma are generally managed, though never cured, with chemotherapy or radiation therapy as well as palliative care measures, and these are also used as adjuvant therapies post-surgically in cases where resection has been successful. Some areas of ongoing medical research in cholangiocarcinoma include the use of newer targeted therapies (such as erlotinib) or photodynamic therapy for treatment, and the concentration of byproducts of cancer stromal cell formation in the blood for diagnosis.

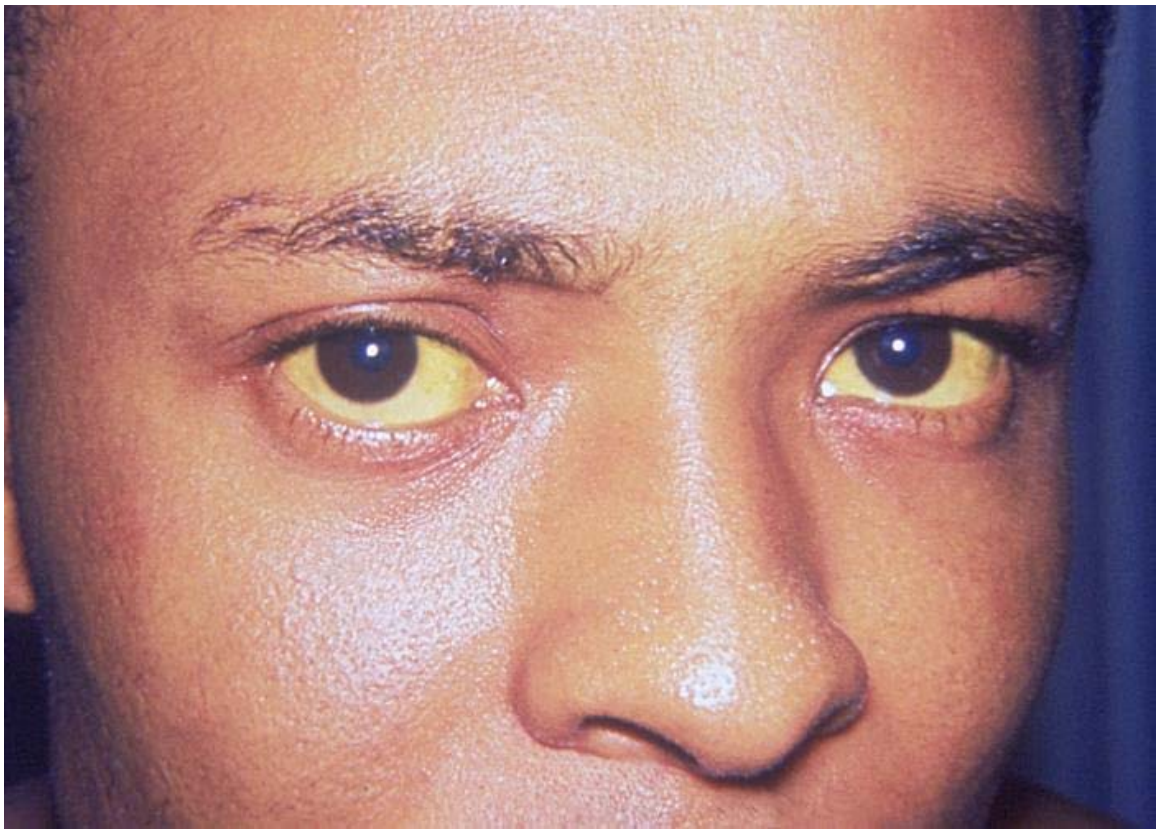
Staging

Although there are at least three staging systems for cholangiocarcinoma (e.g. Bismuth, Blumgart, American Joint Committee on Cancer) none have been shown to be useful in predicting survival. The most important staging issue is whether the tumor can be surgically removed, or whether it is too advanced or invasive for surgical treatment. Often, this determination can only be made at the time of surgery.

General guidelines for operability include:

- Absence of lymph node or liver metastases
- Absence of involvement of the portal vein
- Absence of direct invasion of adjacent organs
- Absence of widespread metastatic disease

Signs and symptoms



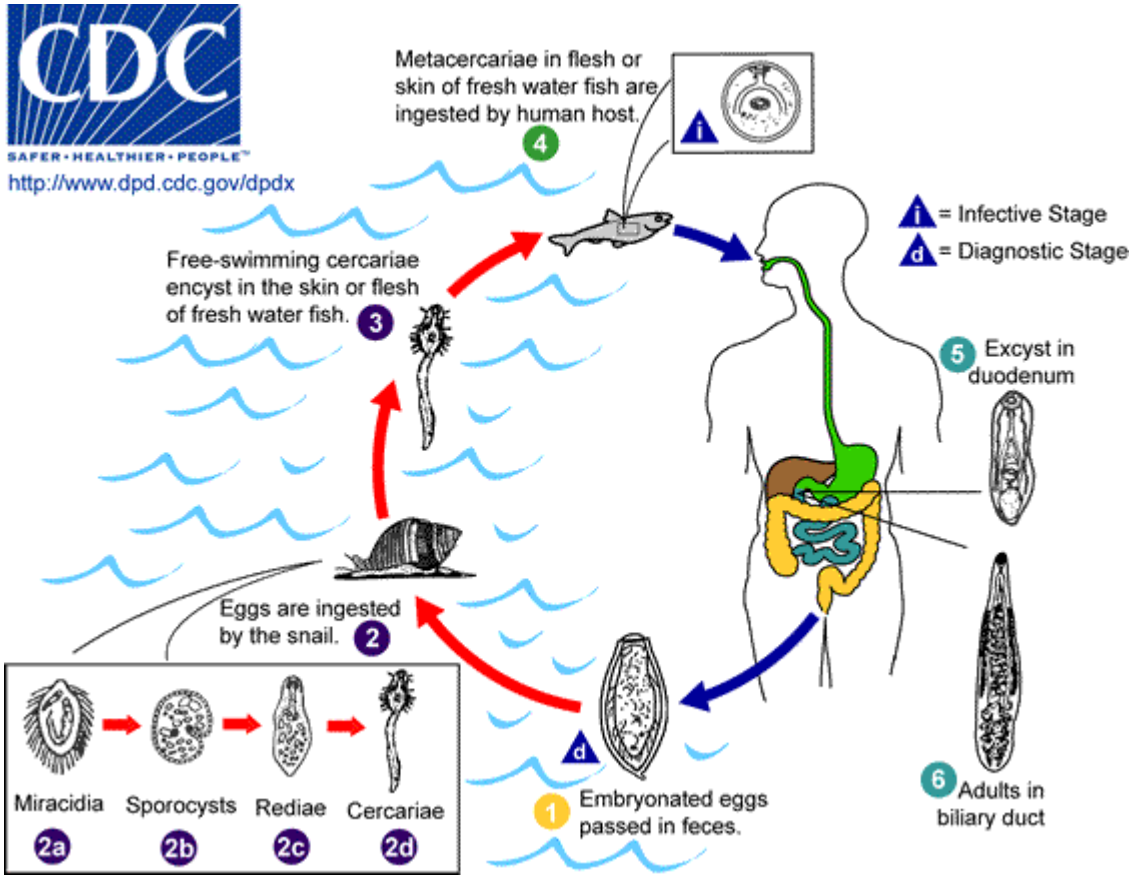
Yellowing of the skin and eyes (jaundice)

The most common physical indications of cholangiocarcinoma are abnormal liver function tests, jaundice (yellowing of the eyes and skin), which occurs only, when bile ducts are blocked by the tumor, abdominal pain (30%–50%), generalized itching (66%), weight loss (30%–50%), fever (up to 20%), or changes in stool or urine color. To some

extent, the symptoms depend upon the location of the tumor: Patients with cholangiocarcinoma in the extrahepatic bile ducts (outside the liver) are more likely to have jaundice, while those with tumors of the bile ducts within the liver often have pain without jaundice.

Blood tests of liver function in patients with cholangiocarcinoma often reveal a so-called "obstructive picture," with elevated bilirubin, alkaline phosphatase, and gamma glutamyl transferase levels, and relatively normal transaminase levels. Such laboratory findings suggest obstruction of the bile ducts, rather than inflammation or infection of the liver, as the primary cause of the jaundice. CA19-9 is elevated in most cases.

Risk factors



Life cycle of *Clonorchis sinensis*, a liver fluke associated with cholangiocarcinoma

Although most patients present without any known risk factors evident, a number of risk factors for the development of cholangiocarcinoma have been described; in the Western world, the most common of these is primary sclerosing cholangitis (PSC), an inflammatory disease of the bile ducts which is in turn closely associated with ulcerative colitis (UC). Epidemiologic studies have suggested that the lifetime risk of developing cholangiocarcinoma for a person with PSC is 10%–15%, although autopsy series have

found rates as high as 30% in this population. The mechanism by which PSC increases the risk of cholangiocarcinoma is not well understood.

Certain parasitic liver diseases may be risk factors as well. Colonization with the liver flukes *Opisthorchis viverrini* (found in Thailand, Laos, and Malaysia) or *Clonorchis sinensis* (found in Japan, Korea, and Vietnam) has been associated with the development of cholangiocarcinoma. Patients with chronic liver disease, whether in the form of viral hepatitis (e.g. hepatitis B or C), alcoholic liver disease, or cirrhosis from other causes, are at increased risk of cholangiocarcinoma. HIV infection was also identified in one study as a potential risk factor for cholangiocarcinoma, although it was unclear whether HIV itself or correlated factors (e.g. hepatitis C infection) were responsible for the association.

Congenital liver abnormalities, such as Caroli's syndrome or choledochal cysts, have been associated with an approximately 15% lifetime risk of developing cholangiocarcinoma. The rare inherited disorders Lynch syndrome II and biliary papillomatosis are associated with cholangiocarcinoma. The presence of gallstones (cholelithiasis) is not clearly associated with cholangiocarcinoma. However, intrahepatic stones (so-called hepatolithiasis), which are rare in the West but common in parts of Asia, have been strongly associated with cholangiocarcinoma. Exposure to Thorotrast, a form of thorium dioxide which was used as a radiologic contrast medium, has been linked to the development of cholangiocarcinoma as late as 30–40 years after exposure; Thorotrast was banned in the United States in the 1950s due to its carcinogenicity.

Pathophysiology

Cholangiocarcinoma can affect any area of the bile ducts, either within or outside the liver. Tumors occurring in the bile ducts within the liver are referred to as *intrahepatic*, those occurring in the ducts outside the liver are *extrahepatic*; and tumors occurring at the site where the bile ducts exit the liver may be referred to as *perihilar*. A cholangiocarcinoma occurring at the junction where the left and right hepatic ducts meet to form the common bile duct may be referred to eponymously as a Klatskin tumor.

Although cholangiocarcinoma is known to be an adenocarcinoma of the epithelial cells lining the biliary tract, the actual cell of origin is unknown, although recent evidence has suggested that it may arise from a pluripotent hepatic stem cell. Cholangiocarcinoma is thought to develop through a series of stages — from early hyperplasia and metaplasia, through dysplasia, to the development of frank carcinoma — in a process similar to that seen in the development of colon cancer. Chronic inflammation and obstruction of the bile ducts, and the resulting impaired bile flow, are thought to play a role in this progression.

Histologically, cholangiocarcinomas may vary from undifferentiated to well-differentiated. They are often surrounded by a brisk fibrotic or desmoplastic tissue response; in the presence of extensive fibrosis, it can be difficult to distinguish well-differentiated cholangiocarcinoma from normal reactive epithelium. There is no entirely specific immunohistochemical stain that can distinguish malignant from benign biliary

ductal tissue, although staining for cytokeratins, carcinoembryonic antigen, and mucins may aid in diagnosis. Most tumors (>90%) are adenocarcinomas.

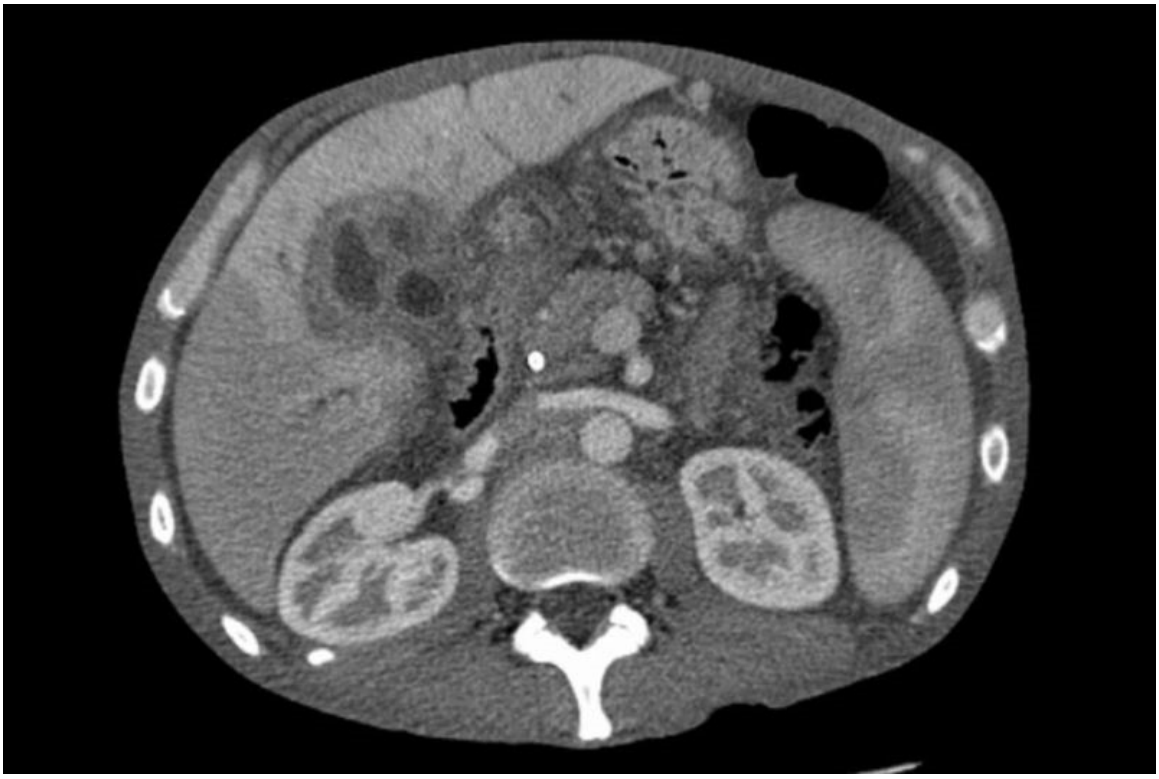
Diagnosis

Cholangiocarcinoma is definitively diagnosed from tissue, i.e. it is proven by biopsy or examination of the tissue excised at surgery. It may be suspected in a patient with obstructive jaundice. Considering it as the working diagnosis may be challenging in patients with primary sclerosing cholangitis (PSC); such patients are at high risk of developing cholangiocarcinoma, but the symptoms may be difficult to distinguish from those of PSC. Furthermore, in patients with PSC, such diagnostic clues as a visible mass on imaging or biliary ductal dilatation may not be evident.

Blood tests

There are no specific blood tests that can diagnose cholangiocarcinoma by themselves. Serum levels of carcinoembryonic antigen (CEA) and CA19-9 are often elevated, but are not sensitive or specific enough to be used as a general screening tool. However, they may be useful in conjunction with imaging methods in supporting a suspected diagnosis of cholangiocarcinoma.

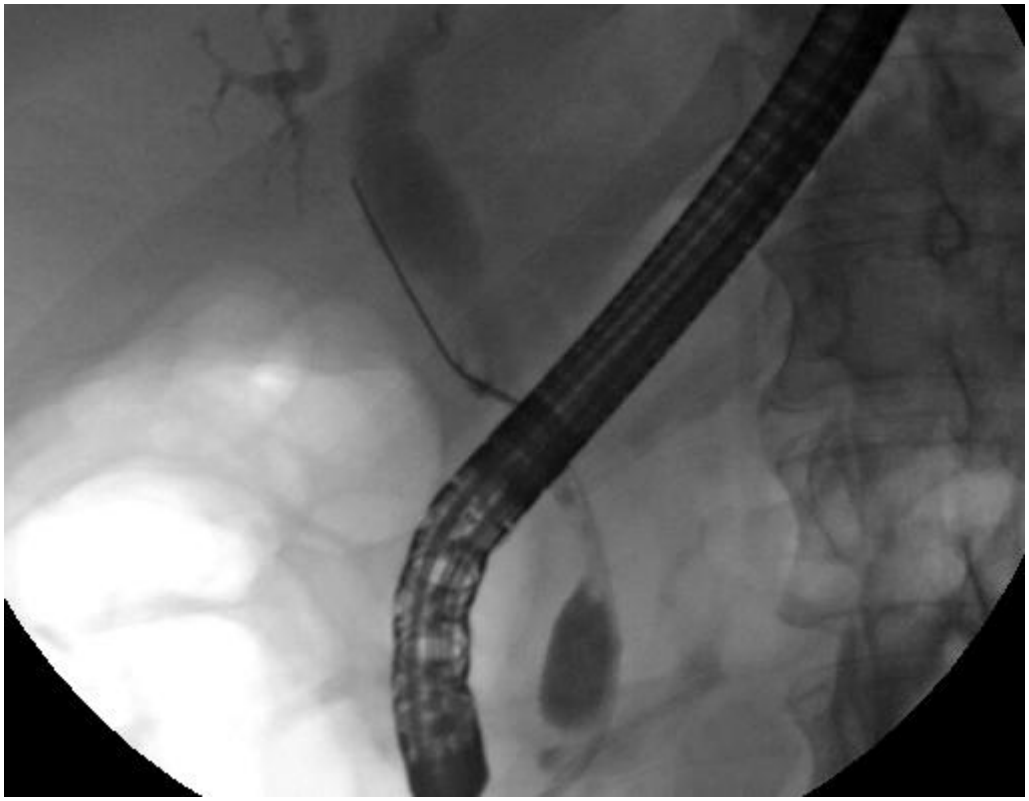
Abdominal imaging



CT scan showing cholangiocarcinoma

Ultrasound of the liver and biliary tree is often used as the initial imaging modality in patients with suspected obstructive jaundice. Ultrasound can identify obstruction and ductal dilatation and, in some cases, may be sufficient to diagnose cholangiocarcinoma. Computed tomography (CT) scanning may also play an important role in the diagnosis of cholangiocarcinoma.

Imaging of the biliary tree



ERCP image of cholangiocarcinoma, showing common bile duct stricture and dilation of the proximal common bile duct

While abdominal imaging can be useful in the diagnosis of cholangiocarcinoma, direct imaging of the bile ducts is often necessary. Endoscopic retrograde cholangiopancreatography (ERCP), an endoscopic procedure performed by a gastroenterologist or specially trained surgeon, has been widely used for this purpose. Although ERCP is an invasive procedure with attendant risks, its advantages include the ability to obtain biopsies and to place stents or perform other interventions to relieve biliary obstruction. Endoscopic ultrasound can also be performed at the time of ERCP and may increase the accuracy of the biopsy and yield information on lymph node invasion and operability. As an alternative to ERCP, percutaneous transhepatic cholangiography (PTC) may be utilized. Magnetic resonance cholangiopancreatography (MRCP) is a non-invasive alternative to ERCP. Some authors have suggested that MRCP should supplant ERCP in the diagnosis of biliary cancers, as it may more accurately define the tumor and avoids the risks of ERCP.

Surgery

Surgical exploration may be necessary to obtain a suitable biopsy and to accurately stage a patient with cholangiocarcinoma. Laparoscopy can be used for staging purposes and may avoid the need for a more invasive surgical procedure, such as laparotomy, in some patients. Surgery is also the only curative option for cholangiocarcinoma, although it is limited to patients with early-stage disease.

Pathology

Histologically, cholangiocarcinomas are classically well to moderately differentiated. Immunohistochemistry is useful in the diagnosis and can be used to differentiate a cholangiocarcinoma primary tumour from metastasis of most other gastrointestinal tumours. Cytological scrapings are often nondiagnostic.

Treatment

Cholangiocarcinoma is considered to be an incurable and rapidly lethal disease unless all the tumors can be fully resected (that is, cut out surgically). Since the operability of the tumor can only be assessed during surgery in most cases, a majority of patients undergo exploratory surgery unless there is already a clear indication that the tumor is inoperable.

Adjuvant therapy followed by liver transplantation may have a role in treatment of certain unresectable cases.

Adjuvant chemotherapy and radiation therapy

If the tumor can be removed surgically, patients may receive adjuvant chemotherapy or radiation therapy after the operation to improve the chances of cure. If the tissue margins are negative (i.e. the tumor has been totally excised), adjuvant therapy is of uncertain benefit. Both positive and negative results have been reported with adjuvant radiation therapy in this setting, and no prospective randomized controlled trials have been conducted as of March 2007. Adjuvant chemotherapy appears to be ineffective in patients with completely resected tumors. The role of combined chemoradiotherapy in this setting is unclear. However, if the tumor tissue margins are positive, indicating that the tumor was not completely removed via surgery, then adjuvant therapy with radiation and possibly chemotherapy is generally recommended based on the available data.

Treatment of advanced disease

The majority of cases of cholangiocarcinoma present as inoperable (unresectable) disease in which case patients are generally treated with palliative chemotherapy, with or without radiotherapy. Chemotherapy has been shown in a randomized controlled trial to improve quality of life and extend survival in patients with inoperable cholangiocarcinoma. There is no single chemotherapy regimen which is universally used, and enrollment in clinical trials is often recommended when possible. Chemotherapy agents used to treat

cholangiocarcinoma include 5-fluorouracil with leucovorin, gemcitabine as a single agent, or gemcitabine plus cisplatin, irinotecan, or capecitabine. A small pilot study suggested possible benefit from the tyrosine kinase inhibitor erlotinib in patients with advanced cholangiocarcinoma.

Photodynamic therapy, an experimental approach in which patients are injected with a light-sensitizing agent and light is then applied endoscopically directly to the tumor, has shown promising results compared to supportive care in two small randomized controlled trials. However, its ultimate role in the management of cholangiocarcinoma is unclear at present. Photodynamic Therapy has been shown to improve survival and quality of life

Prognosis

Surgical resection offers the only potential chance of cure in cholangiocarcinoma. For non-resectable cases, the 5-year survival rate is 0% where the disease is inoperable because distal lymph nodes show metastases, and less than 5% in general. Overall median duration of survival is less than 6 months in inoperable, untreated, otherwise healthy patients with tumors involving the liver by way of the intrahepatic bile ducts and hepatic portal vein.

For surgical cases, the odds of cure vary depending on the tumor location and whether the tumor can be completely, or only partially, removed. Distal cholangiocarcinomas (those arising from the common bile duct) are generally treated surgically with a Whipple procedure; long-term survival rates range from 15%–25%, although one series reported a five-year survival of 54% for patients with no involvement of the lymph nodes. Intrahepatic cholangiocarcinomas (those arising from the bile ducts within the liver) are usually treated with partial hepatectomy. Various series have reported survival estimates after surgery ranging from 22%–66%; the outcome may depend on involvement of lymph nodes and completeness of the surgery. Perihilar cholangiocarcinomas (those occurring near where the bile ducts exit the liver) are least likely to be operable. When surgery is possible, they are generally treated with an aggressive approach often including removal of the gallbladder and potentially part of the liver. In patients with operable perihilar tumors, reported 5-year survival rates range from 20%–50%.

The prognosis may be worse for patients with primary sclerosing cholangitis who develop cholangiocarcinoma, likely because the cancer is not detected until it is advanced. Some evidence suggests that outcomes may be improving with more aggressive surgical approaches and adjuvant therapy.

Epidemiology

Cholangiocarcinoma is an adenocarcinoma of the biliary tract, along with pancreatic cancer (which occurs about 20 times more frequently), gall bladder cancer (which occurs twice as often), and cancer of the ampulla of Vater. Treatments and clinical trials for pancreatic cancer, being far more prevalent, are often taken as a starting point for managing cholangiocarcinoma, even though the biologies are different enough that

chemotherapies can put pancreatic cancer into permanent remission whereas there are no reports in the literature of long-term survival due to chemotherapy or radiation applied to an inoperable cholangiocarcinoma case.

Country	*IC (men/women)*	*EC (men/women)*
U.S.A.	0.60 / 0.43	0.70 / 0.87
Japan	0.23 / 0.10	5.87 / 5.20
Australia	0.70 / 0.53	0.90 / 1.23
England/Wales	0.83 / 0.63	0.43 / 0.60
Scotland	1.17 / 1.00	0.60 / 0.73
France	0.27 / 0.20	1.20 / 1.37
Italy	0.13 / 0.13	2.10 / 2.60

Age-standardized mortality rates from intrahepatic (IC) and extrahepatic (EC) cholangiocarcinoma for men and women, by country.

Cholangiocarcinoma is a relatively rare form of cancer; each year, approximately 2,000 to 3,000 new cases are diagnosed in the United States, translating into an annual incidence of 1–2 cases per 100,000 people. Autopsy series have reported a prevalence of 0.01% to 0.46%. There is a higher prevalence of cholangiocarcinoma in Asia, which has been attributed to endemic chronic parasitic infestation. The incidence of cholangiocarcinoma increases with age, and the disease is slightly more common in men than in women (possibly due to the higher rate of primary sclerosing cholangitis, a major risk factor, in men). The prevalence of cholangiocarcinoma in patients with primary sclerosing cholangitis may be as high as 30%, based on autopsy studies.

Multiple studies have documented a steady increase in the incidence of intrahepatic cholangiocarcinoma over the past several decades; increases have been seen in North America, Europe, Asia, and Australia. The reasons for the increasing occurrence of cholangiocarcinoma are unclear; improved diagnostic methods may be partially responsible, but the prevalence of potential risk factors for cholangiocarcinoma, such as HIV infection, has also been increasing during this time frame.

Chapter 14

Immunology

Immunology is a broad branch of biomedical science that covers the study of all aspects of the immune system in all organisms. It deals with the physiological functioning of the immune system in states of both health and disease; malfunctions of the immune system in immunological disorders (autoimmune diseases, hypersensitivities, immune deficiency, transplant rejection); the physical, chemical and physiological characteristics of the components of the immune system in vitro, in situ, and in vivo. Immunology has applications in several disciplines of science, and as such is further divided.

Histological examination of the immune system

Even before the concept of immunity (from *immunis*, Latin for "exempt") was developed, numerous early physicians characterized organs that would later prove to be part of the immune system. The key primary lymphoid organs of the immune system are like thymus and bone marrow, and secondary lymphatic tissues such as spleen, tonsils, lymph vessels, lymph nodes, adenoids, and skin and liver. When health conditions warrant, immune system organs including the thymus, spleen, portions of bone marrow, lymph nodes and secondary lymphatic tissues can be surgically excised for examination while patients are still alive.

Many components of the immune system are actually cellular in nature and not associated with any specific organ but rather are embedded or circulating in various tissues located throughout the body.

Classical immunology

Classical immunology ties in with the fields of epidemiology and medicine. It studies the relationship between the body systems, pathogens, and immunity. The earliest written mention of immunity can be traced back to the plague of Athens in 430 BCE. Thucydides noted that people who had recovered from a previous bout of the disease could nurse the sick without contracting the illness a second time. Many other ancient societies have references to this phenomenon, but it was not until the 19th and 20th centuries before the concept developed into scientific theory.

The study of the molecular and cellular components that comprise the immune system, including their function and interaction, is the central science of immunology. The

immune system has been divided into a more primitive innate immune system, and acquired or adaptive immune system of vertebrates, the latter of which is further divided into humoral and cellular components.

The humoral (antibody) response is defined as the interaction between antibodies and antigens. Antibodies are specific proteins released from a certain class of immune cells (B lymphocytes). Antigens are defined as anything that elicits generation of antibodies, hence they are **Anti**body **Gen**erators. Immunology itself rests on an understanding of the properties of these two biological entities. However, equally important is the cellular response, which can not only kill infected cells in its own right, but is also crucial in controlling the antibody response. Put simply, both systems are highly interdependent.

In the 21st century, immunology has broadened its horizons with much research being performed in the more specialized niches of immunology. This includes the immunological function of cells, organs and systems not normally associated with the immune system, as well as the function of the immune system outside classical models of immunity (Yemeserach 2010).

Clinical immunology

Clinical immunology is the study of diseases caused by disorders of the immune system (failure, aberrant action, and malignant growth of the cellular elements of the system). It also involves diseases of other systems, where immune reactions play a part in the pathology and clinical features.

The diseases caused by disorders of the immune system fall into two broad categories: immunodeficiency, in which parts of the immune system fail to provide an adequate response (examples include chronic granulomatous disease), and autoimmunity, in which the immune system attacks its own host's body (examples include systemic lupus erythematosus, rheumatoid arthritis, Hashimoto's disease and myasthenia gravis). Other immune system disorders include different hypersensitivities, in which the system responds inappropriately to harmless compounds (asthma and other allergies) or responds too intensely.

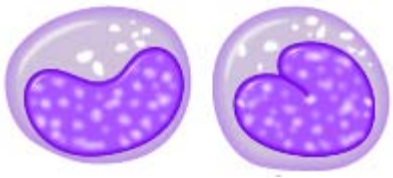
The most well-known disease that affects the immune system itself is AIDS, caused by HIV. AIDS is an immunodeficiency characterized by the lack of CD4+ ("helper") T cells and macrophages, which are destroyed by HIV.

Clinical immunologists also study ways to prevent transplant rejection, in which the immune system attempts to destroy allografts os

Developmental immunology

The body’s capability to react to antigen depends on a person's age, antigen type, maternal factors and the area where the antigen is presented. Neonates are said to be in a state of physiological immunodeficiency, because both their innate and adaptive

immunological responses are greatly suppressed. Once born, a child's immune system responds favorably to protein antigens while not as well to glycoproteins and polysaccharies. In fact, many of the infections acquired by neonates are caused by low virulence organisms like Staphylococcus and Pseudomonas. In neonates, opsonic activity and the ability to activate the complement cascade is very limited. For example, the mean level of C3 in a newborn is approximately 65% of that found in the adult. Phagocytic activity is also greatly impaired in newborns. This is due to lower opsonic activity, as well as diminished up-regulation of integrin and selectin receptors, which limit the ability of neutrophils to interact with adhesion molecules in the endothelium. Their monocytes are slow and have a reduced ATP production, which also limits the newborns phagocitic activity. Although, the number of total lymphocytes is significantly higher than in adults, the cellular and humoral immunity is also impaired. Antigen presenting cells in newborns have a reduced capability to activate T cells. Also, T cells of a newborn proliferate poorly and produce very small amounts of cytokines like IL-2, IL-4, IL-5, IL-12, and IFN-g which limits their capacity to activate the humoral response as well as the phagocitic activity of macrophage. B cells develop early in gestation but are not fully active.



Monocytes: An Artist's Impression

Maternal factors also play a role in the body's immune response. At birth most of the immunoglobulin is present is maternal IgG. Because IgM, IgD, IgE and IgA don't cross the placenta, they are almost undetectable at birth. Although some IgA is provided in breast milk. These passively acquired antibodies can protect the newborn up to 18 months, but their response is usually short-live and of low affinity. These antibodies can also produce a negative response. If a child is exposed to the antibody for a particular antigen before being exposed to the antigen itself then the child will produce a dampened response. Passively acquired maternal antibodies can suppress the antibody response to active immunization. Similarly the response of T-cells to vaccination differs in children compared to adults, and vaccines that induce Th1 responses in adults do not readily elicit these same responses in neonates. By 6-9 months after birth, a child's immune system begins to respond more strongly to glycoproteins. Not until 12-24 months of age is there a marked improvement in the body's response to polysaccharides. This can be the reason for the specific time frames found in vaccination schedules.

During adolescence the human body undergoes several physical, physiological and immunological changes. These changes are started and mediated by different hormones. Depending on the sex either testosterone or 17-β-oestradiol, act on male and female bodies accordingly, start acting at ages of 12 and 10 years. There is evidence that these steroids act directly not only on the primary and secondary sexual characteristics, but also have an effect on the development and regulation of the immune system. There is an

increased risk in developing autoimmunity for pubescent and post pubescent females and males. There is also some evidence that cell surface receptors on B cells and macrophages may detect sex hormones in the system. The female sex hormone 17-β-oestradiol has been shown to regulate the level of immunological response. Similarly, some male androgens, like testosterone, seem to suppress the stress response to infection; but other androgens like DHEA have the opposite effect, as it increases the immune response instead of down playing it. As in females, the male sex hormones seem to have more control of the immune system during puberty and the time right after than in fully developed adults. Other than hormonal changes physical changes like the involution of the Thymus during puberty will also affect the immunological response of the subject or patient.

Immunotherapy

The use of immune system components to treat a disease or disorder is known as immunotherapy. Immunotherapy is most commonly used in the context of the treatment of cancers together with chemotherapy (drugs) and radiotherapy (radiation). However, immunotherapy is also often used in the immunosuppressed (such as HIV patients) and people suffering from other immune deficiencies or autoimmune diseases.

Diagnostic immunology

The specificity of the bond between antibody and antigen has made it an excellent tool in the detection of substances in a variety of diagnostic techniques. Antibodies specific for a desired antigen can be conjugated with a radiolabel, fluorescent label, or color-forming enzyme and are used as a "probe" to detect it. However, the similarity between some antigens can lead to false positives and other errors in such tests by antibodies cross-reacting with antigens that aren't exact matches.

Evolutionary immunology

Study of the immune system in extant and extinct species is capable of giving us a key understanding of the evolution of species and the immune system.

A development of complexity of the immune system can be seen from simple phagocytotic protection of single celled organisms, to circulating antimicrobial peptides in insects to lymphoid organs in vertebrates. Of course, like much of evolutionary observation, these physical properties are often seen from the anthropocentric aspect. It should be recognized that every organism living today has an immune system absolutely capable of protecting it from most forms of harm; those organisms that did not adapt their immune systems to external threats are no longer around to be observed.

Insects and other arthropods, while not possessing true adaptive immunity, show highly evolved systems of innate immunity, and are additionally protected from external injury (and exposure to pathogens) by their chitinous shells.

Reproductive immunology

This area of the immunology is devoted to the study of immunological aspects of the reproductive process including fetus acceptance. The term has also been used by fertility clinics to address fertility problems, recurrent miscarriages, premature deliveries, and dangerous complications such as pre-eclampsia.

Immunologist

Immunologist	
Occupation	
Type	Profession, Specialty
Activity sectors	Science, Laboratory, Medicine
Description	
Education required	Doctor of Philosophy, Medical Doctor
Fields of employment	Hospitals, Clinics, Academia
Related jobs	Physician, Research scientist

According to the American Academy of Allergy, Asthma, and Immunology (AAAAI), "an immunologist is a research scientist who investigates the immune system of vertebrates (including the human immune system). Immunologists include research scientists (Ph.D.) who work in laboratories. Immunologists also include physicians who, for example, treat patients with immune system disorders. Some immunologists are physician-scientists who combine laboratory research with patient care."

Chapter 15

Immunotherapy

Immunotherapy is a medical term defined as "treatment of disease by inducing, enhancing, or suppressing an immune response".

Immunotherapies designed to elicit or amplify an immune response are classified as **activation immunotherapies.**

Immunotherapies designed to reduce, suppress or more appropriately direct an existing immune response, as in cases of autoimmunity or allergy, are classified as **suppression immunotherapies.**

The active agents of immunotherapy are collectively called **immunomodulators**. They are a diverse array of recombinant, synthetic and natural preparations, often cytokines. Some of these substances, such as granulocyte colony-stimulating factor (G-CSF), interferons, imiquimod and cellular membrane fractions from bacteria are already licensed for use in patients. Others including IL-12, various chemokines, synthetic cytosine phosphate-guanosine (CpG), oligodeoxynucleotides and glucans are currently being investigated extensively in clinical and preclinical studies. Immunomodulatory regimens offer an attractive approach as they often have fewer side effects than existing drugs, including less potential for creating resistance in microbial diseases.

Cell based Immunotherapies are proven to be effective for some cancers. Immune effector cells such as lymphocytes, macrophages, dendritic cells, natural killer cells (NK Cell), cytotoxic T lymphocytes (CTL), etc., work together to defend the body against cancer by targeting abnormal antigens expressed on the surface of the tumor due to mutation.

Examples of activation immunotherapies

Cancer

Cancer immunotherapy attempts to stimulate the immune system to reject and destroy tumors. Dr William Coley used Coley's Toxins in the late 1800s as crude immunotherapy with some success. Immuno cell therapy for cancer was first introduced by Rosenberg and his colleagues of National Institute of Health USA. In the late 80s, they published an article in which they reported a low tumor regression rate (2.6–3.3%) in 1205 patients

with metastatic cancer who underwent different types of active specific immunotherapy (ASI), and suggested that immuno cell therapy along with specific chemotherapy is the future of cancer immunotherapy. Initialy Immunotherapy treatments involved administration of cytokines such as Interleukin. Thereafter the adverse effects of such intravenously administered cytokines lead to the extraction of the lymphocytes from the blood and expanding in vitro against tumour antigen before injecting the cells with appropriate stimulatory cytokines. The cells will then specifically target and destroy the tumor expressing antigen against which they have been raised.

The concept of this treatment started in the US in 80s and fully fledged clinical treatments on a routine basis have been in practice in Japan since 1990. Randomized controlled studies in different cancers resulting in significant increase in survival and disease free period have been reported and its efficacy is enhanced by 20 – 30% when cell based immunotherapy is combined with other conventional treatment methods.

BCG immunotherapy for early stage (non-invasive) bladder cancer utilizes *instillation* of attenuated live bacteria into the bladder, and is effective in preventing recurrence in up to two thirds of cases. Topical immunotherapy utilizes an immune enhancement cream (imiquimod) which is an interferon producer causing the patients own killer T cells to destroy warts, actinic keratoses, basal cell cancer, vaginal intraepithelial neoplasia, squamous cell cancer, cutaneous lymphoma, and superficial malignant melanoma,. Injection immunotherapy uses mumps, candida the HPV vaccine or trichophytin antigen injections to treat warts (HPV induced tumors). Lung cancer has been demonstrated to potentially respond to immunotherapy

Dendritic cell based immunotherapy

Dendritic cells can be stimulated to activate a cytotoxic response towards an antigen. Dendritic cells, a type of antigen presenting cell, are harvested from a patient. These cells are then either pulsed with an antigen or transfected with a viral vector. Upon transfusion back into the patient these activated cells present tumour antigen to effector lymphocytes (CD4+ T cells, CD8+ T cells, and B cells). This initiates a cytotoxic response to occur against cells expressing tumour antigens (against which the adaptive response has now been primed). The Dendreon cancer vaccine Provenge is one example of this approach.

T cell based adoptive immunotherapy

Adoptive cell therapy (ACT) using autologous tumor-infiltrating lymphocytes is an effective treatment for patients with metastatic melanoma; this is based on adoptive immunity.

Adoptive cell transfer, or "ACT," uses T cell-based cytotoxic responses to attack cancer. T cells that have a natural or genetically engineered reactivity to a patient's cancer are expanded, made more effective, *in vitro* using a variety of means and then adoptively transferred into a cancer patient.

For example, T cells with a naturally occurring reactivity to a patient's cancer can be found infiltrated in the patient's own tumors. The tumor can be harvested, and these tumor-infiltrating lymphocytes (TIL) can then be expanded, or made more effective, *in vitro* using high concentrations of interleukin-2 (IL-2), anti-CD3 and allo-reactive feeders. These T cells can then be transferred back into the patient along with exogenous administration of IL-2 to further boost their activity.

Thus far, a 51% objective response rate has been observed; and in some patients, tumors shrank to undetectable size.

The initial studies of adoptive cell transfer using TIL, however, revealed that persistence of the transferred cells *in vivo* was too short. Before reinfusion, lymphodepletion of the recipient is required to eliminate regulatory T cells as well as normal endogenous lymphocytes that compete with the transferred cells for homeostatic cytokines. Prior lymphodepletion to transfer of the expanded TIL was made by total body irradiation. The trend for increasing survival as a function of increasing lymphodepletion was highly significant (P=0.007). Transferred cells expanded *in vivo* and persisted in the peripheral blood in many patients, sometimes achieving levels of 75% of all $CD8^+$ T cells at 6–12 months after infusion.

Morgan *et al.* (2006) demonstrated that the adoptive cell transfer of lymphocytes transduced with retrovirus encoding T cell receptors (TCRs) that recognize a cancer antigen can mediate anti-tumor responses in patients with metastatic melanomas.

In such T cell genetic engineering, TCRs that have been identified to have reactivity against tumor-associated antigens are cloned into a replication-incompetent virus that is capable of genomic integration. A patient's own lymphocytes are exposed to these viruses and then expanded non-specifically or stimulated using the engineered TCR. The cells are then transferred back into the patient. This therapy has been demonstrated to result in objective clinical responses in patients with refractory stage IV cancer. The Surgery Branch of the National Cancer Institute (Bethesda, Maryland) is actively investigating this form of cancer treatment for patients suffering aggressive melanomas.

Combination of ACT with such genetic engineering of T cells has opened possibilities for the extension of ACT immunotherapy to patients with a wide variety of cancer types and is a promising new approach to cancer treatment.

In June 2008, it was announced that United States doctors from the Clinical Research Division, led by Dr. Cassian Yee at Fred Hutchinson Cancer Research Center in Seattle had successfully treated a patient with advanced skin cancer by injecting the patient with immune cells cloned from his own immune system. The patient was free from tumours within eight weeks of treatment. Dr. Cassian Yee described the research findings at The Cancer Research Institute International 2008 Symposia Series. Responses, however, were not seen in other patients in this clinical trial.

Larger trials are now under way.

Vaccination

Anti-microbial immunotherapy, which includes vaccination, involves activating the immune system to respond to an infectious agent.

Examples of suppression immunotherapies

Immune suppression dampens an abnormal immune response in autoimmune diseases or reduces a normal immune response to prevent rejection of transplanted organs or cells.

Immune tolerance

Immune tolerance is the process by which the body naturally does not launch an immune system attack on its own tissues. Immune tolerance therapies seeks to reset the immune system so that the body stops mistakenly attacking its own organs or cells in autoimmune disease or accepts foreign tissue in organ transplantation. A brief treatment should then reduce or eliminate the need for life-long immunosuppression and the chances of attendant side effects, in the case of transplantation, or preserve the body's own function, at least in part, in cases of type 1 diabetes or other autoimmune disorders.

Allergies

Immunotherapy is also used to treat allergies. While other allergy treatments (such as antihistamines or corticosteroids) treat only the symptoms of allergic disease, immunotherapy is the only available treatment that can modify the natural course of the allergic disease, by reducing sensitivity to allergens.

A three-to-five-year individually tailored regimen of injections may result in long-term benefits. Recent research suggests that patients who complete immunotherapy may continue to see benefits for years to come. Immunotherapy does not work for everyone and is only partly effective in some people, but it offers allergy sufferers the chance to eventually reduce or stop symptomatic/rescue medication.

The therapy is indicated for people who are extremely allergic or who cannot avoid specific allergens. For example, they may not be able to live a normal life and completely avoid pollen, dust mites, mold spores, pet dander, insect venom, and certain other common triggers of allergic reactions. Immunotherapy is generally not indicated for food or medicinal allergies. Immunotherapy is typically individually tailored and administered by an allergist (allergologist). Injection schedules are available in some healthcare systems and can be prescribed by family physicians. This therapy is particularly useful for people with allergic rhinitis or asthma.

The therapy is particularly likely to be successful if it begins early in life or soon after the allergy develops for the first time. Immunotherapy involves a series of injections (shots) given regularly for several years by a specialist in a hospital clinic. In the past, this was called a serum, but this is an incorrect name. Most allergists now call this mixture an

allergy extract. The first shots contain very tiny amounts of the allergen or antigen to which you are allergic. With progressively increasing dosages over time, your body will adjust to the allergen and become less sensitive to it. This process is called desensitization. A recently approved sublingual tablet (Grazax), containing a grass pollen extract, is similarly effective, with few side effects, and can be self-administered at home, including by those patients who also suffer from allergic asthma, a condition which precludes the use of injection-based desensitization. To read more about this topic, see: allergy and hyposensitization.

Other approaches to immunotherapy

Recent research into the clinical effectiveness of Whipworm ova (*Trichuris suis*) and Hookworm (*Necator americanus*) for the treatment of certain immunological diseases and allergies means that these organisms must be classified as immuno-therapeutic agents. Helminthic therapy is being investigated as a potentially highly effective treatment for the symptoms and or disease process in disorders such as relapsing remitting multiple sclerosis Crohn’s, allergies and asthma.

The precise mechanism of how the helminths modulate the immune response, ensuring their survival in the host and incidentally effectively modulating autoimmune disease processes, is currently unknown. However, several broad mechanisms have been postulated, such as a re-polarisation of the Th1 / Th2 response, and modulation of dendritic cell function by Fujiwara and Carvalho. That helminths modulate host immune response is proven, as the core assertion of the hygiene hypothesis appears to have been, with the recent publication of a study demonstrating that co-evolution with helminths has shaped at least some of the genes associated with Interleukin expression and immunological disorders, like Crohn's, ulcerative colitis and Celiac Disease. Much of the research that has been published now indicates a key role, for what have been traditionally regarded as disease causing organisms, the helminths, in down regulating the pro-inflammatory Th1 cytokines, IL-12 (Interleukin-12), Interferon-Gamma (IFN-γ) and Tumour Necrosis Factor-Alpha (TNF-ά), while promoting the production of regulatory Th2 cytokines such as IL-10 IL-4, IL-5 and IL-13.

Chapter 16

Reproductive Immunology and Immune Tolerance in Pregnancy

Reproductive Immunology

Reproductive immunology refers to a field of medicine that studies interactions (or the absence of them) between the immune system and components related to the reproductive system, such as maternal immune tolerance towards the fetus, or immunological interactions across the blood-testis barrier. The concept has been used by fertility clinics to explain the fertility problems, recurrent miscarriages and pregnancy complications observed when this state of immunological tolerance is not successfully achieved. Immunological therapy is the new up and coming method for treating many cases of previously "unexplained infertility" or recurrent miscarriage.

Between mother and fetus

The fact that the embryo's tissue is half foreign and unlike mismatched organ transplant, it is not normally rejected, suggests that the immunological system of the mother plays an important role in pregnancy. The placenta also plays an important part in protecting the embryo for the immune attack from the mother's system. Studies also propose that proteins in semen may help woman’s immune system prepare for conception and pregnancy. For example, there is substantial evidence for exposure to partner's semen as prevention for pre-eclampsia, largely due to the absorption of several immune modulating factors present in seminal fluid, such as transforming growth factor beta (TGFβ).

Sperm cells within a male

The presence of anti-sperm antibodies in infertile men was first reported in 1954 by Rumke and Wilson. It has been noticed that the number of cases of sperm autoimmunity is higher in the infertile population leading to the idea that autoimmunity could be a cause of infertility. anti sperm antigen has been described as three immunoglobulin isotopes (IgG, IgA, IgM) each of which targets different part of the spermatozoa. If more than 10% of the sperm are bound to anti-sperm antibodies (ASA), then infertility is suspected. The blood-testis barrier separates the immune system and the developing spermatozoa. The tight junction between the Sertoli cells form the blood-testis barrier but it is usually

breached by physiological leakage. Not all sperms are protected by the barrier because spermatogonia and early spermatocytes are located below the junction. They are protected by other means like immunologic tolerance and immunomodulation.

Infertility after anti-sperm antibody binding can be caused by autoagglutination, sperm cytotoxicity, blockage of sperm-ovum interaction, and inadequate motility. Each presents itself depending on the binding site of ASA.

Immunocontraceptive vaccine

Experiments are undergoing to test the effectiveness of an immunocontraceptive vaccine that inhibits the fusing of spermatozoa to the zona pellucida. This vaccine is currently being tested in animals and hopefully will be an effective contraceptive for humans. Normally, spermatozoa fuse with the zona pellucida surrounding the mature oocyte; the resulting acrosome reaction breaks down the egg's tough coating so that the sperm can fertilize the oovum. The mechanism of the vaccine is injection with cloned ZP cDNA, therefore this vaccine is a DNA based vaccine. This results in the production of antibodies against the ZP, which stop the sperm from binding to the zona pellucida and ultimately from fertilizing the oovum.

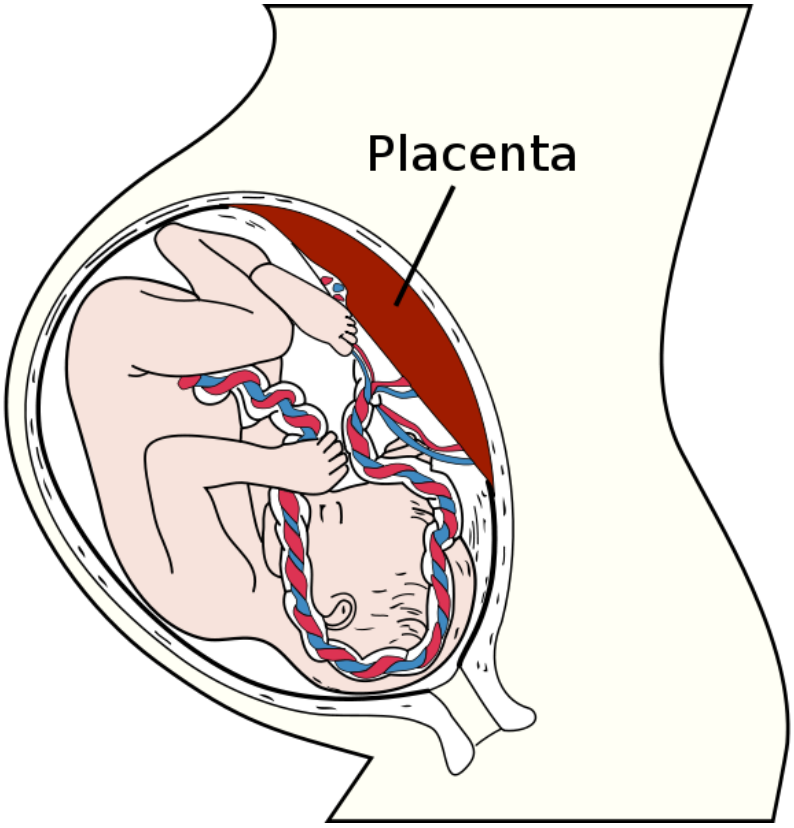
Another vaccine in investigation is one against HCG. This immunization would produce antibodies against hCG and TT. Antibodies against hCG would prevent the maintenance of the uterus for a viable pregnancy therefore preventing contraception. Another vaccine that is utilized is the peptide ß hCG is more specific to hCG and a more rapid and effective response occurs in the absence of LH, FSH, and TSH.

Immune tolerance in pregnancy

Immune tolerance in pregnancy or **gestational/maternal immune tolerance** is the absence of a maternal immune response against the fetus and placenta, which thus may be viewed as unusually successful allografts, since they genetically differ from the mother. In the same way, many cases of spontaneous abortion may be described in the same way as maternal transplant rejection. It is studied within the field of reproductive immunology.

Mechanisms

Placental mechanisms



The placenta functions as an immunological barrier between the mother and the fetus.

The placenta functions as an immunological barrier between the mother and the fetus, creating an immunologically privileged site. For this purpose, it uses several mechanisms:

- It secretes Neurokinin B containing phosphocholine molecules. This is the same mechanism used by parasitic nematodes to avoid detection by the immune system of their host.
- Also, there is presence of small lymphocytic suppressor cells in the fetus that inhibit maternal cytotoxic T cells by inhibiting the response to interleukin 2.
- The placental trophoblast cells do not express the classical MHC class I isotypes HLA-A and HLA-B, unlike most other cells in the body, and this absence is assumed to prevent destruction by maternal cytotoxic T cells, which otherwise would recognize the fetal HLA-A and HLA-B molecules as foreign. On the other hand, they do express the atypical MHC class I isotypes HLA-E and HLA-G, which is assumed to prevent destruction by maternal NK cells, which otherwise destruct cells that do not express any MHC class I. However, trophoblast cells do express the rather typical HLA-C.

- It forms a syncytium without any extracellular spaces between cells in order to limit the exchange of migratory immune cells between the developing embryo and the body of the mother (something an epithelium will not do sufficiently, as certain blood cells are specialized to be able to insert themselves between adjacent epithelial cells). The fusion of the cells is apparently caused by viral fusion proteins from endosymbiotic endogenous retrovirus (ERV). An immunoevasive action was the initial normal behavior of the viral protein, in order to avail for the virus to spread to other cells by simply merging them with the infected one. It is believed that the ancestors of modern viviparous mammals evolved after an infection by this virus, enabling the fetus to better resist the immune system of the mother.

Still, the placenta does allow maternal IgG antibodies to pass from to the fetus to protect it against infections. However, these antibodies do not target fetal cells, unless any fetal material has escaped across the placenta where it can come in contact with maternal B cells and make those B cells start to produce antibodies against fetal targets. The mother does produce antibodies against foreign ABO blood types, where the fetal blood cells are possible targets, but these preformed antibodies are usually of the IgM type, and therefore usually do not cross the placenta. Still, rarely, ABO incompatibility can give rise to IgG antigodies that cross the placenta, and are caused by sentitization of mothers (usually of blood type 0) to antigens in foods or bacteria.

Other mechanisms

Still, the placental barrier is not the sole means to evade the immune system, as foreign fetal cells also persist in the maternal circulation, on the other side of the placental barrier.

In any case, the placenta does not block maternal IgG antibodies, which thereby may pass through the human placenta, providing immune protection to the fetus against infectious diseases.

One model for the induction of tolerance during the very early stages of pregnancy is the Eutherian Fetoembryonic Defense System (eu-FEDS) hypothesis. The basic premise of the eu-FEDS hypothesis is that both soluble and cell surface associated glycoproteins, present in the reproductive system and expressed on gametes, suppress any potential immune responses, and inhibit rejection of the fetus. The eu-FEDS model further suggests that specific carbohydrate sequences (oligosaccharides) are covalently linked to these immunosuppressive glycoproteins and act as “functional groups” that suppress the immune response. The major uterine and fetal glycoproteins that are associated with the eu-FEDS model in the human include alpha-fetoprotein, CA125, and glycodelin-A (also known as placental protein 14 (PP14)).

Regulatory T cells also likely play a role.

Also, a shift from cell-mediated immunity toward humoral immunity is believed to occur.

Insufficient tolerance

Many cases of spontaneous abortion may be described in the same way as maternal transplant rejection, and a chronic insufficient tolerance may cause infertility. Other examples of insufficient immune tolerance in pregnancy are Rh disease and pre-eclampsia:

- **Rh disease** is caused by the mother producing antibodies (including IgG antibodies) against the Rhesus D antigen on her baby's red blood cells. It occurs if the mother is Rh negative and the baby is Rh positive, and a small amount of Rh positive blood from any previous pregnancy has entered the mother's circulation to make her produce IgG antibodies against the Rhesus D antigen. Maternal IgG is able to pass through the placenta into the fetus and if the level of it is sufficient, it will cause destruction of Rhesus D positive fetal red blood cells leading to development Rh disease. Generally Rhesus disease becomes worse with each additional Rhesus incompatible pregnancy.

- One cause of **pre-eclampsia** is an abnormal immune response towards the placenta. There is substantial evidence for exposure to partner's semen as prevention for pre-eclampsia, largely due to the absorption of several immune modulating factors present in seminal fluid.

Pregnancies resulting from egg donation, where the carrier is less genetically similar to the fetus than a biological mother, are associated with a higher incidence of pregnancy-induced hypertension and placental pathology. The local and systemic immunologic changes are also more pronounced than in normal pregnancies, so it has been suggested that the higher frequency of some conditions in egg donation may be caused by reduced immune tolerance from the mother.

Infertility and miscarriage

Immunological responses could be the cause in many cases of infertility and miscarriage. Some immunological reasons that contribute to infertility are reproductive autoimmune failure syndrome, the presence of anti-phospholipid antibodies, and antinuclear antibodies.

Anti-phospholipid antibodies are targeted toward the phospholipids of the cell membrane. Studies have shown that antibodies against phosphatidylserine, phoshphatidylcholine, phosphatidylglycerol, phosphatidylinositol and phosphatidylethanolamine target the pre-embryo. Antibodies against phosphatidylserine and phosphatidylethanolamine are against the trophoblast. These phospholipids are essential in the aiding the cells of the fetus to remain attached to the cells of the uterus with implantation. If a female has antibodies against these phospholipids, they will be destroyed through the immune response and ultimately the fetus will not be able to remain bound to the uterus. These antibodies also jeopardize the health of the uterus by altering the blood flow to the uterus.

Antinuclear antibodies cause an inflammation in the uterus that does not allow it to be a suitable host for implantation of the embryo. Natural killer cells misinterpret the fetal cells as cancer cells and attack them. An individual that presents with reproductive autoimmune failure syndrome has unexplained infertility, endometriosis, and repetitive miscarriages due to elevated levels of antinuclear antibodies circulating. Both the presence of anti-phospholipids antibodies and antinuclear antibodies have toxic effects on the implantation of embryos. This does not apply to anti-thyroid antibodies. Elevated levels do not have a toxic effect, but they are indicative of a risk of miscarriage. Elevated anti-thyroid antibodies act as a marker for females who have T-lymphocyte dysfunction because these levels indicate T cells that are secreting high levels of cytokines that induce inflammation in the uterine wall.

Still, there is currently no drug that has evidence of preventing miscarriage by inhibition of maternal immune responses; aspirin has no effect in this case.

Increased infectious susceptibility

The immunologic changes in pregnancy alters the susceptibility to and severity of infectious diseases. For example, pregnancy may increase susceptibility to toxoplasmosis and listeriosis and may increase severity of illness and increase mortality rates from influenza and varicella.

Interspecific pregnancy

If the mechanisms of rejection-immunity of the fetus could be elucidated, it could avail for interspecific pregnancy, having, for example pigs carry human fetuses to term as an alternative to a human surrogate mother, providing a sober, drug-free and nonsmoking carrier.

Chapter 17

Allergen Immunotherapy

Intervention: *Allergen immunotherapy*	
ICD-10 code:	Z51.6
ICD-9 code:	99.12
MeSH	D003888
Other codes:	

Allergen immunotherapy (also termed **hyposensitization therapy**, **immunologic desensitization**, **hyposensibilization**, or **allergen-specific immunotherapy**) is a form of immunotherapy for allergic disorders in which the patient is vaccinated with increasingly larger doses of an allergen (substances to which they are allergic) with the aim of inducing immunologic tolerance. Allergen specific immunotherapy is the only treatment strategy which treats the underlying cause of the allergic disorder. It is a highly cost-effective treatment strategy which results in an improved quality of life and a reduction in allergic- and allergen-related asthma, as well as a reduction in days off school/work. Immunotherapy has been shown to produce long-term remission of allergic symptoms, reduce severity of associated asthma as well as reduce the chances of new sensitisations to allergens developing. This is achieved via immunotherapy modulating the immune system response to allergens.

Allergen immunotherapy can either reduce the need for medication, severity of symptoms or eliminate hypersensitivity altogether. Therapy can be administered under the tongue (sublingually) or by injections under the skin (subcutaneous). Allergen-specific immunotherapy is the only known treatment option that is known to modify the allergy disease process (with a possible chance of curing the disease), whereas other therapies merely suppress the symptoms. Subcutaneous injection immunotherapy has been shown to be highly efficacious treatment for allergic disease, but due to a rare serious side effect of anaphylaxis, its use is restricted to specialist centers. As a result there has been growing interest in the sublingual therapy which can be safely administered at home.

Background

Allergic rhinitis is an extremely common disorder. For example in the UK 1 in 5 people have allergic rhinitis with approximately 50 percent of those with allergic rhinitis being

allergic to grass pollen. Over half of people receiving symptom based treatments report that they only get partial or a poor benefit from symptomatic based treatments. For these such patients immunodesensitisation therapy can be recommended. Subcutaneous injection based immunotherapy is one effective route but is complicated by rare but serious side effects. As a result of these rare but serious side effects the sublingual route for allergen vaccination immunotherapy is gaining increasing popularity among allergy specialists due to its lack of serious side effects. A product called Grazax is currently recommended for the treatment of grass pollen allergy in the UK for patients who don't respond appropriately to symptomatic based treatment strategies.

Immunotherapy administered through cutaneous injections or sublingually has substantial empirical support. Numerous research articles and several meta-analytic studies support its clinical effectiveness. Immunotherapy can lead to a substantial decline in allergen symptomatology leading to a significant improvement in quality of life for allergy sufferers. Repeated courses of immuno-desensitisation leads to further reduction in allergy disease severity. Immunotherapy is superior to antihistamines and topical steroids in reducing severity of allergy symptoms and has been found to be a cost effective treatment strategy. Immunotherapy results in less time taken off work compared to those who rely solely on symptomatic relieving medications. In the case of grass allergy immunotherapy the pollen from the grass species used has strong cross reactivity between the various grass species thus meaning that treatment leads to desensitisation to all grass species.

Sublingual specific immunotherapy has the benefit of allowing treatment to be carried out in the home environment and has been found to be a cost effective treatment strategy for allergic disorders. Sublingual immunotherapy cost effectiveness is significantly increased due to the reduced number of medical visits compared to those receiving subcutaneous injection based immunotherapy. For example those receiving subcutaneous injections make almost seven times more visits than those receiving home based sublingual immunotherapy versus those receiving subcutaneous injections who require frequent visits to doctors to receive their regular injections. Furthermore the sublingual route appears to be equally as effective as the subcutaneous route in trials of grass allergy.

The term immunotherapy may refer not only to desensitization for allergies but also to a number of other immunomodulator techniques that aim to alter the response of the immune system in order to alleviate or cure autoimmune disease, cancer, and so forth. These include Enzyme Potentiated Desensitization (EPD) and its variant, Ultra Low Dose Enzyme Activated Immunotherapy (LDA), which have replaced "escalating dose" treatments in the U.K. but not in the U.S.

Clinical experience and research

Immunology is a relatively young science that originated in the 19th century. Grass pollens were identified for the first time as the likely trigger of seasonal hay fever in the 1870s. Skin allergy testing became an accepted assessment technique around 1910. IgE was identified in the 1960s. The first scholarly report of immunotherapy for allergy

appeared in 1911 in the medical journal, *The Lancet*, but research lagged behind clinical practice. Whereas clinical lore in medicine generally supports the effectiveness of immunotherapy, sufficient research evidence on the effectiveness and mechanism of immunotherapy began to accumulate in the last 15 years of the 20th century.

Some limited research of sublingual immunotherapy in children has been conducted and shown promise as a generally well tolerated treatment strategy for allergic disorders in children.

Benefits from immunotherapy

Current pharmacotherapies (antihistamines) do not prevent allergic reaction, but instead block the action of histamine in the body, reducing allergic symptoms. Immunotherapy, in contrast, trains the immune system to tolerate allergic triggers by means of gradual exposure to increasing amounts of the offending allergen. The benefits of allergen specific immunotherapy are long lasting unlike symptomatic based treatments. Immunotherapy is most effective for pollen, dust, and animal dander allergies, and may help those with asthma.

Treatment started 10 – 14 weeks before the start of the grass pollen season results in a 34% reduction of rhinoconjunctivitis symptoms and a 54% increase in well days. Continued treatment over 2 years in the case of grass allergy shows progressive immunological changes resulting in progressive desensitisation to the allergen with up to an 73 percent reduction in symptomatology. About 3 in 4 patients with hay fever experience significant improvement with immunotherapy after one year of therapy. However, with continued therapy the number of people benefiting from specific immunotherapy appears to increase to over 4 in 5 people who benefit from specific immunotherapy by the end of the 2nd year of therapy. Sometimes symptoms are reduced rather than abolished. In that case immunotherapy may allow the patient to reduce the quantity of medication required for symptom relief.

Research in children aged from 5 years old to 16 years old shows similar effectiveness in the treatment of grass allergy as seen in adults. Like in adults allergen related asthma also decreases as well as allergic rhinitis symptoms. Recent studies in children suggest that if immunotherapy is commenced soon after allergies first develop, it may actually reduce the risk of developing allergic reactions to other allergens, and even reduce the risk of later developing asthma.

Immunotherapy is also an essential part of managing dangerous allergic reactions (anaphylaxis) to bee and wasp stings. In these cases, the protection against further dangerous allergic reactions to stinging insects is variously quoted at between 80 and 95%.

Mechanism of therapeutic action

The immune system of allergy affected individuals, for reasons not fully understood, misinterprets a usually innocuous substance as a disease agent and begins producing a type of antibody against it, called immunoglobulin E (IgE). This is called the 'primary antibody response.' The IgE produced during this response binds to basophils in the bloodstream and to a similar type of cell called mast cells in the tissues. When the person again encounters the allergen, these basophils and mast cells that have bound to IgE release histamine, prostaglandins, and leukotrienes, which causes inflammation of the surrounding tissues, resulting in allergic symptoms.

Even the most allergic individual can tolerate minuscule amounts of an allergen without experiencing symptoms. Immunotherapy commences with the subcutaneous injection of a tiny amount of offending allergen, and gradually increases the dose until the individual's immune system is essentially 'retrained' to tolerate exposure without producing an allergic response. This process is also known as specific immunotherapy.

Immunotherapy via repeated exposure to a specific allergen via either sublingual or subcutaneous route leads to a desensitisation to the allergen and thus a reduction in allergic symptomatology and use of symptomatic based treatments. The exact mechanism is not fully understood but it is accepted that immunotherapy causes modification of the immune system. This modification leads to changes in IgE synthesis and the production of IgE blocking antibodies which thus reduces the immune systems allergic response to specific allergens. There is also an increase in Th2 to the regulatory T cells. The molecular mechanism of such immunotherapy can be partly interpreted as that there occurs induction of allergen-specific IgG to neutralize the allergen instead of induction of allergen-specific IgE. In bee or wasp venom immunotherapy, immunoglobulin subclass IgG4 has been considered to be particularly important, where IL-4 and IL-13 make the B cells to switch the produced immunoglobulin class from IgE to IgG4. It has been revealed that the mechanism of this immunotherapy consists of some more other components. They include increased production of IL-10 which acts on Th2 or mast cells to become anergic and suppresses Th2 not to release cytokines and prevent histamine from being secreted. It was indicated that osteopontin produced by $CD14^+$ cells induced IL-12 in antigen presenting cells to activate Th1. It was recently shown that a progressive expansion of circulating regulatory T cells was made during venom immunotherapy.

Procedure

For benefits to be felt from either sublingual or injection based allergen specific immunotherapy it needs to be started 2 – 4 months before the start of the allergen season in the case of seasonal allergies. The earlier it is started the better the level of allergy protection. Sublingual immunotherapy is a safe and effective alternative to injection based immunotherapy and can be administered in the home environment. Modest benefits have been demonstrated within the first season of therapy. Treatment needs to be continued for at least 3 years to achieve maximum effectiveness in immune desensitisation to the allergen. In the case of sublingual immunotherapy there is no need

to do a titrated graduated updose and therapy is generally started at the usual clinical dose.

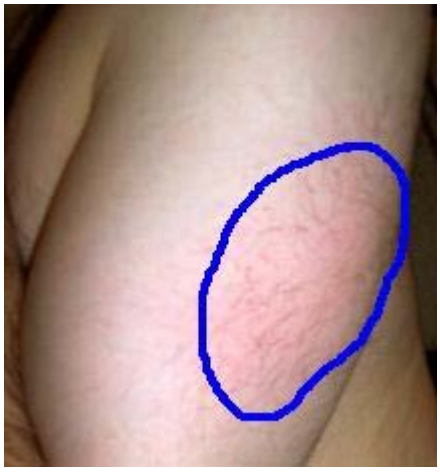
Immunotherapy via the subcutaneous route involves the use of small hypodermic syringes which are used to inject commercial allergen extracts. Injections are normally given into the loose tissue over the back of the upper arm, half way between the shoulder and elbow. Injections are given under the skin ("subcutaneous"). This is the least painful place to inject allergen, as there are few nerve endings in the skin. When given correctly, the injections should be only slightly uncomfortable. They are not normally painful and are usually well tolerated by adults and teenagers. Some doctors may advise you to take an antihistamine a few hours before each injection to reduce the likelihood of local discomfort and other side-effects.

Allergy injections are started at very low doses. The dose is gradually increased on a regular (and usually weekly) basis, until a "maintenance" dose is reached. This usually means four to six months of weekly injections to reach the maintenance dose. Once the maintenance dose is reached, the injections are administered less often (every two to four weeks), still on a regular basis. Maintenance injections are normally given once per month for a few years. Generally, the longer the treatment and the higher the dose, the greater the therapeutic benefit.

After successful completion of immunotherapy, long-term protection can be expected for a period of 3–5 years or more. Therapy can be repeated should symptoms begin to return or if the individual becomes exposed to new allergens that were not included in the previous treatment regiment. This form of treatment is covered by the vast majority of insurance companies in the United States, because allergy vaccine injections have a strong evidence base for clinical effectiveness.

In some countries, particularly in Europe, there is a strong tradition of undertaking immunotherapy using oral vaccines or sublingual drops. While there has been some interesting research in this area in recent years, the effectiveness of this form of treatment is difficult to compare with standard injected immunotherapy. Double-blind, placebo-controlled studies in Europe using high-dose sublingual immunotherapy have shown benefit. Some practitioners in the United States, particularly ENT physicians, offer sublingual immunotherapy as another immunotherapy option.

Side effects and adverse reactions



A relatively large but normative localized reaction to an allergy injection on the upper arm of a patient. This reaction would not cause concern, but larger reactions may require a readjustment of the treatment regimen.

Subcutaneous immuno-therapy

Itchiness, swelling, and redness at the site of injection are expected. Systemic reactions such as hives or anaphylaxis occur rarely and need to be treated immediately. If such reactions occur, the allergy specialist will adjust the dosage to a safe level. Patients are advised or required to wait in the clinic for 20–30 minutes so that they can be treated immediately in the case that they develop a severe systemic reaction. The risk of a systemic reaction is reduced if the patient avoids exercising or overheating for a few hours before and after the procedure. Some heart and blood pressure medications such as beta-blockers are contraindicated as well.

The physician should be consulted if the patient notices a worsening of allergy symptoms or if he or she is suffering from a cold or has been undergoing a different kind of vaccination procedure. Immunotherapy does not increase the risk of contracting a cold.

Sublingual immuno-therapy

The side effects of sublingual desensitisation therapy are generally mild and limited to local reactions. Common side effects include oral pruritus, edema mouth, ear pruritus, throat irritation, sneezing, mild itching and swelling of the mouth. Side effects which are less common or rare include headache, oral paraesthesia, eye pruritus, conjunctivitis, cough, asthma, pharyngitis, rhinorrhoea, nasal congestion, rhinitis, throat tightness, pruritus and fatigue. In most cases these side effects diminished minutes or hours after immunotherapy and disappeared 1 – 7 days after commencement of therapy. As a precautionary measure against rare but serious side effects e.g. asthma attacks it is recommended that the first sublingual tablet containing the specific allergen for

immunotherapy is administrated whilst under the observation of a medical doctor and observed for 30 minutes for any signs of serious side effects.

Sublingual immunotherapy is contraindicated in patients who have systemic diseases of the immune system, inflammatory conditions of the oral cavity with associated severe symptoms e.g. oral lichen planus with ulcers or severe oral mycosis or individuals with severe and uncontrolled asthma. Immunotherapy tablets are also contraindicated in individuals who are allergic to any of the addition constituents of the tablet.

Chapter 18

Allergen

An **allergen** is a nonparasitic antigen capable of stimulating a type-I hypersensitivity reaction in atopic individuals.

Most humans mount significant Immunoglobulin E (IgE) responses only as a defense against parasitic infections. However, some individuals mount an IgE response against common environmental antigens. This hereditary predisposition is called atopy. In atopic individuals, non-parasitic antigens stimulate inappropriate IgE production, leading to type I hypersensitivity. Sensitivities vary from one person to another and it is possible to be allergic to an extraordinary range of substances.

Types of allergies

Dust mite excretion, pollen and pet dander are all common allergens, but it is possible to be allergic to anything from chlorine to perfume to royal jelly. Food allergies are not as common as food sensitivity, but some foods such as peanuts (a legume), nuts, seafood and shellfish are the cause of serious allergies in many people.

Officially, the United States Food and Drug Administration does recognize eight foods as being common for allergic reactions in a large segment of the sensitive population. These include peanuts, tree nuts, eggs, milk, shellfish, fish, wheat and their derivatives, soy and their derivatives, and sulfites (chemical based, often found in flavors and colors in foods) at 10ppm and over. It should be noted that other countries, due to differences in the genetic profiles of its citizens and different levels of exposure to different foods, the "official" allergen list will change. Canada recognizes all eight of the allergens recognized by the US, and also recognizes sesame seeds.

A few people have been recorded to be allergic to certain chemicals found in almost all water, and even water itself.

Another type of allergen is urushiol, a resin produced by poison ivy and poison oak. It causes the skin rash condition known as urushiol-induced contact dermatitis by changing a skin cell's configuration so that it is no longer recognized by the immune system as part of the body. A little over half of North Americans are known to be allergic to urushiol and repeated exposure can increase one's sensitivity to the allergen.

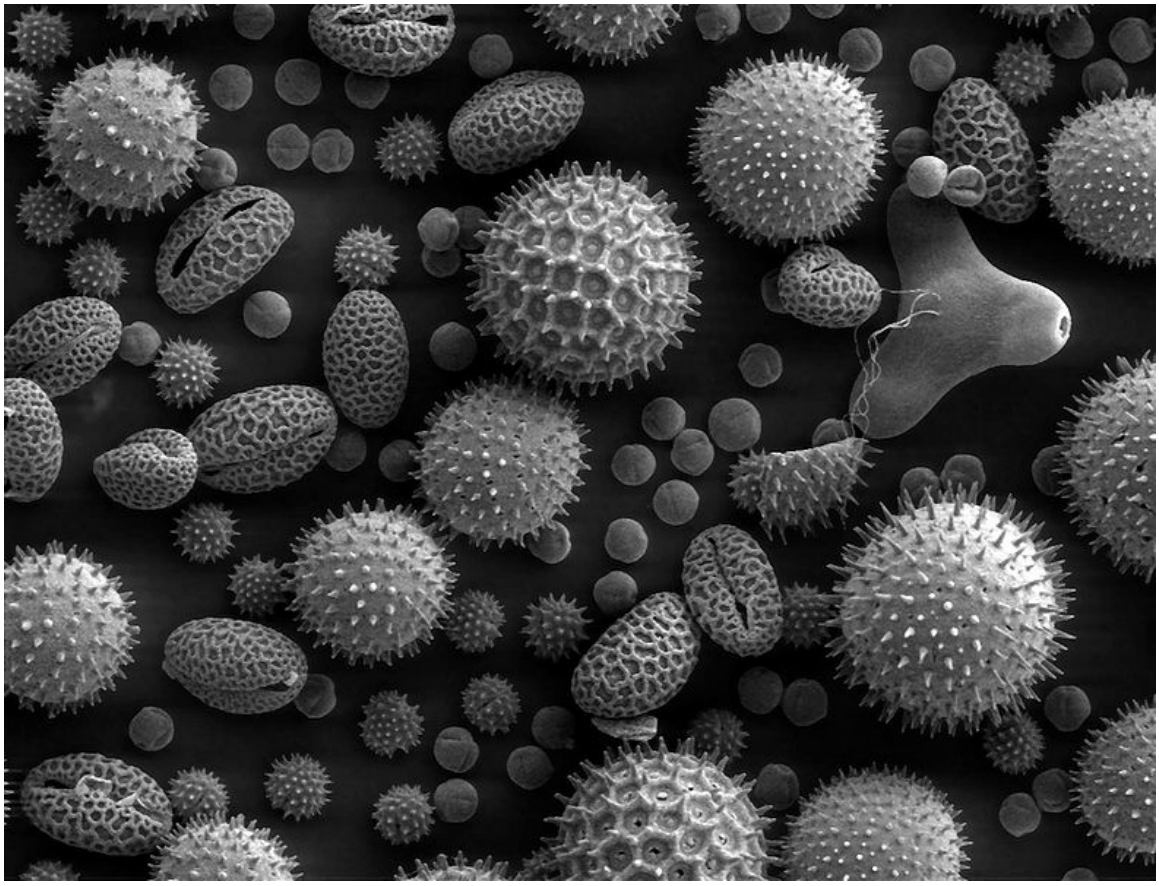
An allergic reaction can be caused by any form of direct contact with the allergen—consuming food or drink one is sensitive to (ingestion), breathing in pollen, perfume or pet dander (inhalation), or brushing a body part against an allergy-causing plant (direct contact, generally resulting in hives). Other common causes of serious allergy are wasp, fire ant and bee stings, penicillin, and latex. An extremely serious form of an allergic reaction is called anaphylaxis. One form of treatment is the administration of sterile epinephrine to the person experiencing anaphylaxis, which suppresses the body's overreaction to the allergen, and allows for the patient to be transported to a medical facility.

Fungal allergens

In 1952 basidiospores were described as being possible airborne allergens and were linked to asthma in 1969. Basidiospore are the dominant airborne fungal allergens. Fungal allergy is associated with seasonal asthma. They are considered to be a major source of airborne allergens. The basidospore family include mushrooms, rusts, smuts, brackets, and puffballs. The airborne spores from mushrooms reach levels comparable to those of mold and pollens. The levels of mushroom respiratory allergy are as high as 30 percent of those with allergic disorder, but it is believed to be less than 1 percent of food allergies. Heavy rainfall (which increases fungal spore release) is associated with increased hospital admissions of children with asthma. A study in New Zealand found that 22 percent of patients with respiratory allergic disorders tested positive for basidiospores allergies. Mushroom spore allergies can cause either immediate allergic symptomatology or delayed allergic reactions. Those with asthma are more likely to have immediate allergic reactions and those with allergic rhinitis are more likely to have delayed allergic responses. A study found that 27 percent of patients were allergic to basidiomycete mycelia extracts and 32 percent were allergic to basidiospore extracts, thus demonstrating the high incidence of fungal sensitisation in individuals with suspected allergies. It has been found that of basidiomycete cap, mycelia, and spore extracts that spore extracts are the most reliable extract for diagnosing basidiomycete allergy.

In Canada, 8% of children attending allergy clinics were found to be allergic to Ganoderma which is a basidiospore. Pleurotus ostreatus, cladosporium, and calvatia cyathiformis are significant airborne spores. Other significant fungal allergens include aspergillus and alternaria-penicillin families. In India fomes pectinatis is a predominant air bourne allergen effecting up to 22 percent of patients with respiratory allergies. Some fungal air-bourne allergens such as coprinus comatus are associated with worsening of eczematous skin lesions. Children who are born during autumn months (during fungal spore season) are more likely to develop asthmatic symptoms later in life.

Common allergens



SEM of miscellaneous plant pollens. Pollens are very common allergens.

The house dust mite, its feces and chitin are common allergens around the home

In addition to foreign proteins found in foreign serum (from blood transfusions) and vaccines, common allergens include:

- Animal products
  - Fel d 1 (cat allergy)
  - fur and dander
  - cockroach calyx
  - wool
  - dust mite excretion
- Drugs
  - penicillin
  - sulfonamides
  - salicylates (also found naturally in numerous fruits)
  - local anaesthetics
- Foods
  - celery and celeriac
  - corn or maize
  - eggs (typically albumen, the white)
  - fruit
    - pumpkin
  - legumes
    - beans
    - peas

        - peanuts
        - soybeans
    - milk
    - seafood
    - sesame
    - soy
    - tree nuts
        - pecans
        - almonds
    - wheat
- Insect stings
    - bee sting venom
    - wasp sting venom
    - mosquito stings
- Mold spores
- Other
    - latex
    - metal
- Plant pollens (hay fever)
    - grass — ryegrass, timothy-grass
    - weeds — ragweed, plantago, nettle, artemisia vulgaris, chenopodium album, sorrel
    - trees — birch, alder, hazel, hornbeam, aesculus, willow, poplar, platanus, tilia, olea, Ashe juniper

Seasonal allergies

Seasonal allergy symptoms are commonly experienced part of the year, usually during spring, summer or fall when certain trees or grasses pollinate. This depends on the kind of tree or grass. For instance, some trees such as oak, elm, and maple pollinate in the spring, while grasses such as Bermuda, timothy and orchard pollinate in the summer.

Grass allergy is generally linked to hay fever because their symptoms and causes are somehow similar to each other. Symptoms include rhinitis, which causes sneezing and a runny nose, as well as allergic conjunctivitis, which includes watering and itchy eyes. Also an initial tickle on the roof of the mouth or in the back of the throat may be experienced.

Also, depending on the season, the symptoms may be more severe and people may experience coughing, wheezing, and irritability. A few people even become depressed, lose their appetite, or have problems sleeping. Moreover, since the sinuses may also become congested, some people experience headaches.

If both parents suffered from allergies in the past, there is a 66% chance for the individual to suffer from seasonal allergies, and the risk lowers to 60% if just one parent had suffered from allergies. The immune system also has strong influence on seasonal

allergies, since it reacts different to diverse allergens like pollen. When an allergen enters the body of an individual that is predisposed to allergies, it triggers an immune reaction and the production of antibodies. These allergen antibodies migrate to mast cells lining the nose, eyes and lungs. When an allergen drifts into the nose more than once, mast cells release a slew of chemicals or histamines that irritate and inflame the moist membranes lining the nose and produce the symptoms of an allergic reaction: scratchy throat, itching, sneezing and watery eyes. Some facts that differentiate allergies from a cold are the following:

- No fever or muscle ache.
- Mucous secretions are runny and clear.
- Sneezes occurring in rapid and several sequences.
- Itchy throat, ears and nose.
- These symptoms usually last longer that 7–10 days.

Among seasonal allergies, there are some allergens that fuse together and produce a new type of allergy. For instance, grass pollen allergens cross-react with food allergy proteins in vegetables such as onion, lettuce, carrots, celery and corn. Besides, the cousins of birch pollen allergens, like apples, grapes, peaches, celery and apricots, produce severe itching in the ears and throat. The cypress pollen allergy brings a cross reactivity between diverse species like olive, privet, ash and Russian olive tree pollen allergens. In some rural areas there is another form of seasonal grass allergy, combining airborne particles of pollen mixed with mold. Recent research have suggested that humans might develop allergies as a defense to fight off parasites. According to Yale University Immunologist Dr Ruslan Medzhitov, Protease allergens cleave the same sensor proteins that evolved to detect proteases produced by the parasitic worms. Additionally, a new report on seasonal allergies called “Extreme allergies and Global Warming”, have found that many allergy triggers are worsening due to climate change. 16 states in the United States were named as “Allergen Hotspots” for large increases in allergenic tree pollen if global warming pollution keeps increasing as it does lately. Therefore, researchers on this report claimed that Global Warming is bad news for millions of asthmatics in the United States whose asthma attacks are triggered by seasonal allergies. Indeed, seasonal allergies are one of the main triggers for asthma, along with colds or flu, cigarette smoke and exercise. In Canada for example, up to 75% of asthmatics also have seasonal allergies.

Seasonal Allergies Diagnosis

Based on the symptoms seen on the patient, the answers given in terms of symptom evaluation and a physical exam, doctors can make a diagnosis to identify if the patient has a seasonal allergy. After performing the diagnosis, the doctor is able to tell the main cause of the allergic reaction and recommend the treatment to follow. 2 tests have to be done in order to determine the cause: a blood test and a skin test. Allergists do skin tests in one of two ways: either dropping some purified liquid of the allergen onto the skin and pricking the area with a small needle; or injecting a small amount of allergen under the skin. Alternative tools are available to identify seasonal allergies, such as laboratory tests, imaging tests and nasal endoscopy. In the laboratory tests, the doctor will take a nasal

smear and it will be examined microscopically for factors that may indicate a cause: increased numbers of eosinophils, which are white blood cells which indicates an allergic condition. If there is high counts of eosinophil, it may indicate that there is an allergic condition. Another laboratory test is the blood test for IgE (immunoglobulin production), such as the radioallergosorbent Test (RAST), implemented to detect high levels of allergen-specific IgE in response to particular allergens. Although blood tests are less accurate than the skin tests, they can be performed on patients who cannot go skin testing. Imaging tests can be useful to detect sinusitis in people who also suffer from chronic rhinitis, and they can work when other test results are ambiguous. There is also nasal endoscopy, where a tube is inserted through the nose with a small camera to view the passageways and examine any irregularities in the nose structure. Endoscopy can be used for some cases of chronic or unresponsive seasonal rhinitis.

Treatment

Treatment includes over-the-counter medications, antihistamines, nasal decongestants, allergy shots, and alternative medicine.

In the case of nasal symptoms, antihistamines are normally the first option. They may be taken together with pseudoephedrine to help relieve a stuffy nose and they can stop the itching and sneezing. Some over-the-counter options are Benadryl and Tavist. However, these antihistamines may cause extreme drowsiness, therefore, people are advised to not operate heavy machinery or drive while taking this kind of medication. Other side effects include dry mouth, blurred vision, constipation, difficulty with urination, confusion, and light-headedness.

There is also a newer second generation of antihistamines which are generally classified as the "non-sedating antihistamines" or anti-drowsy which include cetirizine, loratadine, and fexofenadine.

An example of nasal decongestants is pseudoephedrine and its side effects include insomnia, restlessness, and difficulty urinating. Some other nasal sprays are available by prescription, and they include: Azelastine, and Ipratropium. Some of their side effects include drowsiness. For eye symptoms it is important to first bath the eyes with plain eyewashes to reduce the irritation. People should not wear contact lenses during episodes of conjunctivitis.

Allergy shots, also called immunotherapy, are also available and are especially recommended for people who cannot tolerate allergy medications or who experience severe symptoms, and also for those who develop asthma during pollen season.

Immunotherapy contains a small amount of the substance that triggers the allergic reactions and it should start after the pollen season to get prepared for the next season.

Natural remedies are another option that patients look for relief. One of the most popular recently is the European herb butterbur (Petasites hybridus). The British Medical Journal

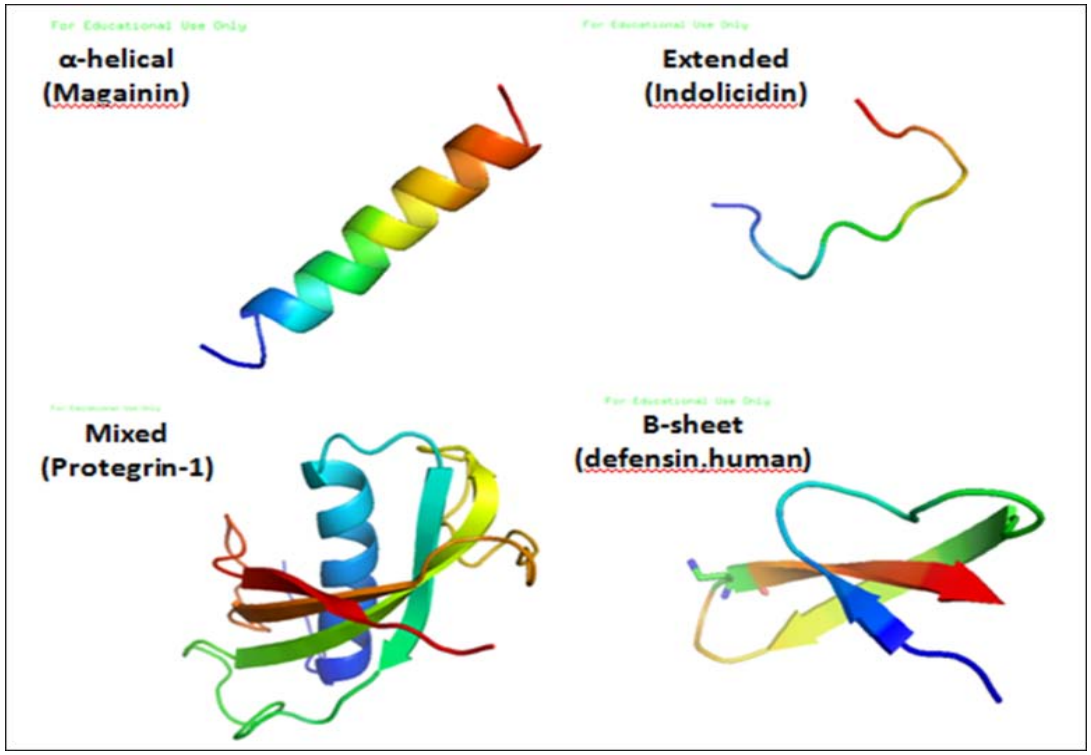
published a study where Swiss researchers proved how one tablet of butterbur four times daily was as effective as an antihistamine in controlling symptoms of hay fever. On a second study, a group of British researchers gave their approval to butterbur's effectiveness in battling symptoms of grass allergy. Other herbal supplements that function as remedies, include freeze-dried nettles and a tonic made from the goldenseal herb, which doctors recommend it additionally to saline nasal spray (another natural remedy).

Chapter 19

Antimicrobial Peptides

Antimicrobial peptides (also called host defence peptides) are an evolutionarily conserved component of the innate immune response and are found among all classes of life. Fundamental differences exist between prokaryotic and eukaryotic cells that may represent targets for antimicrobial peptides. These peptides are potent, broad spectrum antibiotics which demonstrate potential as novel therapeutic agents. Antimicrobial peptides have been demonstrated to kill Gram negative and Gram positive bacteria (including strains that are resistant to conventional antibiotics), mycobacteria (including *Mycobacterium tuberculosis*), enveloped viruses, fungi and even transformed or cancerous cells. Unlike the majority of conventional antibiotics it appears as though antimicrobial peptides may also have the ability to enhance immunity by functioning as immunomodulators.

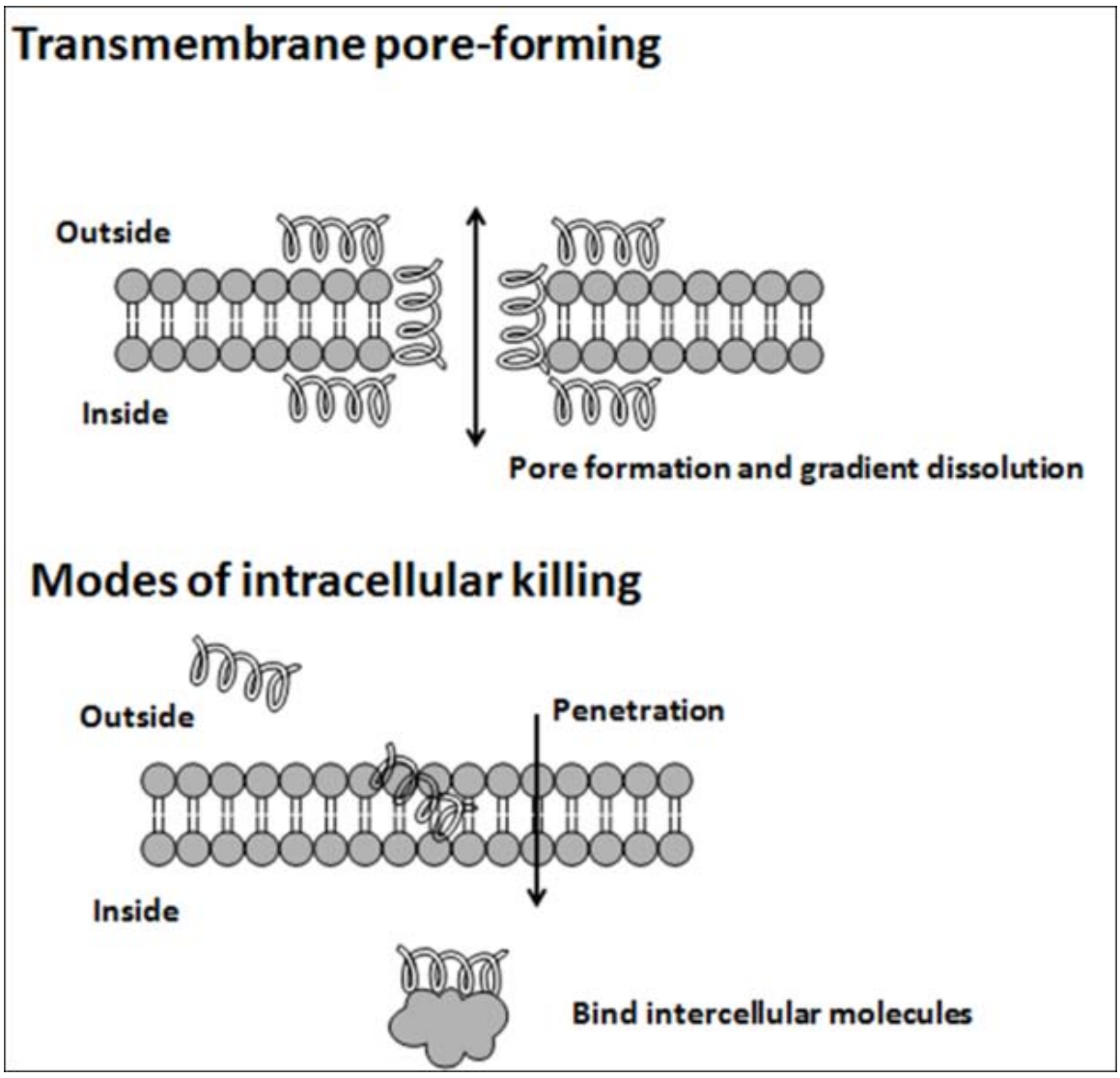
Structure



Various structures of antimicrobial peptides

Antimicrobial peptides are a unique and diverse group of molecules, which are divided into subgroups on the basis of their amino acid composition and structure. Antimicrobial peptides are generally between 12 and 50 amino acids. These peptides include two or more positively charged residues provided by arginine, lysine or, in acidic environments, histidine, and a large proportion (generally >50%) of hydrophobic residues. The secondary structures of these molecules follow 4 themes, including i) α-helical, ii) β-stranded due to the presence of 2 or more disulfide bonds, iii) β-hairpin or loop due to the presence of a single disulfide bond and/or cyclization of the peptide chain, and iv) extended. Many of these peptides are unstructured in free solution, and fold into their final configuration upon partitioning into biological membranes. It contains hydrophilic amino acid residues aligned along one side and hydrophobic amino acid residues aligned along the opposite side of a helical molecule. This amphipathicity of the antimicrobial peptides allows to partition into the membrane lipid bilayer. The ability to associate with membranes is a definitive feature of antimicrobial peptides although membrane permeabilisation is not necessary. These peptides have a variety of antimicrobial activities ranging from membrane permeabilization to action on a range of cytoplasmic targets.

Type	characteristic	AMPs
Anionic peptides	rich in glutamic and aspartic acids	Maximin H5 from amphibians, Dermcidin from humans
Linear cationic α-helical peptides	lack in cysteine	Cecropins, andropin, moricin, ceratotoxin and melittin from insects, Magainin, dermaseptin, bombinin, brevinin-1,esculentins and buforin II from amphibians, CAP18 from rabbits, LL37 from humans
Catioinic peptide enriched for specific amino acid	rich in proline, arginine, phenylalanine, glycine, tryptophan	abaecin, apidaecins from honeybees, prophenin from pigs, indolicidin from cattle.
Anionic and cationic peptides that contain cysteine and form disulfide bonds	contain 1~3 disulphide bond	1 bond:brevinins, 2 bonds:protegrin from pig, tachyplesins from horseshoe crabs, 3 bonds:defensins from humans, more than 3:drosomycin in fruit flies



The modes of action by Antimicrobial peptides

Antimicrobial Activities

The modes of action by which antimicrobial peptides kill bacteria is varied and includes disrupting membranes, interfering with metabolism, and targeting cytoplasmic components. The initial contact between the peptide and the target organism would be electrostatic, as most bacterial surface are anionic. Their amino acid composition, amphipathicity, cationic charge and size allow them to attach to and insert into membrane bilayers to form pores by 'barrel-stave', 'carpet' or 'toroidal-pore' mechanisms. Or allow them to penetrate into the cell to bind intracellular molecules which are crucial to cell living. Intracellular binding model includes inhibition of cell wall synthesis, alteration of cytoplasmic membrane, activation of autolysin, inhibition of DNA, RNA, and protein synthesis, and inhibition of certain enzymes. However, in many cases, the exact mechanism of killing is not known. One emerging technique for the study of such

mechanisms is dual polarisation interferometry,. In contrast to many conventional antibiotics these peptides appear to be bacteriocidal (bacteria killer) instead of bacteriostatic (bacteria growth inhibitor). In general the antimicrobial activity of these peptides is determined by measuring the minimal inhibitory concentration (MIC), which is the lowest concentration of drug that inhibits bacterial growth.

Immunomodulatory Activities

In addition to killing bacteria directly they have been demonstrated to have a number of immunomodulatory functions that may be involved in the clearance of infection, including the ability to alter host gene expression, act as chemokines and/or induce chemokine production, inhibiting lipopolysaccharide induced pro-inflammatory cytokine production, promoting wound healing, and modulating the responses of dendritic cells and cells of the adaptive immune response. Animal models indicate that host defence peptides are crucial for both prevention and clearance of infection. It appears as though many peptides initially isolated as and termed “antimicrobial peptides” have been shown to have more significant alternative functions in vivo (e.g. hepcidin).

Determination of AMP action

Several methods have been used to determine the mechanisms of antimicrobial peptide activity.

Methods	Applications
Microscopy	to visualize the effects of antimicrobial peptides on microbial cells.
Fluorescent dyes	to measure antimicrobial peptides to permeabilize membrane vesicles.
Ion channel formation	to assess the formation and stability of an antimicrobial-peptide-induced pore.
Circular dichroism and orientated circular dichroism	to measure the orientation and secondary structure of an antimicrobial peptide bound to a lipid bilayer
Solid-state NMR spectroscopy	to measure the secondary structure, orientation and penetration of antimicrobial peptides into lipid bilayers in the biologically relevant LIQUID-CRYSTALLINE STATE
Neutron diffraction	to measure the diffraction patterns of peptide-induced pores within membranes in oriented multilayers or liquids

Therapeutic Potential

These peptides are excellent candidates for development as novel therapeutic agents and complements to conventional antibiotic therapy because in contrast to conventional antibiotics they do not appear to induce antibiotic resistance while they generally have a

broad range of activity, are bacteriocidal as opposed to bacteriostatic and require a short contact time to induce killing. A number of naturally occurring peptides and their derivatives have been developed as novel anti-infective therapies for conditions as diverse as oral mucositis, lung infections associated with cystic fibrosis (CF), cancer, and topical skin infections. Pexiganan has been shown to be useful to treat infection related diabetic foot ulcer.

Models of Antimicrobial Peptides

Computer simulations have recently provided atomistic resolution pictures of how antimicrobial peptides interact with membranes. Biophysical studies utilizing solid-state NMR experiments have provided an atomic-level resolution explanation of membrane disruption by antimicrobial peptides.

Selectivity of antimicrobial peptides

In the competition of bacterial cells and host cells with the antimicrobial peptides, antimicrobial peptides will preferentially interact with the bacterial cell to the mammalian cells, which enables them to kill microorganisms without being significantly toxic to mammalian cells. Selectivity is a very important feature of the antimicrobial peptides and it can guarantee their function as antibiotics in host defense systems.

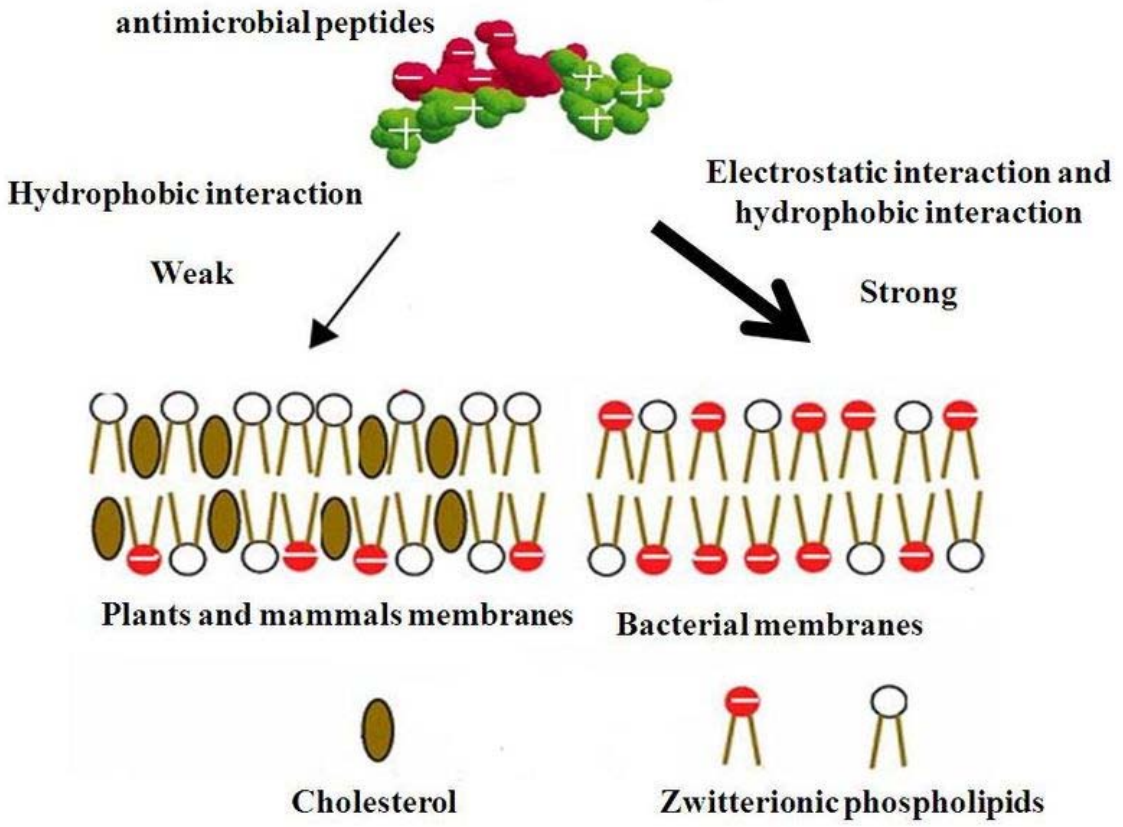
Factors that determine the selectivity of antimicrobial peptides

There are some factors that are closely related to the selectivity property of antimicrobial peptides, among which the cationic property contributes most. Since the surface of the bacterial membranes is more negatively charged than mammalian cells, antimicrobial peptides will show different affinities towards the bacterial membranes and mammalian cell membranes.

In addition, there are also other factors that will affect the selectivity. It’s well known that cholesterol is normally widely distributed in the mammalian cell membranes as a membrane stabilizing agents but absent in bacterial cell membranes; and the presence of these cholesterols will also generally reduce the activities of the antimicrobial peptides, due either to stabilization of the lipid bilayer or to interactions between cholesterol and the peptide. So the cholesterol in mammalian cells will protect the cells from attack by the antimicrobial peptides.

Besides, the transmembrane potential is well-known to affect peptide-lipid interactions. There's an inside-negative transmembrane potential existing from the outer leaflet to the inner leaflet of the cell membranes and this inside-negative transmembrane potential will facilitate membrane permeabilization probably by facilitating the insertion of positively charged peptides into membranes. By comparison, the transmembrane potential of bacterial cells is more negative than that of normal mammalian cells, so bacterial membrane will be prone to be attacked by the positively charged antimicrobial peptides.

Similarly, it is also believed that increasing ionic strength, which in general reduces the activity of most antimicrobial peptides, contributes partially to the selectivity of the antimicrobial peptides by weakening the electrostatic interactions required for the initial interaction.



Molecular Basis of Cell Selectivity of Antimicrobial Peptides

Mechanism of the selectivity

The cell membranes of bacteria are rich in acidic phospholipids, such as phosphatidylglycerol and cardiolipin. These phospholipid headgroups are heavily negatively charged. Therefore, the outmost leaflets of the bilayer which is exposed to the outside of the bacterial membranes are more attractive to the attack of the positively charged antimicrobial peptides. So the interaction between the positive charges of antimicrobial peptides and the negatively charged bacterial membranes is mainly the electrostatic interactions, which is the major driving force for cellular association. Besides, since antimicrobial peptides form structures with a positively charged face as well as a hydrophobic face, there are also some hydrophobic interactions between the hydrophobic regions of the antimicrobial peptides and the zwitterionic phospholipids (electrically neutral) surface of the bacterial membranes, which act only as a minor effect in this case.

In contrast, the outer part of the membranes of the plants and mammals is mainly composed of lipid without any net charges since most of the lipids with negatively charged headgroups are principally sequestered into the inner leaflet of the plasma membranes. Thus in the case of mammals cells, the outer surfaces of the membranes are usually made of zwitterionic phosphatidylcholine and sphingomyelin, even though a small portion of the membranes outer surfaces contain some negatively charged gangliosides. So the hydrophobic interaction between the hydrophobic face of amphipathic antimicrobial peptides and the zwitterionic phospholipids on the cell surface of mammalian cell membranes plays a major role in the formation of peptide-cell binding. However, the hydrophobic interaction is relatively weak when compared to the electrostatic interaction, thus, the antimicrobial peptides will preferentialy interact with the bacterial membranes.

Dual polarisation interferometry has been used *in vitro* to study and quantify the association to headgroup, insertion into the bilayer, pore formation and eventual disruption of the membrane.

Methods to control the selectivity of antimicrobial peptides

A lot of efforts have been tried to control the cell selectivities. For example, Katsumi tried to modify and optimize the physicochemical parameters of the peptides to control the selectivities, including net charge, helicity, hydrophobicity per residue (H), hydrophobic moment (μ) and the angle subtended by the positively charged polar helix face (Φ). Besides, other methods like the introduction of D-amino acids and fluorinated amino acids in the hydrophobic face is believed to break the secondary structures and thus to reduce hydrophobic interaction that's necessary for interaction with mammalian cells. Wan L Z, el also found that Pro→Nlys substitution in Pro-containing β-turn antimicrobial peptides is a promising strategy for the design of new short bacterial cell-selective antimicrobial peptides with intracellular mechanisms of action. Nadezhda V el suggested that direct attachment of magainin to the substrate surface decreased nonspecific cell binding as well as led to improved detection limit for bacterial cells such as Salmonella and E. coli.

Bacterial Resistance

Bacteria use various resistance strategies to avoid antimicrobial peptide killing. Some microorganisms alter net surface charges. *Staphylococcus aureus* transports D-alanine from the cytoplasm to the surface teichoic acid to reduce the net negative charge by introducing basic amino groups. *S. aureus* also modifies its anionic membranes via MprF with L-lysine, increasing the positive net charge. The interaction of antimicrobial peptides with membrane targets can be limited by capsule polysaccharide of *Klebsiella pneumoniae*. Alterations occur in Lipid A. *Salmonella* species reduce the fluidity of their outer membrane by increasing hydrophobic interactions between an increased number of Lipid A acyl tails by adding myristate to Lipid A with 2-hydroxymyristate and forming hepta-acylated Lipid A by adding palmitate. The increased hydrophobic moment is though to retard or abolish antimicrobial peptide insertion and pore formation. The

residues undergo alteration in membrane proteins. In some Gram-negative bacteria, alteration in the production of outer membrane proteins correlates with resistance to killing by antimicrobial peptides. ATP-binding cassette transporters import antimicrobial peptides and the resistance-nodulation cell-division efflux pump exports antimicrobial peptides. Both transporters have been associated with antimicrobial peptide resistance. Bacteria produce proteolytic enzymes,which may degrade antimicrobial peptides leading to their resistance.

Examples

Examples of antimicrobal peptides include magainins, alamethicin, pexiganan or MSI-78, and other MSI peptides like MSI-843 and MSI-594, polyphemusin, human antimicrobial peptide, LL-37, defensins and protegrins.

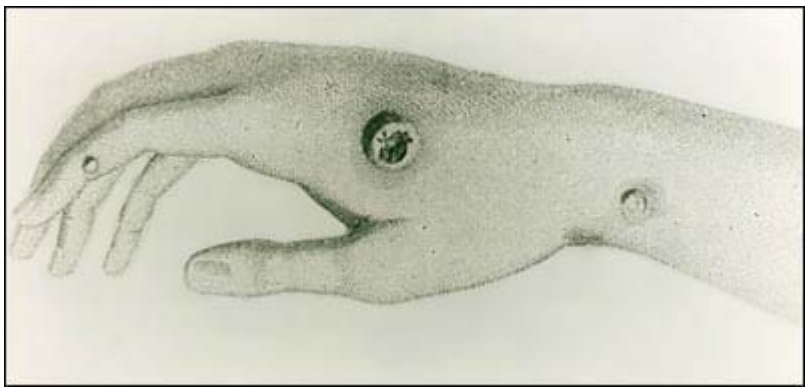
Chapter 20

Artificial Induction of Immunity

Artificial induction of immunity is the artificial (by human agency, artifice and skill) induction (production, generation and initiation) of immunity (the state of not being susceptible) to specific diseases - making people immune to disease by means other than waiting for them to catch the disease. The purpose is to reduce the risk of death and suffering. Immunity against infections that can cause serious illness is generally beneficial. Since Pasteur provided support for a germ theory of infectious disease, we have increasingly **induced immunity** against a widening range of diseases to prevent the associated risks from the wild infections. It is hoped that further understanding of the molecular basis of immunity will translate to improved clinical practice in the future.

Here we, places the development of techniques in historical and logical sequence and points to detailed articles on each of the main topics.

Variolation and smallpox



Typical site of inoculation in Europe and the British colonies

The earliest recorded artificial induction of immunity in humans was by variolation or inoculation, which is the controlled infection of a subject with a less lethal natural form of smallpox (known as Variola Minor) to make him or her immune to re-infection with the more lethal natural form, Variola Major. This was practiced in ancient times in China and India, and imported into Europe, via Turkey, around 1720 by Lady Montagu and perhaps others. From England, the technique spread rapidly to the Colonies, and was also spread by African slaves arriving into Boston.

Variolation had the disadvantage that the inoculating agent used was still an active form of smallpox and, although less potent, could still kill the inoculee or spread in its full form to others nearby. However, as the risk of death from inoculation with Variola Minor was just 1% to 2%, as compared to the 20% risk of death from the natural form of smallpox, the risks of inoculation were generally considered acceptable.

Vaccination

In 1796, Edward Jenner, a doctor and scientist who had practiced variolation, performed an experiment based on the folk-knowledge that infection with cowpox, a disease with minor symptoms which was never fatal, also conferred immunity to smallpox. Jenner induced cowpox infection by transferring material from a lesion on one patient to another, thus infecting the second patient with cowpox. He then demonstrated that the latter was immune by exposing him to smallpox. The principle had been demonstrated some years earlier by Benjamin Jesty, who had not publicized his discovery. Jenner described and generalised the process and then arranged to propagate cowpox for therapeutic use and he is credited with the discovery. Vaccination took over from variolation.

Jenner, like all members of the Royal Society in those days, was an empiricist. The theory to support further advances in vaccination came later.

Germ theory

Louis Pasteur perfected experiments which disproved the then-popular theory of spontaneous generation and from which he derived the modern germ theory of (infectious) disease. Using experiments based on this theory, which posited that specific microorganisms cause specific diseases, Pasteur isolated the infectious agent from anthrax. He then derived a vaccine by altering the infectious agent so as to make it harmless and then introducing this inactivated form of the infectious agents into farm animals, which then proved to be immune to the disease.

Pasteur also isolated a crude preparation of the infectious agent for rabies. In a brave piece of rapid medicine development, he probably saved the life of a person who had been bitten by a clearly rabid dog by performing the same inactivating process upon his rabies preparation and then inoculating the patient with it. The patient, who was expected to die, lived, and thus was the first person successfully vaccinated against rabies.

Anthrax is now known to be caused by a bacterium, and rabies is known to be caused by a virus. The microscopes of the time could reasonably be expected to show bacteria, but imaging of viruses had to wait until the development of electron microscopes with their greater resolving power in the 20th century.

Toxoids

Some diseases, such as tetanus, cause disease not by bacterial growth but by bacterial production of a toxin. Tetanus toxin is so lethal that humans cannot develop immunity to a natural infection, as the amount of toxin and time required to kill a person is much less than is required by the immune system to recognize the toxin and produce antibodies against it. However the tetanus toxin is easily denatured losing its ability to produce disease, but leaving it able to induce immunity to tetanus when injected into subjects. The denatured toxin is called a toxoid.

Adjuvants

The use of simple molecules such as toxoids for immunization tends to produce a low response by the immune system, and thus poor immune memory. However, adding certain substances to the mixture, for example adsorbing tetanus toxoid onto alum, greatly enhances the immune response. These substances are known as adjuvants. Several different adjuvants have been used in vaccine preparation. Adjuvants are also used in other ways in researching the immune system.

A more contemporary approach for "boosting" the immune response to simpler immunogenic molecules (known as antigens) is to **conjugate** the antigens. Conjugation is the attachment to the antigen of another substance which also generates an immune response, thus amplifying the overall response and causing a more robust immune memory to the antigen. For example, a toxoid might be attached to a polysaccharide from the capsule of the bacteria responsible for most lobar pneumonia.

Temporarily-induced immunity

Platypus: monotremes lack placental transfer of immunity

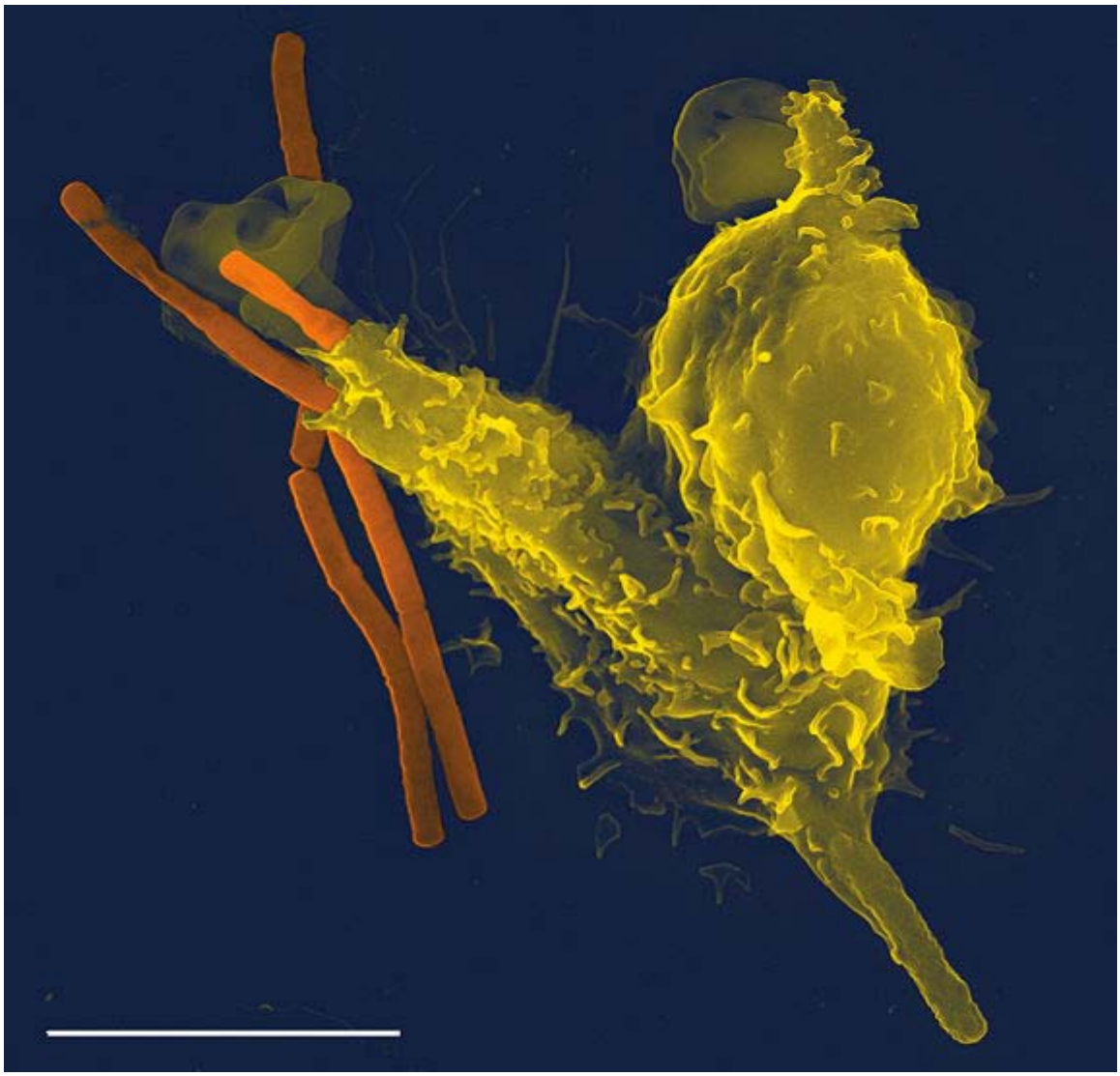
Temporary immunity to a specific infection can be induced in a subject by providing the subject with externally produced immune molecules, known as antibodies or immunoglobulins. This was first performed (and is still sometimes performed) by taking blood from a subject who is already immune, isolating the fraction of the blood which contains antibodies (known as the serum), and injecting this serum into the person for whom immunity is desired. This is known as passive immunity, and the serum that is isolated from one subject and injected into another is sometimes called antiserum. Antiserum from other mammals, notably horses, has been used in humans with generally good and often life-saving results, but there is some risk of anaphylactic shock and even death from this procedure because the human body sometimes recognizes antibodies from other animals as foreign proteins.

Passive immunity is temporary, because the antibodies which are transferred have a lifespan of only about 3–6 months. Every placental mammal (including humans) has experienced temporarily-induced immunity by transfer of homologous antibodies from its mother across the placenta, giving it passive immunity to whatever its mother was immune to. This allows some protection for the young while its own immune system is developing.

Synthetic (recombinant or cell-clone) human immunoglobulins can now be made, and for several reasons (including the risk of prion contamination of biological materials) are likely to be used more and more often. However, they are expensive to produce and are not in large-scale production as of 2006. In the future it might be possible to artificially design antibodies to fit specific antigens, then produce them in large quantities to induce temporary immunity in people in advance of exposure to a specific pathogen, such as a bacterium, a virus, or a prion. At present, the science to understand this process is available but not the technology to perform it.

Chapter 21

Immune System



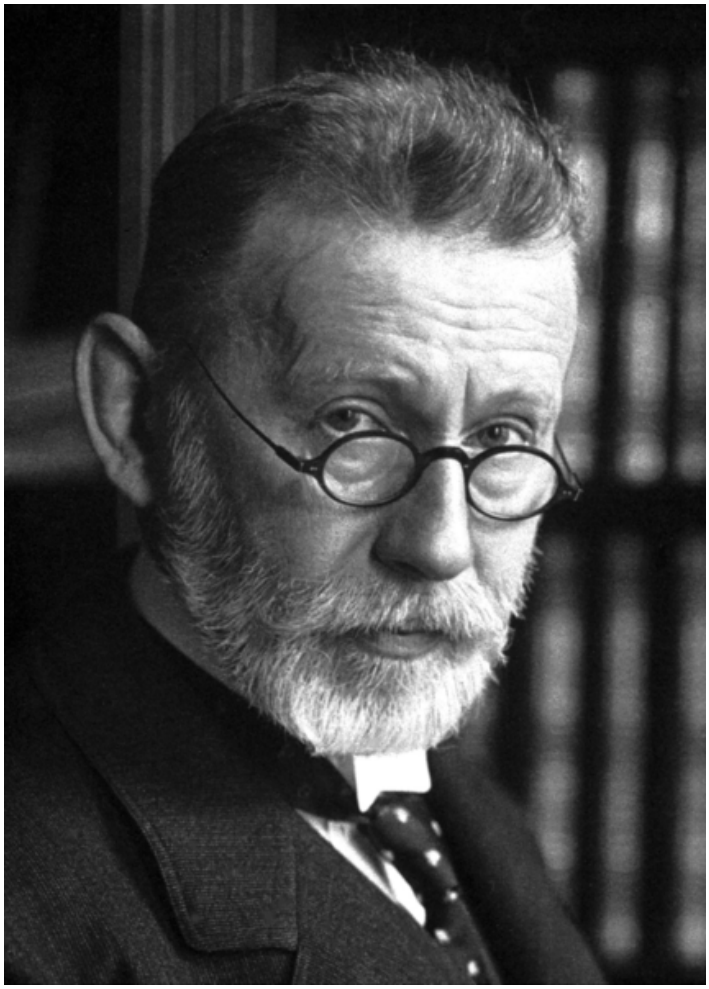
A scanning electron microscope image of a single neutrophil (yellow), engulfing anthrax bacteria (orange)

An **immune system** is a system of biological structures and processes within an organism that protects against disease by identifying and killing pathogens and tumor cells. It detects a wide variety of agents, from viruses to parasitic worms, and needs to distinguish them from the organism's own healthy cells and tissues in order to function properly. Detection is complicated as pathogens can evolve rapidly, and adapt to avoid the immune system and allow the pathogens to successfully infect their hosts.

To survive this challenge, multiple mechanisms evolved that recognize and neutralize pathogens. Even simple unicellular organisms such as bacteria possess enzyme systems that protect against viral infections. Other basic immune mechanisms evolved in ancient eukaryotes and remain in their modern descendants, such as plants and insects. These mechanisms include antimicrobial peptides called defensins, phagocytosis, and the complement system. Jawed vertebrates, including humans, have even more sophisticated defense mechanisms. The typical vertebrate immune system consists of many types of proteins, cells, organs, and tissues that interact in an elaborate and dynamic network. As part of this more complex immune response, the human immune system adapts over time to recognize specific pathogens more efficiently. This adaptation process is referred to as "adaptive immunity" or "acquired immunity" and creates immunological memory. Immunological memory, created from a primary response to a specific pathogen, provides an enhanced response to secondary encounters with that same, specific pathogen. This process of acquired immunity is the basis of vaccination. Primary response can take 2 days and up to 2 weeks to develop. After the body gains immunity towards a certain pathogen, when infection by that pathogen occurs again, the immune response is called the secondary response.

Disorders in the immune system can result in disease, including autoimmune diseases, inflammatory diseases and cancer. Immunodeficiency diseases occur when the immune system is less active than normal, resulting in recurring and life-threatening infections. Immunodeficiency can either be the result of a genetic disease, such as severe combined immunodeficiency, or be produced by pharmaceuticals or an infection, such as the acquired immune deficiency syndrome (AIDS) that is caused by the retrovirus HIV. In contrast, autoimmune diseases result from a hyperactive immune system attacking normal tissues as if they were foreign organisms. Common autoimmune diseases include Hashimoto's thyroiditis, rheumatoid arthritis, diabetes mellitus type 1, and lupus erythematosus. Immunology covers the study of all aspects of the immune system, having significant relevance to health and diseases. Further investigation in this field is expected to play a serious role in promotion of health and treatment of diseases.

History of immunology



Paul Ehrlich

Immunology is a science that examines the structure and function of the immune system. It originates from medicine and early studies on the causes of immunity to disease. The earliest known mention of immunity was during the plague of Athens in 430 BC. Thucydides noted that people who had recovered from a previous bout of the disease could nurse the sick without contracting the illness a second time. In the 18th century, Pierre-Louis Moreau de Maupertuis made experiments with scorpion venom and observed that certain dogs and mice were immune to this venom. This and other observations of acquired immunity was later exploited by Louis Pasteur in his development of vaccination and his proposed germ theory of disease. Pasteur's theory was in direct opposition to contemporary theories of disease, such as the miasma theory. It was not until Robert Koch's 1891 proofs, for which he was awarded a Nobel Prize in 1905, that microorganisms were confirmed as the cause of infectious disease. Viruses were confirmed as human pathogens in 1901, with the discovery of the yellow fever virus by Walter Reed.

Immunology made a great advance towards the end of the 19th century, through rapid developments, in the study of humoral immunity and cellular immunity. Particularly important was the work of Paul Ehrlich, who proposed the side-chain theory to explain the specificity of the antigen-antibody reaction; his contributions to the understanding of humoral immunity were recognized by the award of a Nobel Prize in 1908, which was jointly awarded to the founder of cellular immunology, Elie Metchnikoff.

Layered defense

The immune system protects organisms from infection with layered defenses of increasing specificity. In simple terms, physical barriers prevent pathogens such as bacteria and viruses from entering the organism. If a pathogen breaches these barriers, the innate immune system provides an immediate, but non-specific response. Innate immune systems are found in all plants and animals. If pathogens successfully evade the innate response, vertebrates possess a third layer of protection, the adaptive immune system, which is activated by the innate response. Here, the immune system adapts its response during an infection to improve its recognition of the pathogen. This improved response is then retained after the pathogen has been eliminated, in the form of an immunological memory, and allows the adaptive immune system to mount faster and stronger attacks each time this pathogen is encountered.

Components of the immune system	
Innate immune system	**Adaptive immune system**
Response is non-specific	Pathogen and antigen specific response
Exposure leads to immediate maximal response	Lag time between exposure and maximal response
Cell-mediated and humoral components	Cell-mediated and humoral components
No immunological memory	Exposure leads to immunological memory
Found in nearly all forms of life	Found only in jawed vertebrates

Both innate and adaptive immunity depend on the ability of the immune system to distinguish between self and non-self molecules. In immunology, *self* molecules are those components of an organism's body that can be distinguished from foreign substances by the immune system. Conversely, *non-self* molecules are those recognized as foreign molecules. One class of non-self molecules are called antigens (short for *anti*body *gen*erators) and are defined as substances that bind to specific immune receptors and elicit an immune response.

Surface barriers

Several barriers protect organisms from infection, including mechanical, chemical, and biological barriers. The waxy cuticle of many leaves, the exoskeleton of insects, the shells and membranes of externally deposited eggs, and skin are examples of mechanical barriers that are the first line of defense against infection. However, as organisms cannot

be completely sealed against their environments, other systems act to protect body openings such as the lungs, intestines, and the genitourinary tract. In the lungs, coughing and sneezing mechanically eject pathogens and other irritants from the respiratory tract. The flushing action of tears and urine also mechanically expels pathogens, while mucus secreted by the respiratory and gastrointestinal tract serves to trap and entangle microorganisms.

Chemical barriers also protect against infection. The skin and respiratory tract secrete antimicrobial peptides such as the β-defensins. Enzymes such as lysozyme and phospholipase A2 in saliva, tears, and breast milk are also antibacterials. Vaginal secretions serve as a chemical barrier following menarche, when they become slightly acidic, while semen contains defensins and zinc to kill pathogens. In the stomach, gastric acid and proteases serve as powerful chemical defenses against ingested pathogens.

Within the genitourinary and gastrointestinal tracts, commensal flora serve as biological barriers by competing with pathogenic bacteria for food and space and, in some cases, by changing the conditions in their environment, such as pH or available iron. This reduces the probability that pathogens will be able to reach sufficient numbers to cause illness. However, since most antibiotics non-specifically target bacteria and do not affect fungi, oral antibiotics can lead to an "overgrowth" of fungi and cause conditions such as a vaginal candidiasis (a yeast infection). There is good evidence that re-introduction of probiotic flora, such as pure cultures of the lactobacilli normally found in unpasteurized yoghurt, helps restore a healthy balance of microbial populations in intestinal infections in children and encouraging preliminary data in studies on bacterial gastroenteritis, inflammatory bowel diseases, urinary tract infection and post-surgical infections.

Innate

Microorganisms or toxins that successfully enter an organism will encounter the cells and mechanisms of the innate immune system. The innate response is usually triggered when microbes are identified by pattern recognition receptors, which recognize components that are conserved among broad groups of microorganisms, or when damaged, injured or stressed cells send out alarm signals, many of which (but not all) are recognized by the same receptors as those that recognize pathogens. Innate immune defenses are non-specific, meaning these systems respond to pathogens in a generic way. This system does not confer long-lasting immunity against a pathogen. The innate immune system is the dominant system of host defense in most organisms.

Inflammation

Inflammation is one of the first responses of the immune system to infection. The symptoms of inflammation are redness and swelling, which are caused by increased blood flow into a tissue. Inflammation is produced by eicosanoids and cytokines, which are released by injured or infected cells. Eicosanoids include prostaglandins that produce fever and the dilation of blood vessels associated with inflammation, and leukotrienes that attract certain white blood cells (leukocytes). Common cytokines include interleukins

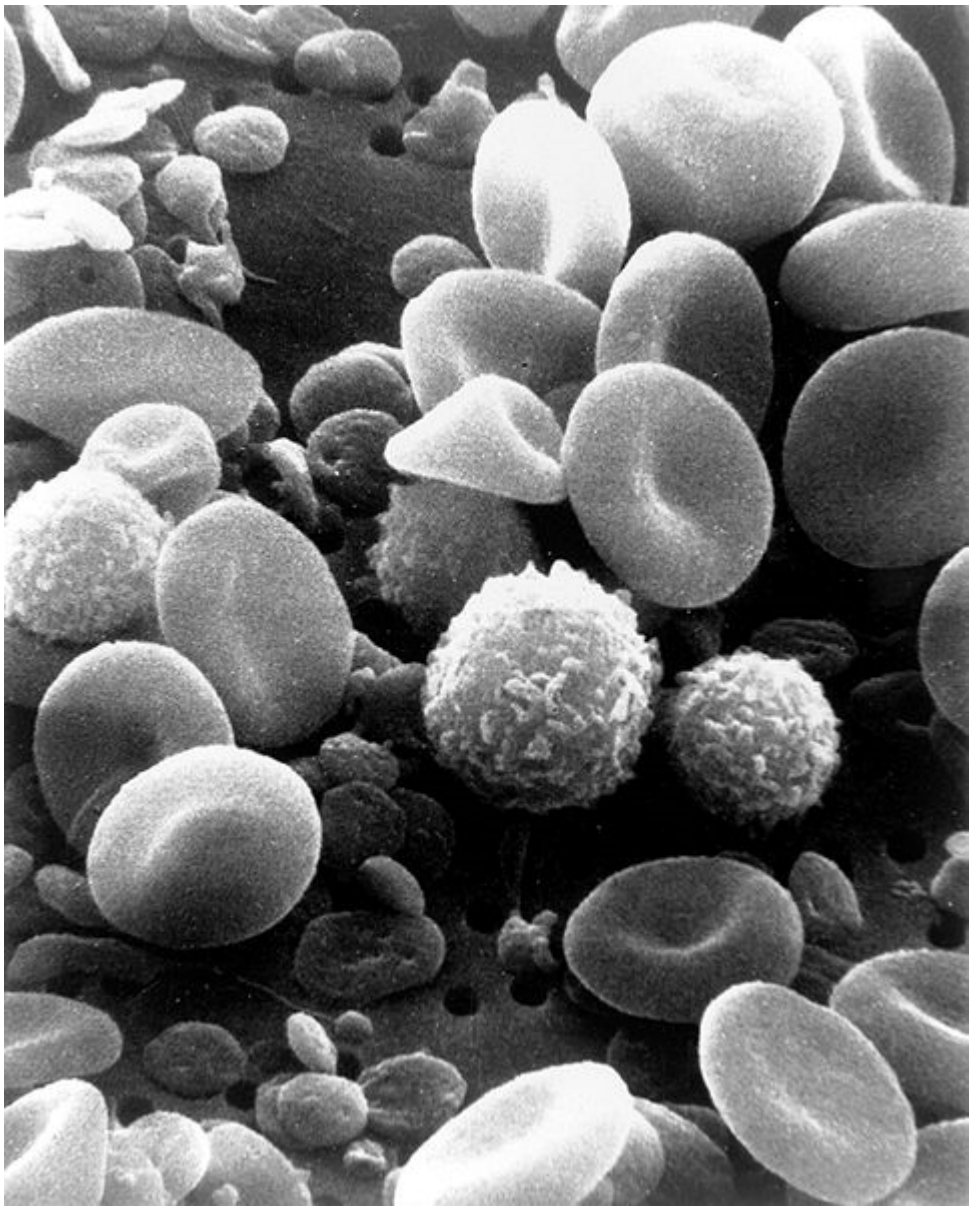
that are responsible for communication between white blood cells; chemokines that promote chemotaxis; and interferons that have anti-viral effects, such as shutting down protein synthesis in the host cell. Growth factors and cytotoxic factors may also be released. These cytokines and other chemicals recruit immune cells to the site of infection and promote healing of any damaged tissue following the removal of pathogens.

Complement system

The complement system is a biochemical cascade that attacks the surfaces of foreign cells. It contains over 20 different proteins and is named for its ability to "complement" the killing of pathogens by antibodies. Complement is the major humoral component of the innate immune response. Many species have complement systems, including non-mammals like plants, fish, and some invertebrates.

In humans, this response is activated by complement binding to antibodies that have attached to these microbes or the binding of complement proteins to carbohydrates on the surfaces of microbes. This recognition signal triggers a rapid killing response. The speed of the response is a result of signal amplification that occurs following sequential proteolytic activation of complement molecules, which are also proteases. After complement proteins initially bind to the microbe, they activate their protease activity, which in turn activates other complement proteases, and so on. This produces a catalytic cascade that amplifies the initial signal by controlled positive feedback. The cascade results in the production of peptides that attract immune cells, increase vascular permeability, and opsonize (coat) the surface of a pathogen, marking it for destruction. This deposition of complement can also kill cells directly by disrupting their plasma membrane.

Cellular barriers



A scanning electron microscope image of normal circulating human blood. One can see red blood cells, several knobby white blood cells including lymphocytes, a monocyte, a neutrophil, and many small disc-shaped platelets.

Leukocytes (white blood cells) act like independent, single-celled organisms and are the second arm of the innate immune system. The innate leukocytes include the phagocytes (macrophages, neutrophils, and dendritic cells), mast cells, eosinophils, basophils, and natural killer cells. These cells identify and eliminate pathogens, either by attacking larger pathogens through contact or by engulfing and then killing microorganisms. Innate cells are also important mediators in the activation of the adaptive immune system.

Phagocytosis is an important feature of cellular innate immunity performed by cells called 'phagocytes' that engulf, or eat, pathogens or particles. Phagocytes generally patrol the body searching for pathogens, but can be called to specific locations by cytokines. Once a pathogen has been engulfed by a phagocyte, it becomes trapped in an intracellular vesicle called a phagosome, which subsequently fuses with another vesicle called a lysosome to form a phagolysosome. The pathogen is killed by the activity of digestive enzymes or following a respiratory burst that releases free radicals into the phagolysosome. Phagocytosis evolved as a means of acquiring nutrients, but this role was extended in phagocytes to include engulfment of pathogens as a defense mechanism. Phagocytosis probably represents the oldest form of host defense, as phagocytes have been identified in both vertebrate and invertebrate animals.

Neutrophils and macrophages are phagocytes that travel throughout the body in pursuit of invading pathogens. Neutrophils are normally found in the bloodstream and are the most abundant type of phagocyte, normally representing 50% to 60% of the total circulating leukocytes. During the acute phase of inflammation, particularly as a result of bacterial infection, neutrophils migrate toward the site of inflammation in a process called chemotaxis, and are usually the first cells to arrive at the scene of infection. Macrophages are versatile cells that reside within tissues and produce a wide array of chemicals including enzymes, complement proteins, and regulatory factors such as interleukin 1. Macrophages also act as scavengers, ridding the body of worn-out cells and other debris, and as antigen-presenting cells that activate the adaptive immune system.

Dendritic cells (DC) are phagocytes in tissues that are in contact with the external environment; therefore, they are located mainly in the skin, nose, lungs, stomach, and intestines. They are named for their resemblance to neuronal dendrites, as both have many spine-like projections, but dendritic cells are in no way connected to the nervous system. Dendritic cells serve as a link between the bodily tissues and the innate and adaptive immune systems, as they present antigen to T cells, one of the key cell types of the adaptive immune system.

Mast cells reside in connective tissues and mucous membranes, and regulate the inflammatory response. They are most often associated with allergy and anaphylaxis. Basophils and eosinophils are related to neutrophils. They secrete chemical mediators that are involved in defending against parasites and play a role in allergic reactions, such as asthma. Natural killer (NK cells) cells are leukocytes that attack and destroy tumor cells, or cells that have been infected by viruses.

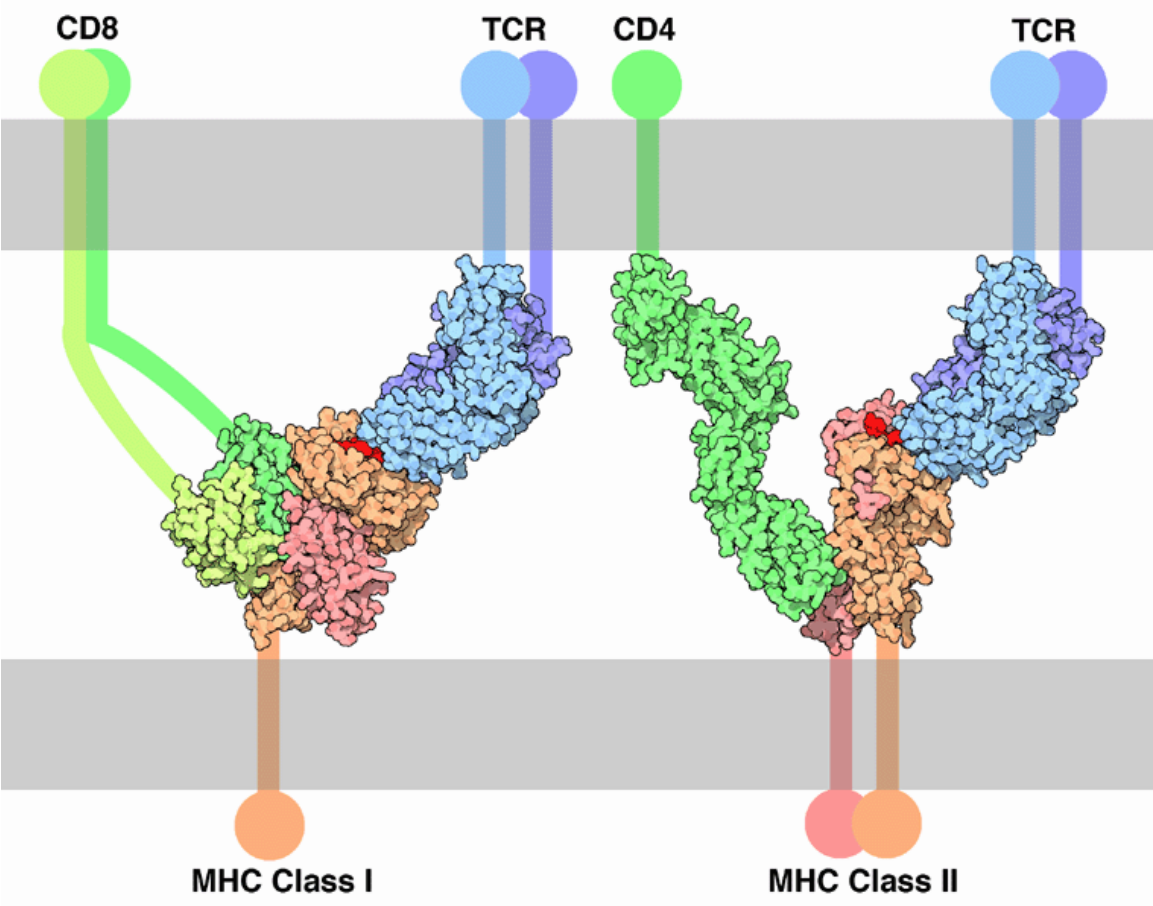
Adaptive

The adaptive immune system evolved in early vertebrates and allows for a stronger immune response as well as immunological memory, where each pathogen is "remembered" by a signature antigen. The adaptive immune response is antigen-specific and requires the recognition of specific “non-self” antigens during a process called antigen presentation. Antigen specificity allows for the generation of responses that are tailored to specific pathogens or pathogen-infected cells. The ability to mount these

tailored responses is maintained in the body by "memory cells". Should a pathogen infect the body more than once, these specific memory cells are used to quickly eliminate it.

Lymphocytes

The cells of the adaptive immune system are special types of leukocytes, called lymphocytes. B cells and T cells are the major types of lymphocytes and are derived from hematopoietic stem cells in the bone marrow. B cells are involved in the humoral immune response, whereas T cells are involved in cell-mediated immune response.

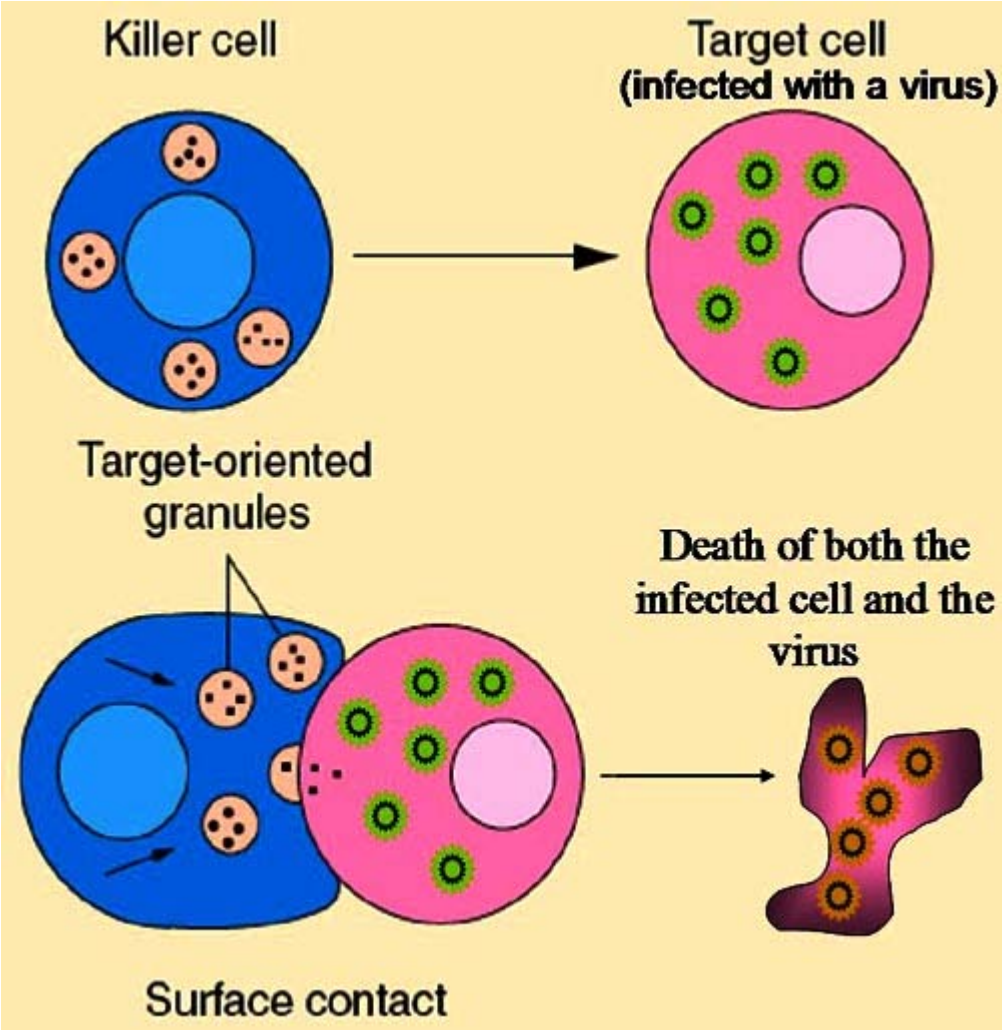


Association of a T cell with MHC class I or MHC class II, and antigen (in red)

Both B cells and T cells carry receptor molecules that recognize specific targets. T cells recognize a “non-self” target, such as a pathogen, only after antigens (small fragments of the pathogen) have been processed and presented in combination with a “self” receptor called a major histocompatibility complex (MHC) molecule. There are two major subtypes of T cells: the killer T cell and the helper T cell. Killer T cells only recognize antigens coupled to Class I MHC molecules, while helper T cells only recognize antigens coupled to Class II MHC molecules. These two mechanisms of antigen presentation reflect the different roles of the two types of T cell. A third, minor subtype are the γδ T cells that recognize intact antigens that are not bound to MHC receptors.

In contrast, the B cell antigen-specific receptor is an antibody molecule on the B cell surface, and recognizes whole pathogens without any need for antigen processing. Each lineage of B cell expresses a different antibody, so the complete set of B cell antigen receptors represent all the antibodies that the body can manufacture.

Killer T cells

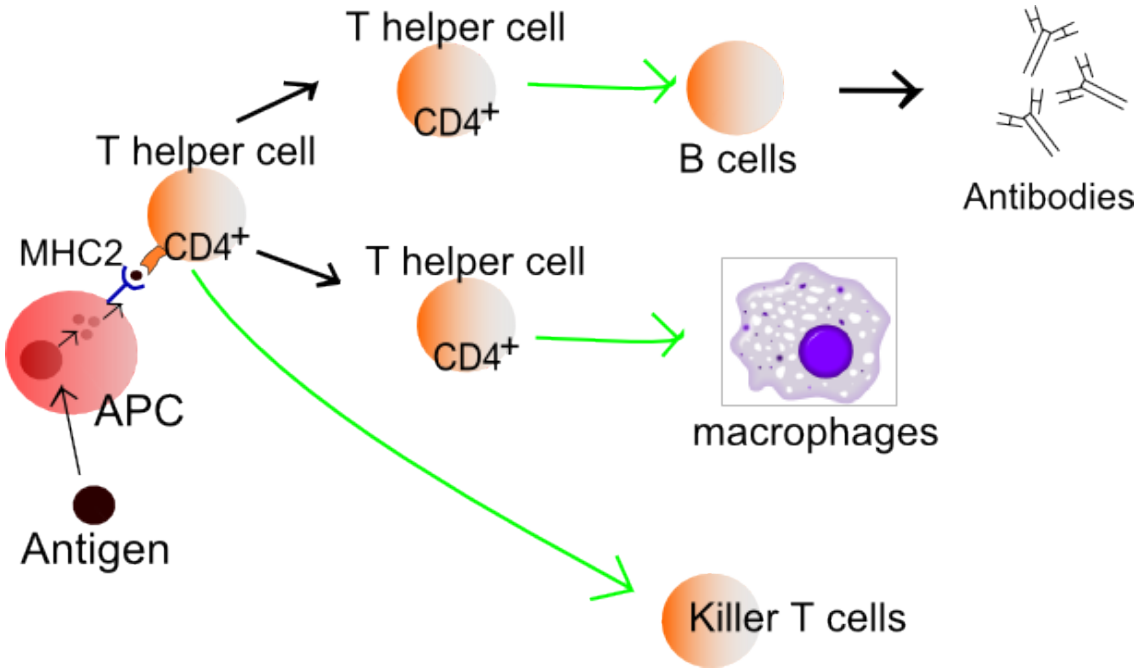


Killer T cells directly attack other cells carrying foreign or abnormal antigens on their surfaces.

Killer T cell are a sub-group of T cells that kill cells that are infected with viruses (and other pathogens), or are otherwise damaged or dysfunctional. As with B cells, each type of T cell recognises a different antigen. Killer T cells are activated when their T cell receptor (TCR) binds to this specific antigen in a complex with the MHC Class I receptor of another cell. Recognition of this MHC:antigen complex is aided by a co-receptor on the T cell, called CD8. The T cell then travels throughout the body in search of cells where the MHC I receptors bear this antigen. When an activated T cell contacts such cells, it releases cytotoxins, such as perforin, which form pores in the target cell's plasma

membrane, allowing ions, water and toxins to enter. The entry of another toxin called granulysin (a protease) induces the target cell to undergo apoptosis. T cell killing of host cells is particularly important in preventing the replication of viruses. T cell activation is tightly controlled and generally requires a very strong MHC/antigen activation signal, or additional activation signals provided by "helper" T cells (see below).

Helper T cells



Function of T helper cells: Antigen-presenting cells (APCs) present antigen on their Class II MHC molecules (MHC2). Helper T cells recognize these, with the help of their expression of CD4 co-receptor (CD4+). The activation of a resting helper T cell causes it to release cytokines and other stimulatory signals (green arrows) that stimulate the activity of macrophages, killer T cells and B cells, the latter producing antibodies. The stimulation of B cells and macrophages succeeds a proliferation of T helper cells.

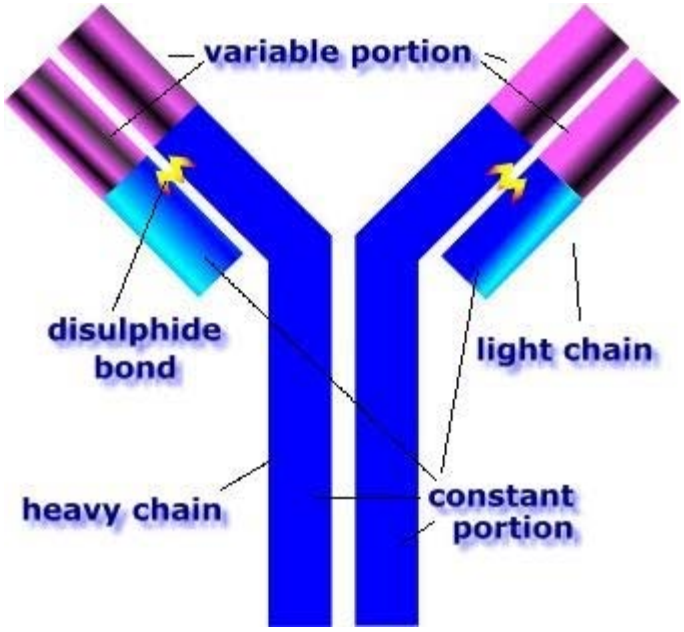
Helper T cells regulate both the innate and adaptive immune responses and help determine which types of immune responses the body will make to a particular pathogen. These cells have no cytotoxic activity and do not kill infected cells or clear pathogens directly. They instead control the immune response by directing other cells to perform these tasks.

Helper T cells express T cell receptors (TCR) that recognize antigen bound to Class II MHC molecules. The MHC:antigen complex is also recognized by the helper cell's CD4 co-receptor, which recruits molecules inside the T cell (e.g., Lck) that are responsible for the T cell's activation. Helper T cells have a weaker association with the MHC:antigen complex than observed for killer T cells, meaning many receptors (around 200–300) on the helper T cell must be bound by an MHC:antigen in order to activate the helper cell, while killer T cells can be activated by engagement of a single MHC:antigen molecule.

Helper T cell activation also requires longer duration of engagement with an antigen-presenting cell. The activation of a resting helper T cell causes it to release cytokines that influence the activity of many cell types. Cytokine signals produced by helper T cells enhance the microbicidal function of macrophages and the activity of killer T cells. In addition, helper T cell activation causes an upregulation of molecules expressed on the T cell's surface, such as CD40 ligand (also called CD154), which provide extra stimulatory signals typically required to activate antibody-producing B cells.

γδ T cells

γδ T cells possess an alternative T cell receptor (TCR) as opposed to CD4+ and CD8+ (αβ) T cells and share the characteristics of helper T cells, cytotoxic T cells and NK cells. The conditions that produce responses from γδ T cells are not fully understood. Like other 'unconventional' T cell subsets bearing invariant TCRs, such as CD1d-restricted Natural Killer T cells, γδ T cells straddle the border between innate and adaptive immunity. On one hand, γδ T cells are a component of adaptive immunity as they rearrange TCR genes to produce receptor diversity and can also develop a memory phenotype. On the other hand, the various subsets are also part of the innate immune system, as restricted TCR or NK receptors may be used as pattern recognition receptors. For example, large numbers of human Vγ9/Vδ2 T cells respond within hours to common molecules produced by microbes, and highly restricted Vδ1+ T cells in epithelia will respond to stressed epithelial cells.



An antibody is made up of two heavy chains and two light chains. The unique variable region allows an antibody to recognize its matching antigen.

B lymphocytes and antibodies

A B cell identifies pathogens when antibodies on its surface bind to a specific foreign antigen. This antigen/antibody complex is taken up by the B cell and processed by proteolysis into peptides. The B cell then displays these antigenic peptides on its surface MHC class II molecules. This combination of MHC and antigen attracts a matching helper T cell, which releases lymphokines and activates the B cell. As the activated B cell then begins to divide, its offspring (plasma cells) secrete millions of copies of the antibody that recognizes this antigen. These antibodies circulate in blood plasma and lymph, bind to pathogens expressing the antigen and mark them for destruction by complement activation or for uptake and destruction by phagocytes. Antibodies can also neutralize challenges directly, by binding to bacterial toxins or by interfering with the receptors that viruses and bacteria use to infect cells.

Alternative adaptive immune system

Although the classical molecules of the adaptive immune system (e.g., antibodies and T cell receptors) exist only in jawed vertebrates, a distinct lymphocyte-derived molecule has been discovered in primitive jawless vertebrates, such as the lamprey and hagfish. These animals possess a large array of molecules called variable lymphocyte receptors (VLRs) that, like the antigen receptors of jawed vertebrates, are produced from only a small number (one or two) of genes. These molecules are believed to bind pathogenic antigens in a similar way to antibodies, and with the same degree of specificity.

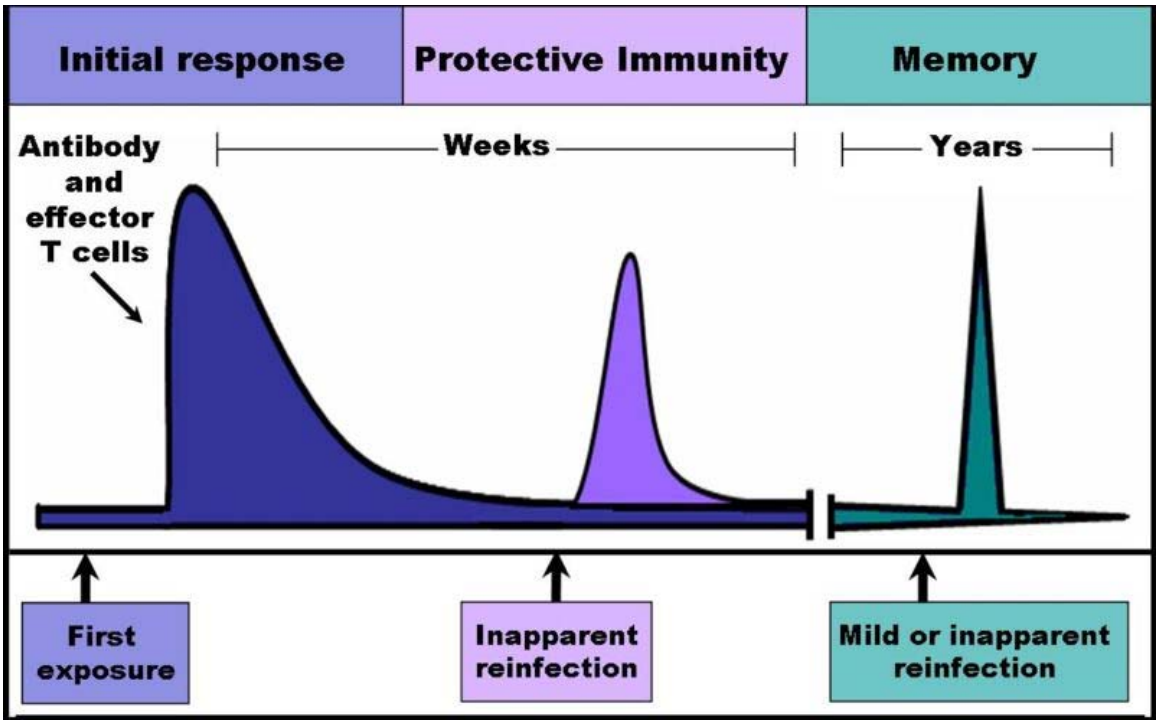
Immunological memory

When B cells and T cells are activated and begin to replicate, some of their offspring will become long-lived memory cells. Throughout the lifetime of an animal, these memory cells will remember each specific pathogen encountered and can mount a strong response if the pathogen is detected again. This is "adaptive" because it occurs during the lifetime of an individual as an adaptation to infection with that pathogen and prepares the immune system for future challenges. Immunological memory can be in the form of either passive short-term memory or active long-term memory.

Passive memory

Newborn infants have no prior exposure to microbes and are particularly vulnerable to infection. Several layers of passive protection are provided by the mother. During pregnancy, a particular type of antibody, called IgG, is transported from mother to baby directly across the placenta, so human babies have high levels of antibodies even at birth, with the same range of antigen specificities as their mother. Breast milk or colostrum also contains antibodies that are transferred to the gut of the infant and protect against bacterial infections until the newborn can synthesize its own antibodies. This is passive immunity because the fetus does not actually make any memory cells or antibodies—it only borrows them. This passive immunity is usually short-term, lasting from a few days

up to several months. In medicine, protective passive immunity can also be transferred artificially from one individual to another via antibody-rich serum.



The time-course of an immune response begins with the initial pathogen encounter, (or initial vaccination) and leads to the formation and maintenance of active immunological memory.

Active memory and immunization

Long-term *active* memory is acquired following infection by activation of B and T cells. Active immunity can also be generated artificially, through vaccination. The principle behind vaccination (also called immunization) is to introduce an antigen from a pathogen in order to stimulate the immune system and develop specific immunity against that particular pathogen without causing disease associated with that organism. This deliberate induction of an immune response is successful because it exploits the natural specificity of the immune system, as well as its inducibility. With infectious disease remaining one of the leading causes of death in the human population, vaccination represents the most effective manipulation of the immune system mankind has developed.

Most viral vaccines are based on live attenuated viruses, while many bacterial vaccines are based on acellular components of micro-organisms, including harmless toxin components. Since many antigens derived from acellular vaccines do not strongly induce the adaptive response, most bacterial vaccines are provided with additional adjuvants that activate the antigen-presenting cells of the innate immune system and maximize immunogenicity.

Disorders of human immunity

The immune system is a remarkably effective structure that incorporates specificity, inducibility and adaptation. Failures of host defense do occur, however, and fall into three broad categories: immunodeficiencies, autoimmunity, and hypersensitivities.

Immunodeficiencies

Immunodeficiencies occur when one or more of the components of the immune system are inactive. The ability of the immune system to respond to pathogens is diminished in both the young and the elderly, with immune responses beginning to decline at around 50 years of age due to immunosenescence. In developed countries, obesity, alcoholism, and drug use are common causes of poor immune function. However, malnutrition is the most common cause of immunodeficiency in developing countries. Diets lacking sufficient protein are associated with impaired cell-mediated immunity, complement activity, phagocyte function, IgA antibody concentrations, and cytokine production. Deficiency of single nutrients such as iron; copper; zinc; selenium; vitamins A, C, E, and B_6; and folic acid (vitamin B_9) also reduces immune responses. Additionally, the loss of the thymus at an early age through genetic mutation or surgical removal results in severe immunodeficiency and a high susceptibility to infection.

Immunodeficiencies can also be inherited or 'acquired'. Chronic granulomatous disease, where phagocytes have a reduced ability to destroy pathogens, is an example of an inherited, or congenital, immunodeficiency. AIDS and some types of cancer cause acquired immunodeficiency.

Autoimmunity

Overactive immune responses comprise the other end of immune dysfunction, particularly the autoimmune disorders. Here, the immune system fails to properly distinguish between self and non-self, and attacks part of the body. Under normal circumstances, many T cells and antibodies react with “self” peptides. One of the functions of specialized cells (located in the thymus and bone marrow) is to present young lymphocytes with self antigens produced throughout the body and to eliminate those cells that recognize self-antigens, preventing autoimmunity.

Hypersensitivity

Hypersensitivity is an immune response that damages the body's own tissues. They are divided into four classes (Type I – IV) based on the mechanisms involved and the time course of the hypersensitive reaction. Type I hypersensitivity is an immediate or anaphylactic reaction, often associated with allergy. Symptoms can range from mild discomfort to death. Type I hypersensitivity is mediated by IgE, which triggers degranulation of mast cells and basophils when cross-linked by antigen. Type II hypersensitivity occurs when antibodies bind to antigens on the patient's own cells, marking them for destruction. This is also called antibody-dependent (or cytotoxic)

hypersensitivity, and is mediated by IgG and IgM antibodies. Immune complexes (aggregations of antigens, complement proteins, and IgG and IgM antibodies) deposited in various tissues trigger Type III hypersensitivity reactions. Type IV hypersensitivity (also known as cell-mediated or *delayed type hypersensitivity*) usually takes between two and three days to develop. Type IV reactions are involved in many autoimmune and infectious diseases, but may also involve *contact dermatitis* (poison ivy). These reactions are mediated by T cells, monocytes, and macrophages.

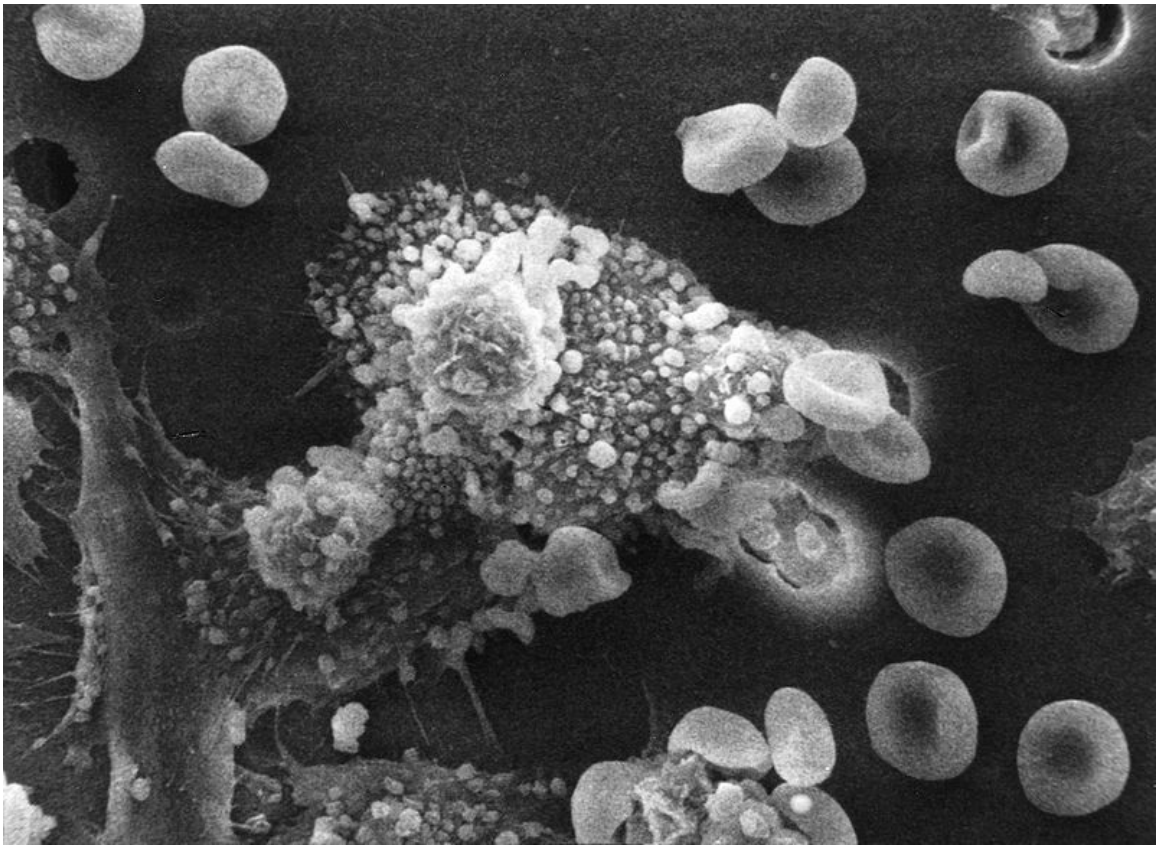
Other mechanisms

It is likely that a multicomponent, adaptive immune system arose with the first vertebrates, as invertebrates do not generate lymphocytes or an antibody-based humoral response. Many species, however, utilize mechanisms that appear to be precursors of these aspects of vertebrate immunity. Immune systems appear even in the structurally most simple forms of life, with bacteria using a unique defense mechanism, called the restriction modification system to protect themselves from viral pathogens, called bacteriophages. Prokaryotes also possess acquired immunity, through a system that uses CRISPR sequences to retain fragments of the genomes of phage that they have come into contact with in the past, which allows them to block virus replication through a form of RNA interference.

Pattern recognition receptors are proteins used by nearly all organisms to identify molecules associated with pathogens. Antimicrobial peptides called defensins are an evolutionarily conserved component of the innate immune response found in all animals and plants, and represent the main form of invertebrate systemic immunity. The complement system and phagocytic cells are also used by most forms of invertebrate life. Ribonucleases and the RNA interference pathway are conserved across all eukaryotes, and are thought to play a role in the immune response to viruses.

Unlike animals, plants lack phagocytic cells, but many plant immune responses involve systemic chemical signals that are sent through a plant. Individual plant cells respond to molecules associated with pathogens known as Pathogen-associated molecular patterns or PAMPs. When a part of a plant becomes infected, the plant produces a localized hypersensitive response, whereby cells at the site of infection undergo rapid apoptosis to prevent the spread of the disease to other parts of the plant. Systemic acquired resistance (SAR) is a type of defensive response used by plants that renders the entire plant resistant to a particular infectious agent. RNA silencing mechanisms are particularly important in this systemic response as they can block virus replication.

Tumor immunology



Macrophages have identified a cancer cell (the large, spiky mass). Upon fusing with the cancer cell, the macrophages (smaller white cells) will inject toxins that kill the tumor cell. Immunotherapy for the treatment of cancer is an active area of medical research.

Another important role of the immune system is to identify and eliminate tumors. The *transformed cells* of tumors express antigens that are not found on normal cells. To the immune system, these antigens appear foreign, and their presence causes immune cells to attack the transformed tumor cells. The antigens expressed by tumors have several sources; some are derived from oncogenic viruses like human papillomavirus, which causes cervical cancer, while others are the organism's own proteins that occur at low levels in normal cells but reach high levels in tumor cells. One example is an enzyme called tyrosinase that, when expressed at high levels, transforms certain skin cells (e.g. melanocytes) into tumors called melanomas. A third possible source of tumor antigens are proteins normally important for regulating cell growth and survival, that commonly mutate into cancer inducing molecules called oncogenes.

The main response of the immune system to tumors is to destroy the abnormal cells using killer T cells, sometimes with the assistance of helper T cells. Tumor antigens are presented on MHC class I molecules in a similar way to viral antigens. This allows killer T cells to recognize the tumor cell as abnormal. NK cells also kill tumorous cells in a similar way, especially if the tumor cells have fewer MHC class I molecules on their

surface than normal; this is a common phenomenon with tumors. Sometimes antibodies are generated against tumor cells allowing for their destruction by the complement system.

Clearly, some tumors evade the immune system and go on to become cancers. Tumor cells often have a reduced number of MHC class I molecules on their surface, thus avoiding detection by killer T cells. Some tumor cells also release products that inhibit the immune response; for example by secreting the cytokine TGF-β, which suppresses the activity of macrophages and lymphocytes. In addition, immunological tolerance may develop against tumor antigens, so the immune system no longer attacks the tumor cells.

Paradoxically, macrophages can promote tumor growth when tumor cells send out cytokines that attract macrophages, which then generate cytokines and growth factors that nurture tumor development. In addition, a combination of hypoxia in the tumor and a cytokine produced by macrophages induces tumor cells to decrease production of a protein that blocks metastasis and thereby assists spread of cancer cells.

Physiological regulation

Hormones can act as immunomodulators, altering the sensitivity of the immune system. For example, female sex hormones are known immunostimulators of both adaptive and innate immune responses. Some autoimmune diseases such as lupus erythematosus strike women preferentially, and their onset often coincides with puberty. By contrast, male sex hormones such as testosterone seem to be immunosuppressive. Other hormones appear to regulate the immune system as well, most notably prolactin, growth hormone and vitamin D.

Part of the mechanism by which vitamin D acts to modulate the immune system is by working with "naive" T-cells and activating them so they can attack foreign pathogens. When a T-cell encounters a foreign pathogen like an invading virus or harmful bacteria, the first thing that it does is look around for vitamin D. Once the T-cell finds vitamin D, it binds to it to "activate" itself and become a killer T-cell. Without this activation, T-cells may detect the pathogen, but will not respond with an attack and instead remain "naive". In this way vitamin D acts as the "on" switch for this critical part of the immune system.

It is conjectured that a progressive decline in hormone levels with age is partially responsible for weakened immune responses in aging individuals. Conversely, some hormones are regulated by the immune system, notably thyroid hormone activity. The age-related decline in immune function is also related to dropping vitamin D levels in the elderly. As people age, two things happen that negatively affect their vitamin D levels. First, they stay indoors more due to decreased activity levels. This means that they get less sun and therefore produce less cholecalciferol via UVB radiation. Second, as a person ages the skin becomes less adept at producing vitamin D.

The immune system is affected by sleep and rest, and sleep deprivation is detrimental to immune function. Complex feedback loops involving cytokines, such as interleukin-1 and

tumor necrosis factor-α produced in response to infection, appear to also play a role in the regulation of non-rapid eye movement (REM) sleep. Thus the immune response to infection may result in changes to the sleep cycle, including an increase in slow-wave sleep relative to REM sleep.

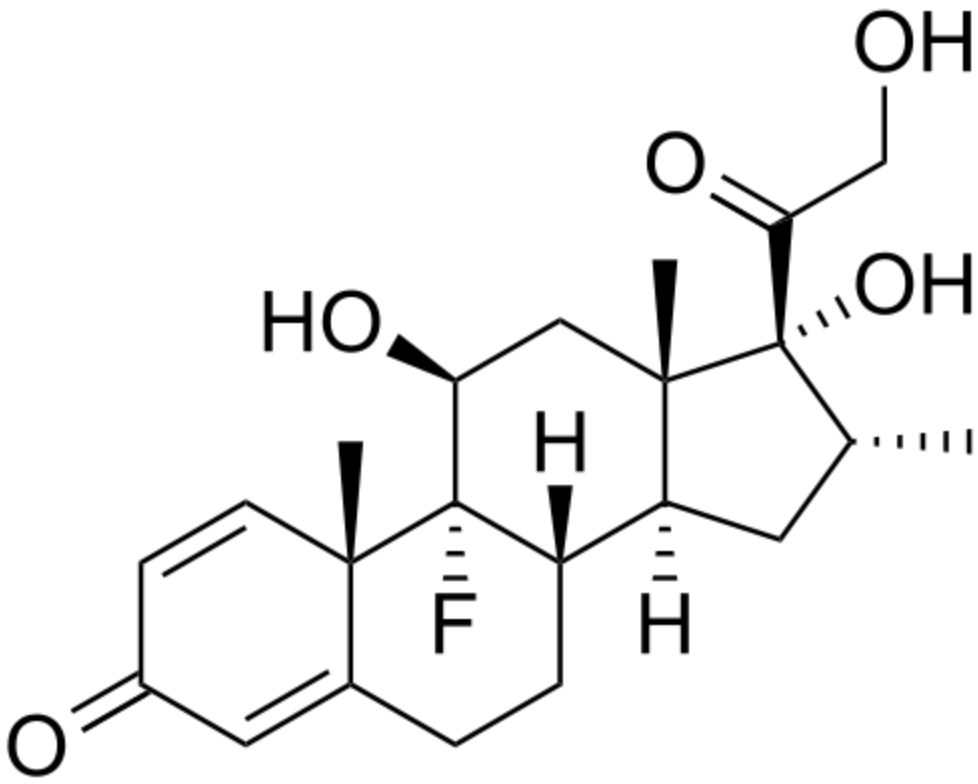
Nutrition and diet

The functioning of the immune system, like most systems in the body, is dependent on proper nutrition. It has been long known that severe malnutrition leads to immunodeficiency. Overnutrition is also associated with diseases such as diabetes and obesity, which are known to affect immune function. More moderate malnutrition, as well as certain specific trace mineral and nutrient deficiencies, can also compromise the immune response.

Specific foods may also affect the immune system; for example, fresh fruits, vegetables, and foods rich in certain fatty acids may foster a healthy immune system. Likewise, fetal undernourishment can cause a lifelong impairment of the immune system. In traditional medicine, some herbs are believed to stimulate the immune system, such as echinacea, licorice, ginseng, astragalus, sage, garlic, elderberry, and hyssop, as well as honey although further research is needed to understand their mode of action.

Medicinal mushrooms like Shiitake, Lingzhi mushrooms, the Turkey tail mushroom, *Agaricus blazei*, and Maitake have shown some evidence of immune system up-regulation in *in vitro* and *in vivo* studies, as well as in a limited number of clinical studies. Research suggests that the compounds in medicinal mushrooms most responsible for up-regulating the immune system are a diverse collection of polysaccharides, particularly beta-glucans, and to a lesser extent, alpha-glucans (such as Active Hexose Correlated Compound isolated from Shiitake). The mechanisms by which beta-glucans stimulate the immune system is only partially understood. One mechanism by which beta-glucans are thought affect immune function is through interaction with the complement receptor 3 (CD18), which is expressed on several types of immune cells. Other receptors–such as Toll-like receptor 2, Dectin-1, lactosylceramide, and scavenger receptors–have also been identified as being able to receive signals from beta-glucans.

Manipulation in medicine



The immunosuppressive drug dexamethasone

The immune response can be manipulated to suppress unwanted responses resulting from autoimmunity, allergy, and transplant rejection, and to stimulate protective responses against pathogens that largely elude the immune system. Immunosuppressive drugs are used to control autoimmune disorders or inflammation when excessive tissue damage occurs, and to prevent transplant rejection after an organ transplant.

Anti-inflammatory drugs are often used to control the effects of inflammation. The glucocorticoids are the most powerful of these drugs; however, these drugs can have many undesirable side effects (*e.g.*, central obesity, hyperglycemia, osteoporosis) and their use must be tightly controlled. Therefore, lower doses of anti-inflammatory drugs are often used in conjunction with cytotoxic or immunosuppressive drugs such as methotrexate or azathioprine. Cytotoxic drugs inhibit the immune response by killing dividing cells such as activated T cells. However, the killing is indiscriminate and other constantly dividing cells and their organs are affected, which causes toxic side effects. Immunosuppressive drugs such as ciclosporin prevent T cells from responding to signals correctly by inhibiting signal transduction pathways.

Larger drugs (>500 Da) can provoke a neutralizing immune response, particularly if the drugs are administered repeatedly, or in larger doses. This limits the effectiveness of drugs based on larger peptides and proteins (which are typically larger than 6000 Da). In

some cases, the drug itself is not immunogenic, but may be co-administered with an immunogenic compound, as is sometimes the case for Taxol. Computational methods have been developed to predict the immunogenicity of peptides and proteins, which are particularly useful in designing therapeutic antibodies, assessing likely virulence of mutations in viral coat particles, and validation of proposed peptide-based drug treatments. Early techniques relied mainly on the observation that hydrophilic amino acids are overrepresented in epitope regions than hydrophobic amino acids; however, more recent developments rely on machine learning techniques using databases of existing known epitopes, usually on well-studied virus proteins, as a training set. A publicly accessible database has been established for the cataloguing of epitopes from pathogens known to be recognizable by B cells. The emerging field of bioinformatics-based studies of immunogenicity is referred to as *immunoinformatics*.

Manipulation by pathogens

The success of any pathogen is dependent on its ability to elude host immune responses. Therefore, pathogens have evolved several methods that allow them to successfully infect a host, while evading detection or destruction by the immune system. Bacteria often overcome physical barriers by secreting enzymes that digest the barrier — for example, by using a type II secretion system. Alternatively, using a type III secretion system, they may insert a hollow tube into the host cell, providing a direct route for proteins to move from the pathogen to the host. These proteins are often used to shut down host defenses.

An evasion strategy used by several pathogens to avoid the innate immune system is to hide within the cells of their host (also called intracellular pathogenesis). Here, a pathogen spends most of its life-cycle inside host cells, where it is shielded from direct contact with immune cells, antibodies and complement. Some examples of intracellular pathogens include viruses, the food poisoning bacterium *Salmonella* and the eukaryotic parasites that cause malaria (*Plasmodium falciparum*) and leishmaniasis (*Leishmania spp.*). Other bacteria, such as *Mycobacterium tuberculosis*, live inside a protective capsule that prevents lysis by complement. Many pathogens secrete compounds that diminish or misdirect the host's immune response. Some bacteria form biofilms to protect themselves from the cells and proteins of the immune system. Such biofilms are present in many successful infections, e.g., the chronic *Pseudomonas aeruginosa* and *Burkholderia cenocepacia* infections characteristic of cystic fibrosis. Other bacteria generate surface proteins that bind to antibodies, rendering them ineffective; examples include *Streptococcus* (protein G), *Staphylococcus aureus* (protein A), and *Peptostreptococcus magnus* (protein L).

The mechanisms used to evade the adaptive immune system are more complicated. The simplest approach is to rapidly change non-essential epitopes (amino acids and/or sugars) on the surface of the pathogen, while keeping essential epitopes concealed. This is called antigenic variation. An example is HIV, which mutates rapidly, so the proteins on its viral envelope that are essential for entry into its host target cell are constantly changing. These frequent changes in antigens may explain the failures of vaccines directed at this virus. The parasite *Trypanosoma brucei* uses a similar strategy, constantly switching one

type of surface protein for another, allowing it to stay one step ahead of the antibody response. Masking antigens with host molecules is another common strategy for avoiding detection by the immune system. In HIV, the envelope that covers the viron is formed from the outermost membrane of the host cell; such "self-cloaked" viruses make it difficult for the immune system to identify them as "non-self" structures.

Chapter 22

Immunologic Adjuvant

In immunology, an **adjuvant** is an agent that may stimulate the immune system and increase the response to a vaccine, without having any specific antigenic effect in itself. The word “adjuvant” comes from the Latin word *adiuvare*, meaning to help or aid. "An immunologic adjuvant is defined as any substance that acts to accelerate, prolong, or enhance antigen-specific immune responses when used in combination with specific vaccine antigens."

Adjuvants have been whimsically called the *dirty little secret* of vaccines in the scientific community. This dates from the early days of commercial vaccine manufacture, when significant variations in the effectiveness of different batches of the same vaccine were observed, correctly assumed to be due to contamination of the reaction vessels. However, it was soon found that more scrupulous attention to cleanliness actually seemed to *reduce* the effectiveness of the vaccines, and that the contaminants – "dirt" – actually enhanced the immune response. There are many known adjuvants in widespread use, including oils, aluminium salts, and virosomes, although precisely how they work is still not entirely understood.

Overview

Adjuvants in immunology are often used to modify or augment the effects of a vaccine by stimulating the immune system to respond to the vaccine more vigorously, and thus providing increased immunity to a particular disease. Adjuvants accomplish this task by mimicking specific sets of evolutionarily conserved molecules, so called PAMPs, which include liposomes, lipopolysaccharide (LPS), molecular cages for antigen, components of bacterial cell walls, and endocytosed nucleic acids such as double-stranded RNA (dsRNA), single-stranded DNA (ssDNA), and unmethylated CpG dinucleotide-containing DNA. Because immune systems have evolved to recognize these specific antigenic moieties, the presence of an adjuvant in conjunction with the vaccine can greatly increase the innate immune response to the antigen by augmenting the activities of dendritic cells (DCs), lymphocytes, and macrophages by mimicking a natural infection. Furthermore, because adjuvants are attenuated beyond any function of virulence, they pose little or no independent threat to a host organism.

Inorganic adjuvants

Aluminium salts

There are many adjuvants, some of which are inorganic (such as alum), that also carry the potential to augment immunogenicity. Two common salts include aluminium phosphate and aluminium hydroxide. These are the most common adjuvants in human vaccines.

Organic adjuvants

While Aluminium salts are popularly used in human vaccines, the organic compound Squalene is also used. However, organic adjuvants are more commonly used in animal vaccines.

Oil-based

Oil-based adjuvants are commonly used in some veterinary vaccines.

Virosomes

Another market-approved adjuvant and carrier system are virosomes. During the last two decades, a variety of technologies have been investigated to improve the widely-used adjuvants based on aluminium salts. These salts are unfavorable, since they develop their effect by inducing local inflammation, which is also the basis for the extended side-effect pattern of this adjuvant. In contrast, the adjuvant capabilities of virosomes are independent of any inflammatory reaction. Virosomes contain a membrane-bound hemagglutinin and neuraminidase derived from the influenza virus, and serve to amplify fusogenic activity and therefore facilitate the uptake into antigen presenting cells (APC) and induce a natural antigen-processing pathway. The delivery of the antigen by virosomes to the immune system in a way that mimics a natural path may be a reason why virosome-based vaccines stand out due to their excellent safety profile.

Experimental adjuvants

An increasing number of vaccines with squalene and phosphate adjuvants are being tested on humans. The compound QS21 is under investigation as a possible immunological adjuvant as is Novartis' (formerly Chiron) MF59.

Adjuvants and the adaptive immune response

One misconception concerning adjuvant function is that an adjuvant-enhanced innate immune response should affect only the transient reaction of the innate immune response and not the more long-lived effects of the adaptive immune response. Although it may appear fitting to separate the two systems, it is however important to realize the interconnected nature of the two systems. When the amount of communication that takes

place between the innate immune response and the adaptive immune response with the onset of infection is considered it becomes difficult to separate the two systems.

In order to understand the links between the innate immune response and the adaptive immune response to help substantiate an adjuvant function in enhancing adaptive immune responses to the specific antigen of a vaccine, the following points should be considered:

- Innate immune response cells such as Dendritic Cells (DCs) engulf pathogens through a process called phagocytosis.
- DCs then migrate to the lymph nodes where T cells (adaptive immune cells) wait for signals to trigger their activation.
- In the lymph nodes, DCs mince the engulfed pathogen and then express the pathogen clippings as antigen on their cell surface by coupling them to a special receptor known as a major histocompatibility complex (MHC).
- T cells can then recognize these clippings and undergo a cellular transformation resulting in their own activation.
- γδ T cells possess characteristics of both the innate and adaptive immune responses.
- Macrophages can also activate T cells in a similar approach (but do not do so naturally).

This process carried out by both DCs and macrophages is termed antigen presentation and represents a physical link between the innate and adaptive immune responses.

Upon activation, mast cells release heparin and histamine to effectively increase trafficking to and seal off the site of infection to allow immune cells of both systems to clear the area of pathogens. In addition, mast cells also release chemokines which result in the positive chemotaxis of other immune cells of both the innate and adaptive immune responses to the infected area.

Due to the variety of mechanisms and links between the innate and adaptive immune response, an adjuvant-enhanced innate immune response results in an enhanced adaptive immune response. Specifically, a recent study has observed that adjuvants may exert their immune-enhancing effects according to five immune-functional activities.

- First, it was found that adjuvants all help in the translocation of antigens to the lymph nodes where they can be recognized by T cells. This will ultimately lead to greater T cell activity resulting in a heightened clearance of pathogen throughout the organism.
- Second, adjuvants provide physical protection to antigens which grants the antigen a prolonged delivery. This means that the organism will be exposed to the antigen for a longer duration, making the immune system more robust as it makes use of the additional time by upregulating the production of B and T cells needed for greater immunological memory in the adaptive immune response.

- Third, adjuvants help to increase the capacity to cause local reactions at the injection site (during vaccination), inducing greater release of danger signals by chemokine releasing cells such as helper T cells and mast cells.
- Fourth, they induce the release of inflammatory cytokines which helps to not only recruit B and T cells at sites of infection but also to increase transcriptional events leading to a net increase of immune cells as a whole.
- Finally, adjuvants are believed to increase the innate immune response to antigen by interacting with pattern recognition receptors (PRRs), specifically Toll-like receptors (TLRs), on accessory cells.

Adjuvants and toll-like receptors

The ability of immune system to recognize molecules that are broadly shared by pathogens is, in part, due to the presence of special Immune receptors called TLRs that are expressed on leukocyte membranes. TLRs were first discovered in drosophila, and are membrane bound pattern recognition receptors (PRRs) responsible for detecting most (although certainly not all) antigen-mediated infections. In fact, some studies have shown that in the absence of TLR, leukocytes become unresponsive (no inflammatory responses) to some microbial components such as LPS. There are at least thirteen different forms of TLR, each with its own characteristic ligand. Prevailing TLR ligands described to date (all of which elicit adjuvant effects) include many evolutionarily conserved molecules such as LPS, lipoproteins, lipopeptides, flagellin, double-stranded RNA, unmethylated CpG islands and various other forms of DNA and RNA classically released by bacteria and viruses.

The binding of ligand - either in the form of adjuvant used in vaccinations or in the form of invasive moieties during times of natural infection - to the TLR marks the key molecular events that ultimately lead to innate immune responses and the development of antigen-specific acquired immunity. The very fact that TLR activation leads to adaptive immune responses to foreign entities explains why so many adjuvants used today in vaccinations are developed to mimic TLR ligands.

It is believed that upon activation, TLRs recruit adapter proteins (proteins that mediate other protein-protein interactions) within the cytosol of the immune cell in order to propagate the antigen-induced signal transduction pathway. To date, four adapter proteins have been well-characterized. These proteins are known as MyD88, Trif, Tram and Tirap (also called Mal). These recruited proteins are then responsible for the subsequent activation of other downstream proteins, including protein kinases (IKKi, IRAK1, IRAK4, and TBK1) that further amplify the signal and ultimately lead to the upregulation or suppression of genes that orchestrate inflammatory responses and other transcriptional events. Some of these events lead to cytokine production, proliferation, and survival, while others lead to greater adaptive immunity. The high sensitivity of TLR for microbial ligands is what makes adjuvants that mimic TLR ligands such a prime candidate for enhancing the overall effects of antigen specific vaccinations on immunological memory.

Finally, the expression of TLRs is vast as they are found on the cell membranes of innate immune cells (DCs, macrophages, natural killer cells), cells of the adaptive immunity (T and B lymphocytes) and non immune cells (epithelial and endothelial cells, fibroblasts).

This further substantiates the importance of administering vaccines with adjuvants in the form of TLR ligands as they will be capable of eliciting their positive effects across the entire spectrum of innate and adaptive immunity. Nevertheless, there are certainly adjuvants whose immune-stimulatory function completely bypasses the putative requisite for TLR signaling. In short, all TLR ligands are adjuvants but not all adjuvants are TLR ligands.

Medical complications

Humans

Aluminium salts used in many human vaccines are generally regarded as safe.

Animals

Aluminum adjuvants have caused motor neuron death in mice and oil-water suspensions have been reported to increase the risk of autoimmune disease in mice. Squalene has caused rheumatoid arthritis in rats already prone to arthritis.

In cats, vaccinations have been linked to sarcomas, at a rate of between 1 and 10 per 10,000 injections. No specific types of vaccines, manufacturers or factors have been associated with sarcomas.

Controversy

Recently, the premise that TLR signaling acts as the key node in antigen-mediated inflammatory responses has been in question as researchers have observed antigen-mediated inflammatory responses in leukocytes in the absence of TLR signaling. One researcher found that in the absence of MyD88 and Trif (essential adapter proteins in TLR signaling), they were still able to induce inflammatory responses, increase T cell activation and generate greater B cell abundancy using conventional adjuvants (alum, Freund’s complete adjuvant, Freund’s incomplete adjuvant, and monophosphoryl-lipid A/trehalose dicorynomycolate (Ribi's adjuvant)).

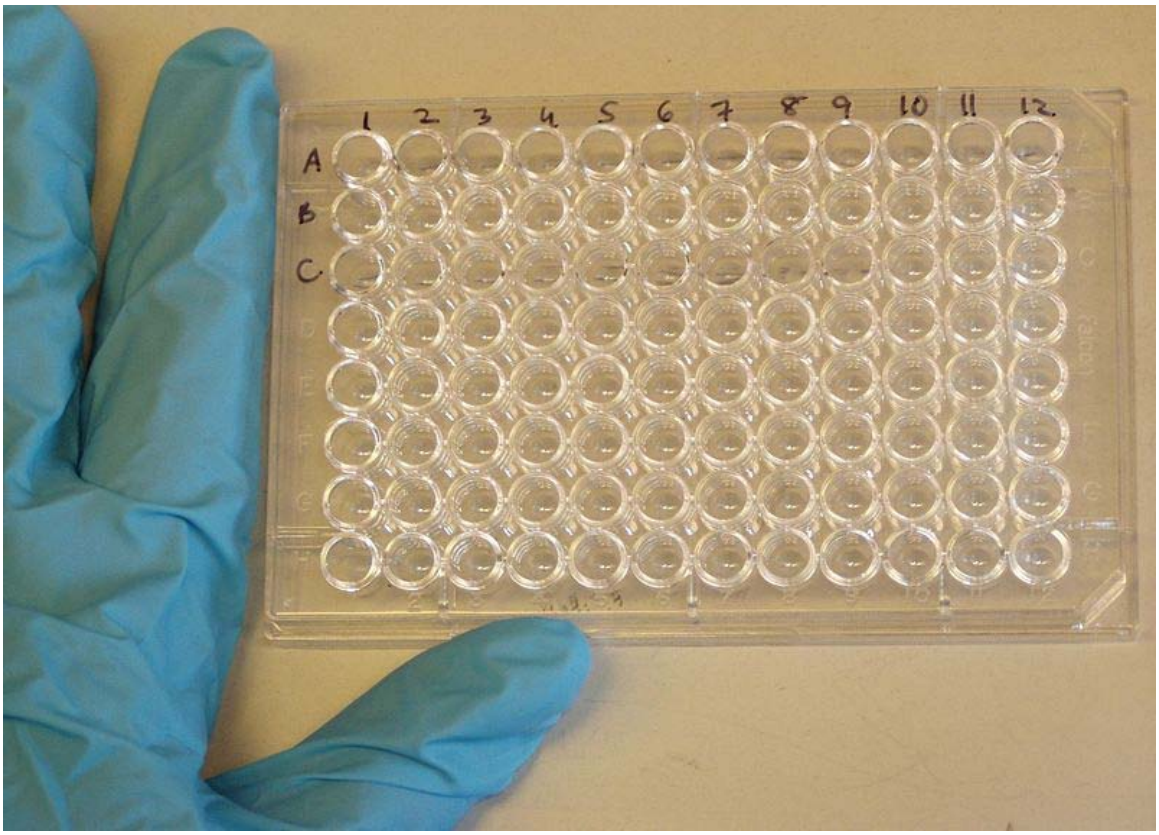
These observations suggest that although TLR activation can lead to increases in antibody responses, TLR activation is not required to induce enhanced innate and adaptive responses to antigens.

Investigating the mechanisms which underlie TLR signaling has been significant in understanding why adjuvants used during vaccinations are so important in augmenting adaptive immune responses to specific antigens. However, with the knowledge that TLR activation is not required for the immune-enhancing effects caused by common

adjuvants, we can conclude that there are, in all likelihood, other receptors besides TLRs that have not yet been characterized, opening the door to future research.

Chapter 23

ELISA



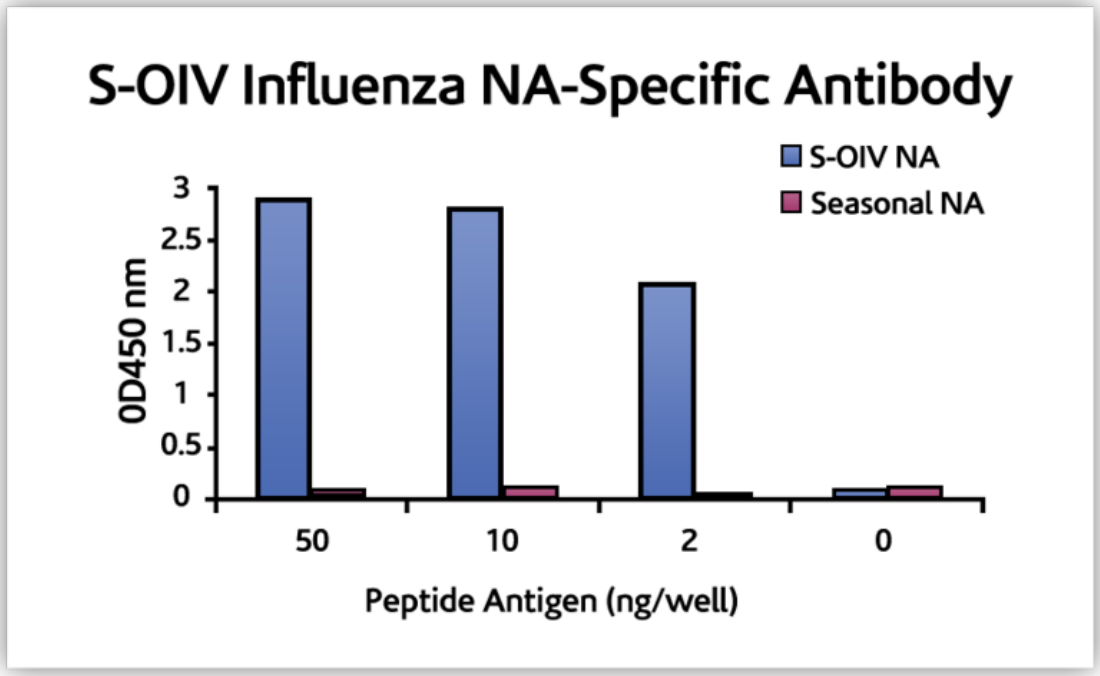
A 96-well microtiter plate being used for ELISA

Enzyme-linked immunosorbent assay (**ELISA**), also known as an **enzyme immunoassay** (**EIA**), is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample. The ELISA has been used as a diagnostic tool in medicine and plant pathology, as well as a quality-control check in various industries. In simple terms, in ELISA, an unknown amount of antigen is affixed to a surface, and then a specific antibody is applied over the surface so that it can bind to the antigen. This antibody is linked to an enzyme, and in the final step a substance is added that the enzyme can convert to some detectable signal, most commonly a colour change in a chemical substrate.

Performing an ELISA involves at least one antibody with specificity for a particular antigen. The sample with an unknown amount of antigen is immobilized on a solid support (usually a polystyrene microtiter plate) either non-specifically (via adsorption to the surface) or specifically (via capture by another antibody specific to the same antigen, in a "sandwich" ELISA). After the antigen is immobilized, the detection antibody is added, forming a complex with the antigen. The detection antibody can be covalently linked to an enzyme, or can itself be detected by a secondary antibody that is linked to an enzyme through bioconjugation. Between each step, the plate is typically washed with a mild detergent solution to remove any proteins or antibodies that are not specifically bound. After the final wash step, the plate is developed by adding an enzymatic substrate to produce a visible signal, which indicates the quantity of antigen in the sample.

Traditional ELISA typically involves chromogenic reporters and substrates that produce some kind of observable color change to indicate the presence of antigen or analyte. Newer ELISA-like techniques utilize fluorogenic, electrochemiluminescent, and real-time PCR reporters to create quantifiable signals. These new reporters can have various advantages including higher sensitivities and multiplexing. In technical terms, newer assays of this type are not strictly ELISAs, as they are not "enzyme-linked" but are instead linked to some non-enzymatic reporter. However, given that the general principles in these assays are largely similar, they are often grouped in the same category as ELISAs.

Applications



ELISA results using S-OIV A neuraminidase antibody at 1 µg/ml to probe the immunogenic and the corresponding seasonal influenza A neuraminidase peptides at 50, 10, 2, and 0 ng/ml.

Because the ELISA can be performed to evaluate either the presence of antigen or the presence of antibody in a sample, it is a useful tool for determining serum antibody concentrations (such as with the HIV test or West Nile Virus). It has also found applications in the food industry in detecting potential food allergens such as milk, peanuts, walnuts, almonds, and eggs. ELISA can also be used in toxicology as a rapid presumptive screen for certain classes of drugs.

The ELISA was the first screening test widely used for HIV because of its high sensitivity. In an ELISA, a person's serum is diluted 400-fold and applied to a plate to which HIV antigens are attached. If antibodies to HIV are present in the serum, they may bind to these HIV antigens. The plate is then washed to remove all other components of the serum. A specially prepared "secondary antibody" — an antibody that binds to other antibodies — is then applied to the plate, followed by another wash. This secondary antibody is chemically linked in advance to an enzyme. Thus, the plate will contain enzyme in proportion to the amount of secondary antibody bound to the plate. A substrate for the enzyme is applied, and catalysis by the enzyme leads to a change in color or fluorescence. ELISA results are reported as a number; the most controversial aspect of this test is determining the "cut-off" point between a positive and a negative result.

A cut-off point may be determined by comparing it with a known standard. If an ELISA test is used for drug screening at workplace, a cut-off concentration, 50 ng/mL, for example, is established, and a sample that contains the standard concentration of analyte will be prepared. Unknowns that generate a signal that is stronger than the known sample are "positive." Those that generate weaker signal are "negative."

History

Before the development of the ELISA, the only option for conducting an immunoassay was radioimmunoassay, a technique using radioactively-labeled antigens or antibodies. In radioimmunoassay, the radioactivity provides the signal, which indicates whether a specific antigen or antibody is present in the sample. Radioimmunoassay was first described in a paper by Rosalyn Sussman Yalow and Solomon Berson published in 1960.

Because radioactivity poses a potential health threat, a safer alternative was sought. A suitable alternative to radioimmunoassay would substitute a non-radioactive signal in place of the radioactive signal. When enzymes (such as peroxidase) react with appropriate substrates (such as ABTS or 3,3’,5,5’-Tetramethylbenzidine), a change in color occurs, which is used as a signal. However, the signal has to be associated with the presence of antibody or antigen, which is why the enzyme has to be linked to an appropriate antibody. This linking process was independently developed by Stratis Avrameas and G.B. Pierce. Since it is necessary to remove any unbound antibody or antigen by washing, the antibody or antigen has to be fixed to the surface of the container; i.e., the *immunosorbent* has to be prepared. A technique to accomplish this was published by Wide and Jerker Porath in 1966.

In 1971, Peter Perlmann and Eva Engvall at Stockholm University in Sweden, and Anton Schuurs and Bauke van Weemen in The Netherlands independently published papers that synthesized this knowledge into methods to perform EIA/ELISA.

Types

"Indirect" ELISA

The steps of "indirect" ELISA follows the mechanism below:-

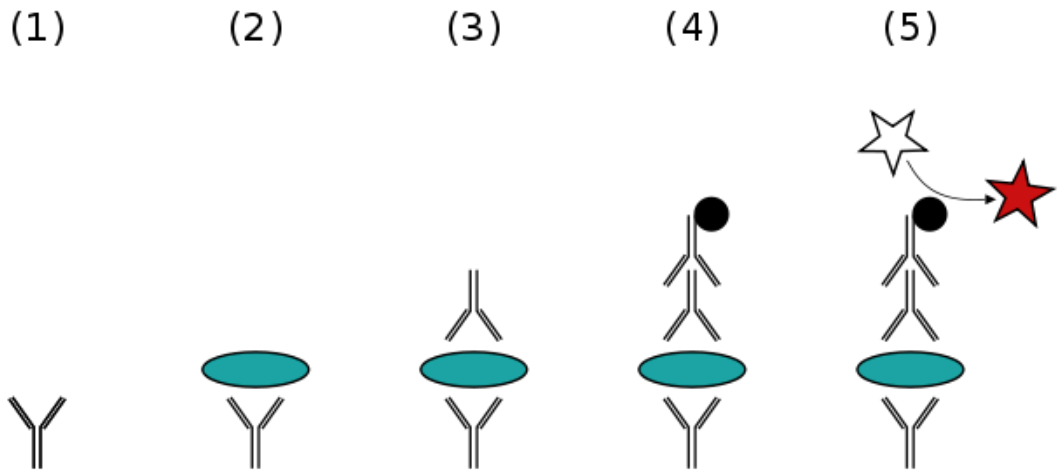
- A buffered solution of the antigen to be tested for is added to each well of a microtiter plate, where it is given time to adhere to the plastic through charge interactions.
- A solution of non-reacting protein, such as bovine serum albumin or casein, is added to block any plastic surface in the well that remains uncoated by the antigen.
- Next the primary antibody is added, which binds specifically to the test antigen that is coating the well. This primary antibody could also be in the serum of a donor to be tested for reactivity towards the antigen.
- Afterwards, a secondary antibody is added, which will bind the primary antibody. This secondary antibody often has an enzyme attached to it, which has a negligible effect on the binding properties of the antibody.
- A substrate for this enzyme is then added. Often, this substrate changes color upon reaction with the enzyme. The color change shows that secondary antibody has bound to primary antibody, which strongly implies that the donor has had an immune reaction to the test antigen. This can be helpful in a clinical setting, and in R&D.
- The higher the concentration of the primary antibody that was present in the serum, the stronger the color change. Often a spectrometer is used to give quantitative values for color strength.

The enzyme acts as an amplifier; even if only few enzyme-linked antibodies remain bound, the enzyme molecules will produce many signal molecules. Within common-sense limitations, the enzyme can go on producing color indefinitely, but the more primary antibody is present in the donor serum the more secondary antibody + enzyme will bind, and the faster color will develop. A major disadvantage of the indirect ELISA is that the method of antigen immobilization is non-specific; when serum is used as the source of test antigen, all proteins in the sample may stick to the microtiter plate well, so small concentrations of analyte in serum must compete with other serum proteins when binding to the well surface. The sandwich or direct ELISA provides a solution to this problem, by using a "capture" antibody specific for the test antigen to pull it out of the serum's molecular mixture.

ELISA may be run in a qualitative or quantitative format. Qualitative results provide a simple positive or negative result (yes or no) for a sample. The cutoff between positive and negative is determined by the analyst and may be statistical. Two or three times the

standard deviation (error inherent in a test) is often used to distinguish positive from negative samples. In quantitative ELISA, the optical density (OD) of the sample is compared to a standard curve, which is typically a serial dilution of a known-concentration solution of the target molecule. For example, if a test sample returns an OD of 1.0, the point on the standard curve that gave OD = 1.0 must be of the same analyte concentration as your sample.

Sandwich ELISA



A sandwich *ELISA*. (1) Plate is coated with a capture antibody; (2) sample is added, and any antigen present binds to capture antibody; (3) detecting antibody is added, and binds to antigen; (4) enzyme-linked secondary antibody is added, and binds to detecting antibody; (5) substrate is added, and is converted by enzyme to detectable form.

A less-common variant of this technique, called "sandwich" ELISA, is used to detect sample antigen. The steps are as follows:

1. Prepare a surface to which a known quantity of capture antibody is bound.
2. Block any nonspecific binding sites on the surface.
3. Apply the antigen-containing sample to the plate.
4. Wash the plate, so that unbound antigen is removed.
5. Apply enzyme linked primary antibodies as detection antibodies that also bind specifically to the antigen.
6. Wash the plate, so that the unbound antibody-enzyme conjugates are removed.
7. Apply a chemical that is converted by the enzyme into a color or fluorescent or electrochemical signal.
8. Measure the absorbency or fluorescence or electrochemical signal (e.g., current) of the plate wells to determine the presence and quantity of antigen.

The image to the right includes the use of a secondary antibody conjugated to an enzyme, though, in the technical sense, this is not necessary if the primary antibody is conjugated

to an enzyme. However, use of a secondary-antibody conjugate avoids the expensive process of creating enzyme-linked antibodies for every antigen one might want to detect. By using an enzyme-linked antibody that binds the Fc region of other antibodies, this same enzyme-linked antibody can be used in a variety of situations. Without the first layer of "capture" antibody, any proteins in the sample (including serum proteins) may competitively adsorb to the plate surface, lowering the quantity of antigen immobilized. Use of the purified specific antibody to attach the antigen to the plastic eliminates a need to purify the antigen from complicated mixtures before the measurement, simplifying the assay, and increasing the specificity and the sensitivity of the assay.

A descriptive animation of the application of sandwich ELISA to home pregnancy testing can be found here.

Competitive ELISA

A third use of ELISA is through competitive binding. The steps for this ELISA are somewhat different than the first two examples:

1. Unlabeled antibody is incubated in the presence of its antigen (Sample).
2. These bound antibody/antigen complexes are then added to an antigen-coated well.
3. The plate is washed, so that unbound antibody is removed. (The more antigen in the sample, the less antibody will be able to bind to the antigen in the well, hence "competition.")
4. The secondary antibody, specific to the primary antibody is added. This second antibody is coupled to the enzyme.
5. A substrate is added, and remaining enzymes elicit a chromogenic or fluorescent signal.

For competitive ELISA, the higher the sample antigen concentration, the weaker the eventual signal. The major advantage of a competitive ELISA is the ability to use crude or impure samples and still selectively bind any antigen that may be present.

(Note that some competitive ELISA kits include enzyme-linked antigen rather than enzyme-linked antibody. The labeled antigen competes for primary antibody binding sites with your sample antigen (unlabeled). The more antigen in the sample the less labeled antigen is retained in the well and the weaker the signal).

It is common that the antigen is not first positioned in the well.

Multiple and Portable ELISA (M&P ELISA)(ELISA Reverse in published papers)

A new technique (EP 1 499 894 B1 in EPO Bulletin 25.02.209 N. 2009/09; USPTO 7510687 in USPTO Bulletin 31.03.2009; ZL 03810029.0 in SIPO PRC Bulletin 08.04.2009) uses a solid phase made up of an immunosorbent polystyrene rod with 8-12

protruding ogives. The entire device is immersed in a test tube containing the collected sample and the following steps (washing, incubation in conjugate and incubation in chromogenous) are carried out by dipping the ogives in microwells of standard microplates pre-filled with reagents.

The advantages of this technique are as follows:

1. The ogives can each be sensitized to a different reagent, allowing the simultaneous detection of different antibodies and/or different antigens for multi-target assays
2. The sample volume can be increased to improve the test sensitivity in clinical (saliva, urine), food (bulk milk, pooled eggs) and environmental (water) samples
3. One ogive is left unsensitized to measure the non-specific reactions of the sample
4. The use of laboratory supplies for dispensing sample aliquots, washing solution and reagents in microwells is not required, facilitating the deveopment of ready-to-use lab-kits and on-site kits.

Chapter 24

Cancer Immunology and Cancer Immunoprevention

Cancer Immunology

Cancer immunology is the study of interactions between the immune system and cancer cells (also called tumors or malignancies). It is also a growing field of research that aims to discover innovative cancer immunotherapies to treat and retard progression of this disease. The immune response, including the recognition of cancer-specific antigens is of particular interest in this field as knowledge gained drives the development of new vaccines and antibody therapies. For instance in 2007, Ohtani published a paper finding tumour infiltrating lymphocytes to be quite significant in human colorectal cancer. The host was given a better chance at survival if the cancer tissue showed infiltration of inflammatory cells, in particular lymphocytic reactions. The results yielded suggest some extent of anti-tumour immunity is present in colorectal cancers in humans.

Over the past 10 years there has been notable progress and an accumulation of scientific evidence for the concept of cancer immunosurveillance and immunoediting based on (i) protection against development of spontaneous and chemically-induced tumors in animal systems and (ii) identification of targets for immune recognition of human cancer.

In 1999, a rat with immunity to cancer was discovered by Dr. Zheng Cui.

Immunosurveillance

Cancer immunosurveillance is a theory formulated in 1957 by Burnet and Thomas, who proposed that lymphocytes act as sentinels in recognizing and eliminating continuously arising, nascent transformed cells. Cancer immunosurveillance appears to be an important host protection process that inhibits carcinogenesis and maintains regular cellular homeostasis. It has also been suggested that immunosurveillance primarily functions as a component of a more general process of cancer immunoediting.

Immunoediting

Immunoediting is a process by which a person is protected from cancer growth and the development of tumour immunogenicity by their immune system. It has three main phases: elimination, equilibrium and escape. The elimination phase consists of the following four phases:

Elimination: Phase 1

The first phase of elimination involves the initiation of antitumor immune response. Cells of the innate immune system recognise the presence of a growing tumor which has undergone stromal remodeling, causing local tissue damage. This is followed by the induction of inflammatory signals which is essential for recruiting cells of the innate immune system (eg. natural killer cells, natural killer T cells, macrophages and dendritic cells) to the tumor site. During this phase, the infiltrating lymphocytes such as the natural killer cells and natural killer T cells are stimulated to produce IFN-gamma.

Elimination: Phase 2

In the second phase of elimination, newly synthesised IFN-gamma induces tumor death (to a limited amount) as well as promoting the production of chemokines CXCL10, CXCL9 and CXCL11. These chemokines play an important role in promoting tumor death by blocking the formation of new blood vessels. Tumor cell debris produced as a result of tumor death is then ingested by dendritic cells, followed by the migration of these dendritic cells to the draining lymph nodes. The recruitment of more immune cells also occurs and is mediated by the chemokines produced during the inflammatory process.

Elimination: Phase 3

In the third phase, natural killer cells and macrophages transactivate one another via the reciprocal production of IFN-gamma and IL-12. This again promotes more tumor killing by these cells via apoptosis and the production of reactive oxygen and nitrogen intermediates. In the draining lymph nodes, tumor-specific dendritic cells trigger the differentiation of Th1 cells which in turn facilitates the development of CD8+ T cells.

Elimination: Phase 4

In the final phase of elimination, tumor-specific CD4+ and CD8+ T cells home to the tumor site and the cytolytic T lymphocytes then destroy the antigen-bearing tumor cells which remain at the site.

Equilibrium and Escape

Tumor cell variants which have survived the elimination phase enter the equilibrium phase. In this phase, lymphocytes and IFN-gamma exert a selection pressure on tumor

cells which are genetically unstable and rapidly mutating. Tumor cell variants which have acquired resistance to elimination then enter the escape phase. In this phase, tumor cells continue to grow and expand in an uncontrolled manner and may eventually lead to malignancies. In the study of cancer immunoeditting, knockout mice have been used for experimentation since human testing is not possible. Tumor infiltration by lymphocytes is seen as a reflection of a tumor-related immune response.

Cancer Immunology and Chemotherapy

Obeid et al. investigate how inducing immunogenic cancer cell death ought to become a priority of anticancer chemotherapy for the reason that, the immune system would be able to play a factor via a 'bystander effect' in eradicating chemotherapy-resistant cancer cells. However, extensive research is still needed on how the immune response is triggered against dying tumour cells.

Professionals in the field have hypothesized that 'apoptotic cell death is poorly immunogenic whereas necrotic cell death is truly immunogenic'. This is perhaps because cancer cells being eradicated via a necrotic cell death pathway induce an immune response by triggering dendritic cells to mature, due to inflammatory response stimulation. On the other hand, apoptosis is connected to slight alterations within the plasma membrane causing the dying cells to be attractive to phagocytic cells.

Thus Obeid *et al.* propose that the way in which cancer cells die during chemotherapy is vital. Anthracyclins produce a beneficial immunogenic environment. The researchers report that when killing cancer cells with this agent uptake and presentation by antigen presenting dendritic cells is encouraged, thus allowing a T-cell response which can shrink tumours. Therefore activating tumour-killing T cells is crucial for immunotherapy success.

However, advanced cancer patients with immunosuppression have left researchers in a dilemma as to how to activate their T cells. The way the host dendritic cells react and uptake tumour antigens to present to CD4+ and CD8+ T cells is the key to success of the treatment.

The role of viruses in cancer development

Various strains of Human Papilloma Virus (HPV) have recently been found to play an important role in the development of cervical cancer. The HPV oncogenes E6 and E7 that these viruses possess have been shown to immortalise some human cells and thus promote cancer development. Although these strains of HPV have not been found in all cervical cancers, they have been found to be the cause in roughly 70% of cases. The study of these viruses and their role in the development of various cancers is still continuing, however a vaccine has been developed that can prevent infection of certain HPV strains, and thus prevent those HPV strains from causing cervical cancer, and possibly other cancers as well.

A virus that has been shown to cause breast cancer in mice is Mouse Mammary Tumour Virus. It is from discoveries such as this and the role of HPV in cervical cancer development that research is currently being undertaken to discover whether or not Human Mammary Tumour Virus is a cause of breast cancer in humans.

Cancer immunoprevention

Cancer immunoprevention is the prevention of cancer onset with immunological means such as vaccines, immunostimulators or antibodies. Cancer immunoprevention is conceptually different from cancer immunotherapy, which aims at stimulating immunity in patients only after tumor onset, however the same immunological means can be used both in immunoprevention and in immunotherapy.

Immunoprevention of tumors caused by viruses

Immunoprevention of tumors caused by viruses or other infectious agents aims at preventing or curing infection before the onset of cancer. Effective vaccines are available for use in humans.
Some tumor types in humans and in animals are the consequence of viral infections. In humans the most frequent viral tumors are liver cancer (also called hepatocellular carcinoma), arising in a small proportion of patients with chronic infection by hepatitis B virus (HBV) or hepatitis C virus (HCV), and carcinoma of the uterine cervix (also called cervical cancer), caused by human papilloma virus (HPV). Altogether these two tumors make 10% of all human cancers, affecting almost one million new patients each year worldwide. The HBV vaccine, now in worldwide use, was shown to reduce the incidence of liver carcinoma. Cancer immunoprevention by the HBV vaccine can be thought of as a beneficial side effect of vaccine developed and used to prevent hepatitis B. This is not the case with HPV vaccines, which were primarily developed for cancer prevention. Clinical trials showed that HPV vaccines can prevent HPV infection and carcinogenesis almost completely; these results led to vaccine approval by regulatory agencies in USA and Europe.

Immunoprevention of non-infectious tumors

Is it possible to devise immunopreventive strategies for tumors not caused by infectious agents? The challenge is to predict in each individual the risk of specific cancer types and to design immune strategies targeting these cancer types. This is not yet feasible in humans, thus immunoprevention of non-infectious tumors is at a preclinical stage of development.
Effective immunoprevention of various types of cancer was obtained in murine models of cancer risk, in particular in transgenic mice harboring activated oncogenes, thus demonstrating that activation of the immune system in healthy hosts can indeed prevent carcinogenesis. Both non-specific immune stimuli, like cytokines and other immunostimulators, and vaccines containing a specific antigen were active in mouse models; combinations of both types of agents yielded the best results, up to an almost

complete, long-term block of carcinogenesis in models of aggressive cancer development.

Immune mechanisms

Two main protective mechanisms elicited by cancer immunoprevention in various mouse models were cytokines released by T cells, in particular gamma-interferon, and cytotoxic antibodies against the target antigen. This is at variance with cancer immunotherapy administered to cure existing tumors, which is mainly based on cytotoxic T lymphocytes (CTL). The lack of a relevant CTL response in long-term immunoprevention is thought to be an advantage, because chronic CTL activation is severely toxic for the host. In contrast circulating antibodies provide long-term protection without toxic side effects. A similar situation happens in viral immunity, acute infections are resolved by CTL, whereas long term immunity from reinfection is provided by antibodies.
Both gamma-interferon and antibodies prevent tumor growth in multiple ways. Gamma-interferon activates T, natural killer and B cells, inhibits angiogenesis and tumor invasiveness, stimulates major histocompatibility complex expression in tumor cells and inhibits cell proliferation. Antibodies binding to antigens on the surface of cells trigger lytic mechanisms mediated by the complement system (complement-mediated cytotoxicity) or by leukocytes carrying Fc receptors (antibody-dependent cell-mediated cytotoxicity, ADCC). Moreover, antibody binding interferes with the cellular functions of the target antigen, causing its internalization or hampering molecular interactions, eventually blocking downstream signaling. If the target antigen controls cell growth (e.g. if it is the product of an oncogene), then a block of signaling can disrupt the carcinogenic process. Surface antigens causally involved in carcinogenesis are called oncoantigens.

Clinical development and risks

The success of cancer immunoprevention in preclinical models suggests that it might have an impact also in humans. The main problems to be solved are the definition of appropriate human applications and of the risks for human health.
Application to the general population, as is being done for vaccines against HBV and HPV, is currently unfeasible, because it would require a precise individual prediction of the risk of cancer. Subgroups at high risk of developing a defined type of tumor, for example families with hereditary cancer or individuals with preneoplastic lesions, are the natural candidates for immunoprevention of non-infectious tumors. It has also been suggested that immunopreventive strategies can have therapeutic effects against metastases, hence early human trials could aim at cancer therapy rather than prevention.
The main risk of prolonged immune stimulation for cancer prevention is the development of autoimmune diseases. Most antitumor immune responses are autoimmune, because most tumor antigens are also expressed by normal cells, but it must be considered that autoimmune responses do not necessarily evolve into autoimmune diseases. The limited autoimmunity triggered by cancer immunoprevention did not cause overt autoimmune diseases in preclinical mouse studies, however this is an issue that will require careful monitoring in early clinical trials.

Chapter 25

Coombs Test

Coombs test (also known as **Coombs' test**, **antiglobulin test** or **AGT**) refers to two clinical blood tests used in immunohematology and immunology. The two Coombs tests are the **direct Coombs test** (also known as **direct antiglobulin test** or **DAT**), and the **indirect Coombs test** (also known as **indirect antiglobulin test** or **IAT**).

The more commonly used test, the Direct Coombs test, is used to test for autoimmune hemolytic anemia.

In certain diseases or conditions an individual's blood may contain IgG antibodies that can specifically bind to antigens on the red blood cell (RBC) surface membrane, and their circulating red blood cells (RBCs) can become coated with IgG alloantibodies and/or IgG autoantibodies. Complement proteins may subsequently bind to the bound antibodies. The **direct Coombs test** is used to detect these antibodies or complement proteins that are bound to the surface of red blood cells; a blood sample is taken and the RBCs are washed (removing the patient's own plasma) and then incubated with antihuman globulin (also known as "Coombs reagent"). If this produces agglutination of RBCs, the direct Coombs test is positive, a visual indication that antibodies (and/or complement proteins) are bound to the surface of red blood cells.

The **indirect Coombs test** is used in prenatal testing of pregnant women, and in testing blood prior to a blood transfusion. It detects antibodies against RBCs that are present unbound in the patient's serum. In this case, serum is extracted from the blood, and the serum is incubated with RBCs of known antigenicity. If agglutination occurs, the indirect Coombs test is positive.

Mechanism

The two Coombs tests are based on the fact that anti-human antibodies, which are produced by immunizing non-human species with human serum, will bind to human antibodies, commonly IgG or IgM. Animal anti-human antibodies will also bind to human antibodies that may be fixed onto antigens on the surface of red blood cells (also referred to as RBCs), and in the appropriate test tube conditions this can lead to agglutination of RBCs. The phenomenon of agglutination of RBCs is important here, because the resulting clumping of RBCs can be visualised; when clumping is seen the test is positive and when clumping is not seen the test is negative.

Common clinical uses of the Coombs test include the preparation of blood for transfusion in cross-matching, screening for atypical antibodies in the blood plasma of pregnant women as part of antenatal care, and detection of antibodies for the diagnosis of immune-mediated haemolytic anemias.

Coombs tests are done on serum from venous blood samples which are taken from patients by venepuncture. The venous blood is taken to a laboratory (or blood bank), where trained scientific technical staff do the Coombs tests. The clinical significance of the result is assessed by the physician who requested the Coombs test, perhaps with assistance from a laboratory-based hematologist.

Direct Coombs test

The direct Coombs test (also known as the **direct antiglobulin test** or DAT) is used to detect if antibodies or complement system factors have bound to RBC surface antigens *in vivo*. The DAT is not currently required for pre-transfusion testing but may be included by some laboratories.

Examples of diseases that give a positive direct Coombs test

The direct Coombs test is used clinically when immune-mediated hemolytic anemia (antibody-mediated destruction of RBCs) is suspected. A positive Coombs test indicates that an immune mechanism is attacking the patient's own RBC's. This mechanism could be autoimmunity, alloimmunity or a drug-induced immune-mediated mechanism.

Examples of alloimmune hemolysis

- Hemolytic disease of the newborn (also known as HDN or erythroblastosis fetalis)
    - Rh D hemolytic disease of the newborn (also known as Rh disease)
    - ABO hemolytic disease of the newborn (the indirect Coombs test may only be weakly positive)
    - Anti-Kell hemolytic disease of the newborn
    - Rh c hemolytic disease of the newborn
    - Rh E hemolytic disease of the newborn
    - Other blood group incompatibility (RhC, Rhe, Kidd, Duffy, MN, P and others)
- Alloimmune hemolytic transfusion reactions

Examples of autoimmune hemolysis

- Warm antibody autoimmune hemolytic anemia
    - Idiopathic
    - Systemic lupus erythematosus
    - Evans' syndrome (antiplatelet antibodies and hemolytic antibodies)

- Cold antibody autoimmune hemolytic anemia
    - Idiopathic cold hemagglutinin syndrome
    - Infectious mononucleosis
    - Paroxysmal cold hemoglobinuria (rare)

Drug-induced immune-mediated hemolysis

- Methyldopa (IgG mediated type II hypersensitivity)
- Penicillin (high dose)
- Quinidine (IgM mediated activation of classical complement pathway and Membrane attack complex, MAC)

(A memory device to remember that the *DAT* tests the RBCs and is used to test infants for *haemolytic disease of the newborn* is: ***R**h **D**isease*; ***R*** = RBCs, ***D*** = DAT.)

Laboratory method

The patient's red blood cells (RBCs) are washed (removing the patient's own serum) and then incubated with antihuman globulin (also known as Coombs reagent). If immunoglobulin or complement factors have been fixed on to the RBC surface in-vivo, the antihuman globulin will agglutinate the RBCs and the direct Coombs test will be positive. (A visual representation of a positive direct Coombs test is shown in the upper half of the schematic).

Indirect Coombs test

The indirect Coombs test (also known as the **indirect antiglobulin test** or IAT) is used to detect in-vitro antibody-antigen reactions. It is used to detect very low concentrations of antibodies present in a patient's plasma/serum prior to a blood transfusion. In antenatal care, the IAT is used to screen pregnant women for antibodies that may cause hemolytic disease of the newborn. The IAT can also be used for compatibility testing, antibody identification, RBC phenotyping, and titration studies.

Examples of clinical uses of the indirect Coombs test

Blood transfusion preparation

The indirect Coombs test is used to screen for antibodies in the preparation of blood for blood transfusion. The donor's and recipient's blood must be ABO and Rh D compatible. Donor blood for transfusion is also screened for infections in separate processes.

- Antibody screening

A blood sample from the recipient and a blood sample from every unit of donor blood are screened for antibodies with the indirect Coombs test. Each sample is incubated against a

wide range of RBCs that together exhibit a full range of surface antigens (i.e. blood types).

- Cross matching

The indirect Coombs test is used to test a sample of the recipient's serum against a sample of the blood donor's RBCs. This is sometimes called cross-matching blood.

Antenatal antibody screening

The indirect Coombs test is used to screen pregnant women for IgG antibodies that are likely to pass through the placenta into the fetal blood and cause haemolytic disease of the newborn.

Laboratory method

The IAT is a two-stage test. (A cross match is shown visually in the lower half of the schematic as an example of an indirect Coombs test).

First stage

Washed test red blood cells (RBCs) are incubated with a test serum. If the serum contains antibodies to antigens on the RBC surface, the antibodies will bind onto the surface of the RBCs.

Second stage

The RBCs are washed three or four times with isotonic saline and then incubated with antihuman globulin. If antibodies have bound to RBC surface antigens in the first stage, RBCs will agglutinate when incubated with the antihuman globulin (also known Coombs reagent) in this stage, and the indirect Coombs test will be positive.

Titrations

By diluting a serum containing antibodies the quantity of the antibody in the serum can be gauged. This is done by using doubling dilutions of the serum and finding the maximum dilution of test serum that is able to produce agglutination of relevant RBCs.

Coombs reagent

Coombs reagent (also known as **Coombs antiglobulin** or **antihuman globulin**) is used in both the direct Coombs test and the indirect Coombs test. Coombs reagent is antihuman globulin. It is made by injecting human globulin into animals, which produce polyclonal antibodies specific for human immunoglobulins and human complement system factors. More specific Coombs reagents or monoclonal antibodies can be used.

Enhancement media

Both IgM and IgG antibodies bind strongly with their antigens. IgG antibodies are most reactive at 37°C. IgM antibodies are easily detected in saline at room temperature as IgM antibodies are able to bridge between RBC's owing to their large size, efficiently creating what is seen as agglutination. IgG antibodies are smaller and require assistance to bridge well enough to form a visual agglutination reaction. Reagents used to enhance IgG detection are referred to as potentiators. RBCs have a net negative charge called zeta potential which causes them to have a natural repulsion for one another. Potentiators reduce the zeta potential of RBC membranes. Common potentiators include low ionic strength solution (LISS), albumin, polyethylene glycol (PEG), and proteolytic enzymes.

History of the Coombs test

The Coombs test was first described in 1945 by Cambridge immunologists Robin Coombs (after whom it is named), Arthur Mourant and Rob Race. Historically, it was done in test tubes. Today, it is commonly done using microarray and gel technology.

Chapter 26

Autoimmunity

Autoimmunity	
ICD-9	279.4
OMIM	109100
DiseasesDB	28805
MeSH	D001327

Autoimmunity is the failure of an organism to recognize its own constituent parts as *self*, which allows an immune response against its own cells and tissues. Any disease that results from such an aberrant immune response is termed an autoimmune disease. Autoimmunity is often caused by a lack of germ development of a target body and as such the immune response acts against its own cells and tissues (Flowers 2009). Prominent examples include Coeliac disease, diabetes mellitus type 1 (IDDM), systemic lupus erythematosus (SLE), Sjögren's syndrome, Churg-Strauss Syndrome, Hashimoto's thyroiditis, Graves' disease, idiopathic thrombocytopenic purpura, and rheumatoid arthritis (RA).

The misconception that an individual's immune system is totally incapable of recognizing *self* antigens is not new. Paul Ehrlich, at the beginning of the twentieth century, proposed the concept of **horror autotoxicus**, wherein a 'normal' body does not mount an immune response against its own tissues. Thus, any autoimmune response was perceived to be abnormal and postulated to be connected with human disease. Now, it is accepted that autoimmune responses are an integral part of vertebrate immune systems (sometimes termed 'natural autoimmunity'), normally prevented from causing disease by the phenomenon of immunological tolerance to self-antigens. Autoimmunity should not be confused with alloimmunity.

Low-level autoimmunity

While a high level of autoimmunity is unhealthy, a low level of autoimmunity may actually be beneficial. First, low-level autoimmunity might aid in the recognition of neoplastic cells by CD8+ T cells, and thus reduce the incidence of cancer.

Second, autoimmunity may have a role in allowing a rapid immune response in the early stages of an infection when the availability of foreign antigens limits the response (i.e., when there are few pathogens present). In their study, Stefanova et al. (2002) injected an anti-MHC Class II antibody into mice expressing a single type of MHC Class II molecule ($H\text{-}2^b$) to temporarily prevent CD4+ T cell-MHC interaction. Naive CD4+ T cells (those that have not encountered any antigens before) recovered from these mice 36 hours post-anti-MHC administration showed decreased responsiveness to the antigen pigeon cytochrome C peptide, as determined by Zap-70 phosphorylation, proliferation, and Interleukin-2 production. Thus Stefanova et al. (2002) demonstrated that self-MHC recognition (which, if too strong may contribute to autoimmune disease) maintains the responsiveness of CD4+ T cells when foreign antigens are absent. This idea of autoimmunity is conceptually similar to play-fighting. The play-fighting of young cubs (TCR and self-MHC) may result in a few scratches or scars (low-level-autoimmunity), but is beneficial in the long-term as it primes the young cub for proper fights in the future.

Immunological tolerance

Pioneering work by Noel Rose and Witebsky in New York, and Roitt and Doniach at University College London provided clear evidence that, at least in terms of antibody-producing B lymphocytes, diseases such as rheumatoid arthritis and thyrotoxicosis are associated with of loss of immunological tolerance, which is the ability of an individual to ignore 'self', while reacting to 'non-self'. This breakage leads to the immune system's mounting an effective and specific immune response against self determinants. The exact genesis of immunological tolerance is still elusive, but several theories have been proposed since the mid-twentieth century to explain its origin.

Three hypotheses have gained widespread attention among immunologists:

- **Clonal Deletion theory**, proposed by Burnet, according to which self-reactive lymphoid cells are destroyed during the development of the immune system in an individual. For their work Frank M. Burnet and Peter B. Medawar were awarded the 1960 Nobel Prize in Physiology or Medicine "for discovery of acquired immunological tolerance".
- **Clonal Anergy theory**, proposed by Nossal, in which self-reactive T- or B-cells become inactivated in the normal individual and cannot amplify the immune response.
- **Idiotype Network theory**, proposed by Jerne, wherein a network of antibodies capable of neutralizing self-reactive antibodies exists naturally within the body.

In addition, two other theories are under intense investigation:

- The so-called "Clonal Ignorance" theory, according to which host immune responses are directed to ignore self-antigens

- The "Suppressor population" or "Regulatory T cell" theories, wherein regulatory T-lymphocytes (commonly $CD4^+FoxP3^+$ cells, among others) function to prevent, downregulate, or limit autoaggressive immune responses in the immune system.

Tolerance can also be differentiated into 'Central' and 'Peripheral' tolerance, on whether or not the above-stated checking mechanisms operate in the central lymphoid organs (Thymus and Bone Marrow) or the peripheral lymphoid organs (lymph node, spleen, etc., where self-reactive B-cells may be destroyed). It must be emphasised that these theories are not mutually exclusive, and evidence has been mounting suggesting that all of these mechanisms may actively contribute to vertebrate immunological tolerance.

A puzzling feature of the documented loss of tolerance seen in spontaneous human autoimmunity is that it is almost entirely restricted to the autoantibody responses produced by B lymphocytes. Loss of tolerance by T cells has been extremely hard to demonstrate, and where there is evidence for an abnormal T cell response it is usually not to the antigen recognised by autoantibodies. Thus, in rheumatoid arthritis there are autoantibodies to IgG Fc but apparently no corresponding T cell response. In systemic lupus there are autoantibodies to DNA, which cannot evoke a T cell response, and limited evidence for T cell responses implicates nucleoprotein antigens. In Celiac disease there are autoantibodies to tissue transglutaminase but the T cell response is to the foreign protein gliadin. This disparity has led to the idea that human autoimmune disease is in most cases (with probable exceptions including type I diabetes) based on a loss of B cell tolerance which makes use of normal T cell responses to foreign antigens in a variety of aberrant ways.

Genetic Factors

Certain individuals are genetically susceptible to developing autoimmune diseases. This susceptibility is associated with multiple genes plus other risk factors. Genetically-predisposed individuals do not always develop autoimmune diseases.

Three main sets of genes are suspected in many autoimmune diseases. These genes are related to:

- Immunoglobulins
- T-cell receptors
- The major histocompatibility complexes (MHC).

The first two, which are involved in the recognition of antigens, are inherently variable and susceptible to recombination. These variations enable the immune system to respond to a very wide variety of invaders, but may also give rise to lymphocytes capable of self-reactivity.

Scientists such as H. McDevitt, G. Nepom, J. Bell and J. Todd have also provided strong evidence to suggest that certain MHC class II allotypes are strongly correlated with

- HLA DR2 is strongly positively correlated with Systemic Lupus Erythematosus, narcolepsy and multiple sclerosis, and negatively correlated with DM Type 1.
- HLA DR3 is correlated strongly with Sjögren's syndrome, myasthenia gravis, SLE, and DM Type 1.
- HLA DR4 is correlated with the genesis of rheumatoid arthritis, Type 1 diabetes mellitus, and pemphigus vulgaris.

Fewer correlations exist with MHC class I molecules. The most notable and consistent is the association between HLA B27 and ankylosing spondylitis. Correlations may exist between polymorphisms within class II MHC promoters and autoimmune disease.

The contributions of genes outside the MHC complex remain the subject of research, in animal models of disease (Linda Wicker's extensive genetic studies of diabetes in the NOD mouse), and in patients (Brian Kotzin's linkage analysis of susceptibility to SLE).

Sex

Ratio of female/male incidence of autoimmune diseases	
Hashimoto's thyroiditis	10/1
Graves' disease	7/1
Multiple sclerosis (MS)	2/1
Myasthenia gravis	2/1
Systemic lupus erythematosus (SLE)	9/1
Rheumatoid arthritis	5/2

A person's sex also seems to have some role in the development of autoimmunity, classifying most autoimmune diseases as *sex-related diseases*. Nearly 75% of the more than 23.5 million Americans who suffer from autoimmune disease are women, although it is less-frequently acknowledged that millions of men also suffer from these diseases. According to the American Autoimmune Related Diseases Association (AARDA), autoimmune diseases that develop in men tend to be more severe. A few autoimmune diseases that men are just as or more likely to develop as women, include: ankylosing spondylitis, type 1 diabetes mellitus, Wegener's granulomatosis, Crohn's disease and psoriasis.

The reasons for the sex role in autoimmunity are unclear. Women appear to generally mount larger inflammatory responses than men when their immune systems are triggered, increasing the risk of autoimmunity. Involvement of sex steroids is indicated by that many autoimmune diseases tend to fluctuate in accordance with hormonal changes, for example, during pregnancy, in the menstrual cycle, or when using oral contraception. A history of pregnancy also appears to leave a persistent increased risk for autoimmune disease. It has been suggested that the slight exchange of cells between mothers and their children during pregnancy may induce autoimmunity. This would tip the gender balance in the direction of the female.

Another theory suggests the female high tendency to get autoimmunity is due to an imbalanced X chromosome inactivation. The X-inactivation skew theory, proposed by Princeton University's Jeff Stewart, has recently been confirmed experimentally in scleroderma and autoimmune thyroiditis. Other complex X-linked genetic susceptibility mechanisms are proposed and under investigation.

Environmental Factors

An interesting inverse relationship exists between infectious diseases and autoimmune diseases. In areas where multiple infectious diseases are endemic, autoimmune diseases are quite rarely seen. The reverse, to some extent, seems to hold true. The hygiene hypothesis attributes these correlations to the immune manipulating strategies of pathogens. Whilst such an observation has been variously termed as spurious and ineffective, according to some studies, parasite infection is associated with reduced activity of autoimmune disease.

The putative mechanism is that the parasite attenuates the host immune response in order to protect itself. This may provide a serendipitous benefit to a host that also suffers from autoimmune disease. The details of parasite immune modulation are not yet known, but may include secretion of anti-inflammatory agents or interference with the host immune signaling.

A paradoxical observation has been the strong association of certain microbial organisms with autoimmune diseases. For example, *Klebsiella pneumoniae* and coxsackievirus B have been strongly correlated with ankylosing spondylitis and diabetes mellitus type 1, respectively. This has been explained by the tendency of the infecting organism to produce super-antigens that are capable of polyclonal activation of B-lymphocytes, and production of large amounts of antibodies of varying specificities, some of which may be self-reactive (see below).

Certain chemical agents and drugs can also be associated with the genesis of autoimmune conditions, or conditions that simulate autoimmune diseases. The most striking of these is the drug-induced lupus erythematosus. Usually, withdrawal of the offending drug cures the symptoms in a patient.

Cigarette smoking is now established as a major risk factor for both incidence and severity of rheumatoid arthritis. This may relate to abnormal citrullination of proteins, since the effects of smoking correlate with the presence of antibodies to citrullinated peptides.

Pathogenesis of autoimmunity

Several mechanisms are thought to be operative in the pathogenesis of autoimmune diseases, against a backdrop of genetic predisposition and environmental modulation. A summary of some of the important mechanisms have been described:

- **T-Cell Bypass** - A normal immune system requires the activation of B-cells by T-cells before the former can produce antibodies in large quantities. This requirement of a T-cell can be bypassed in rare instances, such as infection by organisms producing super-antigens, which are capable of initiating polyclonal activation of B-cells, or even of T-cells, by directly binding to the β-subunit of T-cell receptors in a non-specific fashion.
- **T-Cell-B-Cell discordance** - A normal immune response is assumed to involve B and T cell responses to the same antigen, even if we know that B cells and T cells recognise very different things: conformations on the surface of a molecule for B cells and pre-processed peptide fragments of proteins for T cells. However, there is nothing as far as we know that requires this. All that is required is that a B cell recognising antigen X endocytoses and processes a protein Y (normally =X) and presents it to a T cell. Roosnek and Lanzavecchia showed that B cells recognising IgGFc could get help from any T cell responding to an antigen co-endocytosed with IgG by the B cell as part of an immune complex. In coeliac disease it seems likely that B cells recognising tissue transglutamine are helped by T cells recognising gliadin.
- **Aberrant B cell receptor-mediated feedback** - A feature of human autoimmune disease is that it is largely restricted to a small group of antigens, several of which have known signaling roles in the immune response (DNA, C1q, IgGFc, Ro, Con. A receptor, Peanut agglutinin receptor(PNAR)). This fact gave rise to the idea that spontaneous autoimmunity may result when the binding of antibody to certain antigens leads to aberrant signals being fed back to parent B cells through membrane bound ligands. These ligands include B cell receptor (for antigen), IgG Fc receptors, CD21, which binds complement C3d, Toll-like receptors 9 and 7 (which can bind DNA and nucleoproteins) and PNAR. More indirect aberrant activation of B cells can also be envisaged with autoantibodies to acetyl choline receptor (on thymic myoid cells) and hormone and hormone binding proteins. Together with the concept of T-cell-B-cell discordance this idea forms the basis of the hypothesis of self-perpetuating autoreactive B cells. Autoreactive B cells in spontaneous autoimmunity are seen as surviving because of subversion both of the T cell help pathway and of the feedback signal through B cell receptor, thereby overcoming the negative signals responsible for B cell self-tolerance without necessarily requiring loss of T cell self-tolerance.
- **Molecular Mimicry** - An exogenous antigen may share structural similarities with certain host antigens; thus, any antibody produced against this antigen (which mimics the self-antigens) can also, in theory, bind to the host antigens, and amplify the immune response. The idea of molecular mimicry arose in the context of Rheumatic Fever, which follows infection with Group A beta-haemolytic streptococci. Although rheumatic fever has been attributed to molecular mimicry for half a century no antigen has been formally identified (if anything too many have been proposed). Moreover, the complex tissue distribution of the disease (heart, joint, skin, basal ganglia) argues against a cardiac specific antigen. It remains entirely possible that the disease is due to e.g. an unusual interaction between immune complexes, complement components and endothelium.

- **Idiotype Cross-Reaction** - Idiotypes are antigenic epitopes found in the antigen-binding portion (Fab) of the immunoglobulin molecule. Plotz and Oldstone presented evidence that autoimmunity can arise as a result of a cross-reaction between the idiotype on an antiviral antibody and a host cell receptor for the virus in question. In this case, the host-cell receptor is envisioned as an internal image of the virus, and the anti-idiotype antibodies can react with the host cells.
- **Cytokine Dysregulation** - Cytokines have been recently divided into two groups according to the population of cells whose functions they promote: Helper T-cells type 1 or type 2. The second category of cytokines, which include IL-4, IL-10 and TGF-β (to name a few), seem to have a role in prevention of exaggeration of pro-inflammatory immune responses.
- **Dendritic cell apoptosis** - immune system cells called dendritic cells present antigens to active lymphocytes. Dendritic cells that are defective in apoptosis can lead to inappropriate systemic lymphocyte activation and consequent decline in self-tolerance.
- **Epitope spreading** or **epitope drift** - when the immune reaction changes from targeting the primary epitope to also targeting other epitopes. In contrast to molecular mimicry, the other epitopes need not be structurally similar to the primary one.

The roles of specialized immunoregulatory cell types, such as regulatory T cells, NKT cells, γδ T-cells in the pathogenesis of autoimmune disease are under investigation.

Classification

Autoimmune diseases can be broadly divided into systemic and organ-specific or localised autoimmune disorders, depending on the principal clinico-pathologic features of each disease.

- **Systemic autoimmune diseases** include SLE, Sjögren's syndrome, scleroderma, rheumatoid arthritis, and dermatomyositis. These conditions tend to be associated with autoantibodies to antigens which are not tissue specific. Thus although polymyositis is more or less tissue specific in presentation, it may be included in this group because the autoantigens are often ubiquitous t-RNA synthetases.

- **Local** syndromes which affect a specific organ or tissue:
    - Endocrinologic: Diabetes mellitus type 1, Hashimoto's thyroiditis, Addison's disease
    - Gastrointestinal: Coeliac disease, Pernicious anaemia
    - Dermatologic: Pemphigus vulgaris, Vitiligo
    - Haematologic: Autoimmune haemolytic anaemia, Idiopathic thrombocytopenic purpura
    - Neurological: Myasthenia gravis

Using the traditional “organ specific” and “non-organ specific” classification scheme, many diseases have been lumped together under the autoimmune disease umbrella.

However, many chronic inflammatory human disorders lack the telltale associations of B and T cell driven immunopathology. In the last decade it has been firmly established that tissue "inflammation against self" does not necessarily rely on abnormal T and B cell responses.

This has led to the recent proposal that the spectrum of autoimmunity should be viewed along an “immunological disease continuum,” with classical autoimmune diseases at one extreme and diseases driven by the innate immune system at the other extreme. Within this scheme, the full spectrum of autoimmunity can be included. Many common human autoimmune diseases can be seen to have a substantial innate immune mediated immunopathology using this new scheme. This new classification scheme has implications for understanding disease mechanisms and for therapy development.

Diagnosis

Diagnosis of autoimmune disorders largely rests on accurate history and physical examination of the patient, and high index of suspicion against a backdrop of certain abnormalities in routine laboratory tests (example, elevated C-reactive protein). In several systemic disorders, serological assays which can detect specific autoantibodies can be employed. Localised disorders are best diagnosed by immunofluorescence of biopsy specimens. Autoantibodies are used to diagnose many autoimmune diseases. The levels of autoantibodies are measured to determine the progress of the disease.

Treatments

Treatments for autoimmune disease have traditionally been immunosuppressive, anti-inflammatory, or palliative. Non-immunological therapies, such as hormone replacement in Hashimoto's thyroiditis or Type 1 diabetes mellitus treat outcomes of the autoaggressive response, thus these are palliative treatments. Dietary manipulation limits the severity of celiac disease. Steroidal or NSAID treatment limits inflammatory symptoms of many diseases. IVIG is used for CIDP and GBS. Specific immunomodulatory therapies, such as the TNFα antagonists (e.g. etanercept), the B cell depleting agent rituximab, the anti-IL-6 receptor tocilizumab and the costimulation blocker abatacept have been shown to be useful in treating RA. Some of these immunotherapies may be associated with increased risk of adverse effects, such as susceptibility to infection.

Helminthic therapy is an experimental approach that involves inoculation of the patient with specific parasitic intestinal nematodes (helminths). There are currently two closely-related treatments available, inoculation with either Necator americanus, commonly known as hookworms, or Trichuris Suis Ova, commonly known as Pig Whipworm Eggs.