TRANSPLANT IMMUNITY

The biggest problems

- infection
- genetic
- immunity processes
- immunotherapy

- Implantation – nonbiological material,
- transplantation – biological material,organ on other place
- graft – transplanted organ, tissue

Genetic bases of transplantation

- 20th century – Loeb, Tyzzar, Little – identification of genetic bases of transplantation
- **Genetic match** – condition of success
- Recipient´s IS recognise molecules (genetically based) – h**istocompatibility antigens** – on donor´s cells –
- recipient´s answer is paralel to reaction against external agenses

Among the obstacles that had to be overcome were infection control, the **genetic matching** of donors with hosts, an understanding of the immunologic processes involved, and the development of agents that could inhibit the immune system. The development of antiseptic techniques coupled with antibiotics reduced the risk of infection, while tissue typing and immunosuppressive drugs increased the probability of transplant success.

Presentation of antigen

- <u>Any peptid fragment</u> – produced in cytoplasma or from fagocytosis – is expressed on the surface and presented via <u>MHC I or II</u> molecules and serves as <u>antigen of histocompatibility</u>.

histokompatibility genes encode histokompatibility antigens

- more than 100 locuses
- most strong between them MHC complexes – encode molecules <u>MHC I and II</u>
- Productes of these genes are usually **codominant** expresse always whether in one (heterozygot, hemizygot) or two copies (homozygot)
- other <u>non MHC</u> antigens are not so strictly inherited – many genes scatter on several chromozomes incl. X or Y chromosome

- **MHC I and II**

- **Codominance**
- **Heterozygot**
- **Multiple locuses**
- increase the number of histokompatibility antigens combinations expressed on the cell surfaces
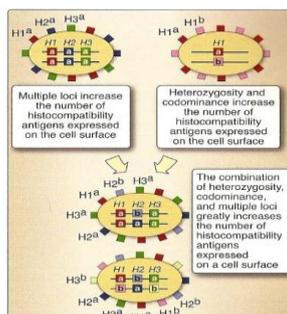
The genetic match (similarity/disparity) between the donor and the host is perhaps the most important factor determining the likelihood of a successful transplant. The recipient's immune system looks for certain genetically encoded molecules (**histocompatibility antigens**) on the surfaces of the donor cells. Thus the response against transplanted cells and tissues has parallels to the body's response to foreign infectious organisms. The structures and functions are known for only a very few of these molecules, namely, the MHC class I and II molecules. Little is known about the other non-MHC histocompatibility antigens except that they include molecules encoded by a large number of genes scattered among all of the chromosomes (including X and Y). In principle, any peptide fragment brought to the cell surface and presented by either MHC class I or II molecules could serve as a histocompatibility.

Histocompatibility genes encode histocompatibility antigens. It is estimated that there are several scores of such loci, probably more than a hundred. Among these are the MHC class I and II molecules encoded within the **major histocompatibility complex** (**MHC**). With the possible exception of a few loci whose expression is not understood, the products of histocompatibility genes are codominantly expressed.

Codominance means that they are expressed whether present as a single copy (heterozygous or hemizygous) or two copies (homozygous). Thus an individual heterozygous at a particular histocompatibility locus (e.g., *H1a/Hb*) would simultaneously express both H1a and H1b molecules on the same surface cell surface. The same would be true for other histocompatibility loci (e.g., *H2a/H2b, H3a/H3*).

Terminology

Localisation
- Orthotopic grafts
- Heterotopic grafts – if technical problems

Donor – recipient
- autotransplantation – the same (transfussions) genetically indentical - syngeneic
- allotransplantation – genetically different from the same genus
- xenotransplantantion – different genus (pig/man)

Transplants may be categorized by location or by the genetic relationship between the recipient and the donor. With respect to location, tissues or organs that are placed in their normal anatomic location are called **orthotopic** grafts. However, many transplanted tissues or organs can function quite well in other sites as well. Grafts that are placed into a site other than their normal one are called **heterotopic** grafts. Heterotopic grafts are especially useful in cases in which orthotopic placement may be technically difficult.

Classification of grafts by the donor-recipient genetic relationship is more complex.
**Autografts** are those transferred from one part of an individual to another location on that same individual.
Syngeneic grafts are those transferred between different individuals who are genetically identical or nearly so (e.g., identical twins or members of an inbred strain).
Allogeneic grafts (or **allografts**) are transferred between two genetically disparate individuals of the same species (e.g., brother and sister, parent and child, or totally unrelated individuals). **Xenogeneic** grafts (or **xenografts**) are those exchanged between members of different species (e.g., the placement of primate hearts into human recipients).

Laws of tansplantation

Depent on individual combination of genetic impairment or match between donor and recipient
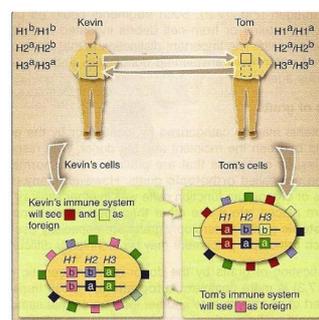

Imbred individuals - experiment

- Moses combined in siblings line in more than 20 generations – 99% of the same genetic material.
- Detection and study of genetic laws:
- Host can recognise as false and react on any histokompatibility antigens, that are not encoded by own cells

The **laws of transplantation** were originally established in experimental studies, particularly in mice, but are applicable to human transplantation as well. Genetic diversity in humans virtually ensures that no two individuals are genetically identical (identical twins are an exception). The histocompatibility antigens of concern in transplantation vary from one case to another, depending upon what specific genetic differences are present in each donor-recipient combination. Experimental animals and plants can be deliberately bred to reduce their genetic heterogeneity so that genetic variability becomes a controlled variable rather than an uncontrolled one. This process, called **inbreeding**, is accomplished by mating of closely related individuals. When laboratory mice are subjected to brothersister matings for 20 or more consecutive generations, **inbred strains** are produced. The animals within a given inbred strain are hypothetically homozygous for more than 99% of their genetic loci and, for practical purposes,

are all genetically identical. Transplants between members of the same inbred strains and between members of different inbred strains were used to deduce the laws of transplantation, which can be summarized as *a host can recognize as foreign, and mount a response against, any histocompatibility antigen not encoded within its own cells*. Grafts exchanged between individuals of the same species who are completely different (homozygous for different alleles) at a histocompatibility locus can potentially be rejected. Such differences do not necessarily cause rejection on every occasion, for a variety of reasons, but the potential is always present. Each member in the exchange will recognize the allelic form of the histocompatibility antigen expressed by the other as foreign. Heterozygous recipients, on the other hand, will see nothing foreign on grafts received from homozygous parental donors. Heterozygous grafts placed onto either type of homozygous parental type recipients will be rejected, as they express histocompatibility antigens that are foreign to one or the other parental recipient.

Heterosygot accepts graft from homozygotic parent.
Homozygotic parent refuses graft from heterozygotic donor
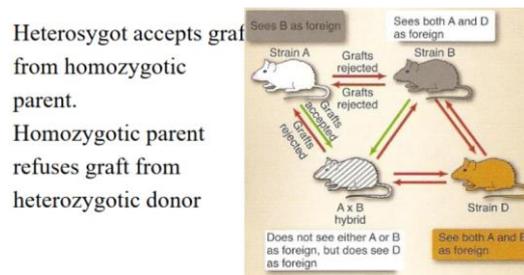
MHC I or II molecules are polymorfic differs also in non MHC locuses

The recipient immune system recognizes peptide fragments presented by MHC class I or II molecules, whether those fragments are derived from infectious organisms or from the degradation of self molecules encoded by host genes. In the case of transplanted tissues, the genes of the engrafted cells may encode nonself molecules that also can be detected by the recipient immune system and function as histocompatibility antigens. T cells can detect and be activated against histocompatibility antigens through two different pathways of recognition: direct or indirect. Direct recognition involves antigen presentation by donor antigen-presenting cells (APCs) to recipient T cells, while indirect recognition involves antigen presentation by recipient APCs to recipient T cells.

Rejection reactions

Recipient reject foreign antigens
- **directly**: donor´s APC and recipient´s T cells
If some MHC I molecules of the donor are idnetical to the recipient´s. APC of the donor present via MHC I peptids and they are bound on T CD8+ of recipient.
APC of the donor process cell debris of the donor and present them via MHC II to recipient´s T CD4+
- **indirectly**: recipient´s APC a donor´s T cells
APC of the recipient process and present peptid fragments from the donor´s cell (his antigens) and present them to T cells of the recipient

Direct recognition can occur only when some of the MHC class I or II molecules on the donor cells are identical to those on recipient cells. Like other cytosolic proteins, MHC class I and II molecules can be degraded by proteasomes and the resulting fragments presented on the cell surface by intact MHC class I molecules. If the donor and recipient have MHC class I molecules in common, APCs of donor origin may be able to present those peptide fragments directly to the TCRs of recipient CD8+ T cells. Because the MHC class I molecules on the donor cells are the same as those present in the host thymus during thymic education, the recipient TCRs are able to recognize and bind the pMHC I molecules on the donor cells. Direct recognition may also occur if donor APCs ingest cellular debris of donor origin and process/present it via MHC class II molecules to recipient CD4+ T cells. **Indirect recognition** occurs

when recipient APCs process and present peptide fragments derived from the ingestion, processing, and presentation of cellular debris from donor cells—debris that contains the donor histocompatibility antigens—and present it to recipient T cells.

Rejection – no acceptation

- **Hyperaccute** – minutes or hrs – preexistence of preformed antibodies against donor (AB0).
- **Accelerated** – days - reactivation of sensibilised T lymphocytes in the 2nd transplantation
- **Accute** – days and weeks – primáry activation of T cells
- **Chronical** – rejection caused by infection or loss of tolerance

Rejection responses fall into three general categories—chronic, acute, and hyperacute—depending upon timing and intensity. Each type involves particular sets of immune responses and is determined in part by the genetic mismatch between donor and recipient.

Chronic rejections are the slowest and the least vigorous type of rejection. The transplanted tissues or organs establish a vascular connection and proceed to function for weeks, months, and even years before signs of deterioration due to immune attack become evident.
Even after the first signs of rejection appear, the graft destruction proceeds slowly and gradually as the graft tissue is replaced by intracellular matrix and scar tissue. Chronic rejections are typical of situations in which the donor and recipient differ by only non-MHC histocompatibility gene differences, although there are exceptions.
Acute rejections occur much sooner after graft emplacement than do chronic rejections. The grafts establish vascular connections and function normally for a relatively short period of time (e.g., two to four weeks) before the first signs of rejection appear. Unlike chronic rejections, acute rejections proceed rapidly once underway. The grafts become edematous and inflamed, with an influx of blood and mononuclear cell infiltrates, and complete destruction and sloughing of the grafted tissues may take only a very few days following the first signs of deterioration. Acute rejections are commonly seen when the donor and recipient differ at MHC histocompatibility genes, especially those involving the MHC class I loci.
Hyperacute rejections are the most rapid type of rejection. They are initiated and completed within a few days of graft placement, usually before the grafted tissue or organs can establish connections with the recipient vasculature. The immune attack is typically directed at the vasculature of the graft and is mediated (in various situations) by complement, natural killer
(NK) cells, and/or preexisting antibodies. Hyperacute rejections have also been called "white grafts" because in the case of skin, the failure to establish a vascular connection gives the engrafted skin a blanched appearance. The term can be misleading; it does not describe the comparable condition of other rejected tissues. A hyperacutely rejected kidney, for example, may be bluish in color owing to the large amount of deteriorating blood trapped within it.
Like responses to infectious organs, immune responses against transplanted tissues or organs can display memory.
Attempts to repeat grafts that have previously been rejected usually result in an accelerated graft rejection, a phenomenon termed **second set rejection**. Grafts that are rejected chronically on the initial occasion may be rejected acutely when repeated. During the initial rejection, activated T and B lymphocytes can generate populations of memory cells that provide the basis for accelerated and heightened secondary responses. Second set responses are therefore simply secondary immune responses directed against histocompatibility antigens.

Terminology

- HvG reaction – host versus graft

immunocompetent recipient recognises foreign antigens of transplanted tissue and reacts by mechanisms that lead to tejection

Survival rise in direction xeno, allo, iso, auto.

Present in
  – MajorHC but also in minorHC.
  – imunological memory and secondaty reaction.

2nd transplantation of the tissue with the same MHC produce more fast rejection. , T memory cells produced during 1st transplantation

Terminology

- GvH reactiona – graft versus host

Imunosupresed recipient receive immunocompetent lymfoid cells of the donor, and they recognise foreign antigens of the host and produce destruction .

Symptoms: diarrhoe, erytema, loss on weight, fever malaise, arthralgy..)

Types of immune reactions – almost any immunological reactions can be present

- **Natural antibodies**:AB0 antigens – transffusions - IgM, produced after mi exposition and cross reactiong
- **Newly generated Ab**: activation of B cells and production of plasma cells and Ig against graft. Longer expositione. Present during 2nd transplantation => ADCC, C´, opsonisation
- **DTH – CTL** – recognision of pMHC I and II
  Activation C´ -
- **NK cells –** recognise the lack of MHC I on transplanted tissue => destruction of graft

While not every type of immune response is necessarily generated for every allograft or xenograft, almost every relevant type of immune response has been observed among various rejection episodes: antibodies, T cell responses, complement, andeven NK cells.Antibodies against graft antigens occur from two primary sources. **Natural antibodies** are preexisting antibodies that are present in the absence of known exposure or immunization. They provide, for example, the basis for transfusion reactions against ABO antigens on red blood cells, a topic that is discussed later in this chapter. Natural antibodies are produced, probably by B-1 B cells, following stimulation by antigenic molecules on the natural flora found in the body. They are of the IgM isotype and are directed against carbohydrate antigens. These antibodies are stimulated by microbial carbohydrate molecules but may cross-react with carbohydrate molecules on eukaryotic cells (e.g., human). Thus, for example, they can act immediately to damage erythrocytes in transfusions that are mismatched for carbohydrate ABO antigens. Similarly, in the case of xenografts, they can bind immediately to some carbohydrate molecules associated with the graft vasculature and initiate fatal damage to the graft.

Immunotherapy

- Basical condition –high genetical identity
- mostly not present
- 2 ways how to influenc:
- **specific immunotolerance** – experimental no in humam
- **immunosupression –** whole-body irradiation, chemotherapy, side iffects (opportune infection)., antibodies against surface molecules of imunocompetent cells (APC, ly, anti MHC I and II molecules, anti CD4, CD8 molecules

Possibilities to influence surviving of transplanted tissues

- Choose the donor
  – MHC identity with recipient – identical sigblings, HLA 95-100%   .....
  – AB0 compatibility must be present
- Preparation of the recipienty
  – skreening for anti HLA Ab donor specific – must be negative,
  – pacient withou infection
+ imunosupressive therapy (cyclosporin – inhibiítion of IL2 synthesis, blocking T cell proliferation), whole body irradiation

Immunosuppressive techniques such as whole-body irradiation or the use of toxic drugs effectively eliminate immune responses that could damage transplanted organs and tissues. The treated recipients, however, are then open to opportunistic infections that can be fatal if not successfully monitored and controlled. Over the past few decades, additional drugs (e.g., cyclosporine, tacrolimus, and rapamycin) have been developed that have more restricted effects on the immune system. Their effects are targeted more closely on cells that react to graft antigens while leaving the remainder of the immune system relatively uninhibited in its ability to deal with infectious agents. They are not without risk, however. Patients must often receive the drugs for an extended period of time. If a significant infection occurs

during this period, the immune cells responding to the infectious agent could be inhibited in the same way as those responding to graft alloantigens. In addition, extended use of these drugs is sometimes associated with damage to organs such as the liver. A second approach to inducing a less than global inhibition of the immune response has been the use of antibodies directed at

molecules on the surface of the cells involved in immune responses, particularly lymphocytes and APCs. Antibodies against MHC class I or class II molecules can inhibit with T cell activation. Antibodies against CD4 or CD8 molecules, when administered during active rejection, have been shown to inhibit or destroy T cells and halt the rejection at least temporarily.

However, antibodies against broad categories of T lymphocytes (e.g., anti-CD3 antibodies) have problems similar to those seen with immunosuppressive drugs, and their long-term use can reduce the body's ability to respond to infectious agents.

Immunesupresive - examples

Agent	Affected Cells	Mode of Action
Azathioprine	Multiple cell types	Inhibition of nucleotide synthesis
Corticosteroids (e.g., prednisone)	Multiple cell types	Inhibition of transcription for numerous cytokines and other products involved in inflammation
Cyclophosphamide	Multiple cell types	Inhibition of nucleotide synthesis
Cyclosporine	Lymphocytes	Inhibition of transcription for multiple cytokines (e.g., IL-2, IL-4)
Mycophenolate mofetil	Lymphocytes	Inhibition of lymphocyte nucleotide synthesis and proliferation
Sirolimus (rapamycin)	T cells	Inhibition of some signal transduction induced by cytokines (e.g., IL-2)
Tacrolimus (FK506)	T cells	Inhibition of gene transcription in lymphocytes, inactivation of calcineurin
Irradiation	Many cell types	Induction of DNA damage, especially in rapidly proliferating cells
Antibodies against lymphocytes or against T cells	Lymphocytes, T cells	Destruction or inhibition of lymphocytes or lymphocyte subsets
Anti-CD4 antibodies, anti-CD8 antibodies	$CD4^+$ T cells, $CD8^+$ T cells	Interference with TCR binding
Anti-MHC I/II antibodies	Antigen-presenting cells	Interference with antigen presentation and T cell activation by blockading

Blood transffusion

- Blood = ery, leu ...., Ery have more than 400 types of antigens – mostly without clinical impact

AB0: surface structures on ery and some epitelial and endotelial cells. – synthesis encoded by H (2alels – for production of H substance) (HH,Hh,hh) a AB0 lokusoch (antigens recognised by natural antibodies - IgM)

Transfussion of blood

Rh: antigen on the surface of ery: Rh- exposed to Rh+ blood produces IgG.

Rh incompatibiliy of pregnant (DD, Dd, = Rh+., dd = Rh-)

Rh-mother can have Rh+fetus and produce antiRh IgG

- 1st. pregnancy – no complication, small amount of IgG
- 2nd and next pregnancy (or aborts) – antibodies circulate in the fetus (Rh+ vs. anti Rh IgG) => hemolytical anemia of newborn– activation of C´, reactive release of inmature erytroblast - erytroblastosis fetalis

Prevention: antiRh Ab after the 12th week of pregnancy, abortuses, transfussions, delivery if Rh-women

Mismatched transfusions (e.g., type A erythrocytes given to a type B recipient) can have serious consequences. The naturally occurring IgM antibodies react almost immediately with the transfused erythrocytes to initiate agglutination and complementmediated

lysis. It is the agglutination that produces the clumping seen in demonstrations of ABO typing commonly performed in laboratories.

ABO mismatching can result in massive destruction of transfused red blood cells (**transfusion reaction**) and, if severe enough, can produce a type of transfusion reaction known as an **acute hemolytic reaction** within 24 hours of transfusion. This reaction is caused by widespread hemolysis within the vasculature from the binding of IgM to erythrocytes and the ensuing complement activation. Clinical signs include fever, chills, shortness of breath, and urticaria. If it is extensive enough, a potentially fatal condition known as disseminated intravascular coagulation can develop. Such situations emphasize the necessity of correct typing and matching of donors and recipients. Type A individuals can safely be given blood of phenotypes A and O, while type B recipients can safely receive blood of phenotypes B or O. Type O recipients should receive erythrocytes only from other type O donors. AB individuals are "universal recipients" and can safely receive transfusions from donors of phenotypes A, B, O, or AB

Rh antigens are encoded by a series of closely linked loci (*D* and *CE*) with dominant alleles (e.g., *D*) and recessive alleles (e.g., *d*), the most important of which is *D*. *DD* or *Dd* individuals have the Rh+ phenotype, while those with *dd* are Rh– .

When the father is Rh+, an Rh– mother may carry an Rh+ fetus. The maternal immune system is exposed to fetal blood as early as the first trimester of pregnancy and begins to generate anti-Rh IgG antibodies. The first Rh+ fetus is rarely at risk because of the time needed for injurious levels of anti-Rh antibodies to develop. However, subsequent Rh+ fetuses are at risk because maternal anti-Rh antibodies can increase rapidly and enter the fetus. Binding to fetal erythrocytes can lead to anemia and damage to other fetal organs. This is called **hemolytic disease of the newborn** (**HDN**) or sometimes **erythroblastosis fetalis**. The Rh antigen is a protein and elicits an IgG response. Every conception between an Rh+ male and an Rh– female has the potential to produce an Rh-incompatible fetus. Aborted (spontaneous or induced) conceptions can also lead to the development of an IgG antibody response to Rh0 (*D*).

Preventive therapy, especially the use of Rh0 (*D*) immune globulin to minimize the risk of the mother becoming sensitized against Rh, is now routinely available for this situation. This involves the injection of a high-titer anti-Rh antibody preparation such as RhoGAM® or MICRhoGAM®. These preparations contain pooled anti-Rh antibodies, prepared from human serum obtained from mothers who have made antibodies to Rh antigens. Rh0 (*D*) immune globulin should be administered after the twelfth gestational week for ongoing pregnancy was well as for spontaneous or induced abortion. Use of Rh0 (*D*) immune globulin may also be appropriate after a blood transfusion of an Rh– female.

Bone marrow transplantation

- Bone marrow contain stem cells for the whole hemotopoesis – source for pationts with deficientcies of cells (risk also).
- Transplantation of immunocompetent cells to immunodeficient recipient
- GvH rejection
- Preparation of bone marrow – T cell elimination before transplantation. Cells of BM undergo posit and negat selection in thymus of recipient
- Time to production of functionnal system – risk of oportunie infection

Immunologically privileged places

- allo and xeno transplantation possible
- Eye – intraoccular liquid of the anterior chamber – nutrition of the eye without blood cells, inhibition of apoptosis = transplantation of cornea
- Lumen testes – closed before development of IS is finished – spermatogonia not recognised as self
- Brain– hematoencefalic barriére – limited interchange of molecules + immunity reactions
- Placenta – existence of nonself histocompatibility antigens without stimulation of mother immunity

Bone marrow transplantation. Immunocompetent T cells in the donor bone marrow may recognize host antigens as foreign and initiate a graft-versus-host (GVH) response. The risk of GVH can be greatly reduced by removing mature T cells from the bone marrow inoculate prior to its introduction.

Some anatomic sites are "permissive" in tolerating genetic mismatches between donor and recipient that would lead to prompt rejection in most parts of the body. Allogeneic and xenogeneic grafts that would be rapidly rejected at most sites in the body can often survive when placed into these areas.

These sites are termed **immune-privileged sites**, and each has features that limit the immune response to cells and molecules within them. The immune-privileged sites include the eye, the testicular tubules, the brain, and perhaps the placenta.

Sources of tissues

Human
- Donors – live or dead
- Stem cells – adult, embryona, self, fetal
- Etic and law

Xenotransplantation

Tissues available for transplantation can come from a variety of different sources. Traditionally, they have been harvested from voluntary living donors or from cadavers.