LECTURE: 06

Title: TRANSPLANTAITON AND REJECTION

LEARNING OBJECTIVES:

The student should be able to:

- **Explain the reason behind phenomenon of graft rejection which occurs between different individuals**
- **Identify the major antigens that cause the graft rejection (e.g., blood group ags, and HLA (MHC) –ags.**
- **Classify the different types of grafts such as:**
  - **Autograft (Autologous antigens).**
  - **Syngeneic graft (Isograft), also syngeneic antigens.**
  - **Allograft (Allogeneic antigens).**
  - **Xenograft (heterograft=heterologous)heterogenic antigens**
- **Explain the mechanisms of graft rejection (cell-mediated & humoral mechanisms).**
- **Compare and contrast the various types of graft rejection:**
  - **Hyper acute rejection.**
  - **Acute rejection.**
  - **Insidious rejection.**
  - **Late rejection.**
- **Enumerate the steps that must be taken before the transplantation to optimize graft survival and minimize the rejections (e.g., ABO blood group compatibility is performed first, tissue typing to identify HLA is performed second, and finally cross-matching).**
- **Explain the effect of the non-specific immunosuppressive drugs.**
- **Compare between the application of the non-specific, and specific immunosuppressive drugs.**
- **List the required serological tests used to phenotype the ABO blood group and HLA-A, B, D, DR.**
- **Explain some special graft situations such as privileged tissues, privileged sites, and graft versus host (GVH).**

LECTURE REFRENCE:

1. TEXTBOOK: ROITT, BROSTOFF, MALE. IMMUNOLOGY. 6th edition. Chapter 25. pp 385-398.

2. TEXTBOOK: CATHERINE SHEEHAN. CLINICAL IMMUNOLOGY. 2ND edition. pp343-349.

3. HANDOUT.

Transplantation and rejection

- **Rejection of transplantation tissues** occurs because the immune system of the recipient recognizes and responds to foreign (tissue) histocompatibility antigens expressed on the graft.
- **The histocompatibility antigens** that are most important are those encoded by the major histocompatibility complex (MHC).
- **T lymphocytes** can directly recognize and respond to foreign MHC molecules.
- **Activated T-helper cells** make lymphokines which drive the activation of many different effector mechanisms of graft destruction.
- **Lymphokines** also act upon the graft to increase the expression of MHC molecules and adhesion molecules, making the graft more susceptible to rejection.
- **Graft rejection responses can be reduced** by matching of donor and recipient MHC molecules, especially to for MHC class II molecules.
- **Specific immunosuppression** will be used in the future, inactivating only those lymphocyte clones which cause graft rejection.

The immunobiology of transplantation is important for many reasons, in terms of both its impact on our understanding of immunological processes and its application in the development of clinical transplantation. It was the study of mouse skin-graft rejection that led to the discovery of the major histocompatibility complex (MHC) molecules, which function in the presentation of antigens to T cells. T cells are pivotal in transplant rejection, and much of our knowledge of T cell physiology and function, of self tolerance and autoimmunity, and of the role of the thymus in T cell destruction, is derived from studies of transplantation. Last, but not least, transplantation of tissues is very important clinically. The need to prevent transplant rejection has led to the development and use of

new tolerance of the grafted tissues. These approaches also have a more general application in the treatment of various immune disorders, such as immune-mediated tissue damage in hypersensitivity and autoimmunity.

In clinical practice, organs are transplanted to make good a functional deficit (**Figure-1**). Unless the donor and recipient are genetically identical, the graft antigens will elicit an immunological rejection response. Transplantation can stimulate all of the various active mechanisms of humoral and cellular immunity, both specific and non-specific. This is a consequence of the recognition by the recipient's T cells of large numbers of foreign and 'neo-self' peptides associated with the foreign MHC molecules on the grafted cells and of graft-derived peptides bound to self MHC **Figure-8**). Also, a transplant can activate all the regulatory mechanisms that control immune responses causing a state of unresponsiveness to the graft. Hence, transplantation immunology encompasses virtually all aspects of immune function.

BARRIERS OF TRANSPLANTATION

Transplantation barriers can be described in terms of the genetic disparity between the donor and the recipient: grafts can be categorized as autografts, isografts, allografts or xenografts (**Figure-2**). Autografts from one part of the body to another are not foreign and therefore do not elicit rejection. Similarly, isografts between isogeneic (genetically identical) individuals, such as monozygotic (identical) twins or mice of the same inbred strain, do not express antigens foreign to the recipient and so do not activate a rejection response. The allograft is the common clinical transplant, where one person donates an organ to a genetically different individual. In this case the graft is llogeneic (i.e. between members of the same species, having allelic variants of certain genes). The cells of the allograft will express alloantigrafts which are recognized as foreign by the recipient.

The maximal genetic disparity is between members of different species, and a xenograft across such a xenogeneic barrier is generally rapidly rejected, either by naturally occurring IgM antibodies in the recipient or by a rapid cell-mediated rejection (see below). If they are treated to reduce their immunogenicity, tissue xenografts that would otherwise be non-viable, such as pig skin, blood vessels or valves, can be grafted to man. Despite this, attempts to transplant whole organs from animal to man have been spectacularly unsuccessful, although some success has been achieved in xenografting between animal species. If the immunological problems of xenografting can be overcome, the use of animal donorscould alleviate the worldwide shortage of human organs for transplantation. Nevertheless, various non-immunological problems remain, including donor organ size, physiological differences, transmission of animal diseases and the ethics of xenografting.

HISTOCOMPATIBILITY ANTIGENS

Histocompatibility antigens are the targets for rejection

The antigens primarily responsible for rejection of genetically different tissues are known as histocompatibility (i.e. tissue compatibility) antigens and the genes coding for these

antigens are referred to as histocompatibility genes. There are more than 30 histocompatibility gene loci, and they cause rejection at different rates. Of these, alloantigens encoded by the genes of the MHC induce particularly strong reactions; these are the molecules that present antigens in a formk recognizable to T cells – all vertebrate species have an MHC. In mice the MHC is called H-2, while in man it is known as the human leucocyte intigaen (HLA) system. The products of allelic variants of the other histocompatibility genes individually cause weaker rejection responses and are consequently known as minor histocompatibility antigens; these antigens are normal cellular constituents. None the less, combinations of several minor antigens can elicit strong rejection responses (**Figure-3**).

MHC haploypes are inherited from both parents and are co-dominantly expressed

The genes of the MHC are subject to simple Mendelian inheritance and are co-dominantly expressed. In other words, each individual has two 'half-sets' (haplotypes) of genes, one haplotypes are expressed equally, so that each cell in the offspring has both maternal and paternal MHC molecules on its surface (**Figure-5**).

MHC molecules are expressed on transplanted tissues ad induced by cytokines

MHC molecules are not equally distributed on all cells of the body. Class I molecules are normally expressed on most nucleated cells (and on erythrocytes and platelets in some species), while class II molecules are restricted to antigen-presenting cells (APCs, e.g. dendritic cells and activated macrophages), B cells and, in some species, activated T cells and vascular endothelial cells. The expression of MHC on cells is controlled by cytokines: interferon-γ (IFNγ) and tumor necrosis factor (TNF) are powerful inducers of MHC expression on many cell types which would otherwise express MHC molecules only weakly. As will be seen, this is important in graft rejection.

THE LAW OF TRANSPLANATION

The transplant situation is unique in that foreign MC molecules can directly activated T cells. Conventional T cell responses against foreign proteins require that such antigens are processed into peptides and presented on the surface of the recipient's APCs in association with MHC molecules.

Host-versus-graft responses cause transplant rejection

The overriding consideration for organ allograft rejection is whether the graft carries any antigens that are not present in the recipient. This principle of host-versus-graft reactions is illustrated in **Figure-6**.

Graft-versus-host reactions result when donor lymphocytes attack the graft recipient

A special situation occurs in bone-marrow transplantation, in which graft-versus-host disease (GVHD) is induced by immunologically competent T cells being transplanted into allogeneic recipients which are unable to reject them. This inability may be due to the genetic differences between the donor and recipient, or because of a lack of immunocompetence (through immunity or immunsuppression) of the recipient. In this situation, the immunocompetent T cell transplanted with the bone marrow can attack the recipient (**Figure-7**). GVHD is a major complication of bone-marrow transplantation, causing severe damage, particularly to the skin and intestine, and is avoided by careful typing, removal of mature T cells from the graft and the use of immunosuppressive drugs.

THE ROLE OF T LYMPHOCYTES IN REJECTION

T-cells are pivotal in graft rejection

Rodents born without a thymus (congenitally athymic or 'nude') have no mature T cells and cannot reject transplants. The same is true of normal rats or mice from which the thymus is removed in the neonatal period, before mature T cells are released to the periphery. Likewise, adult thymectomy (AT) of rats or mice (to stop the production of T cells), followed by irradiation (to remove existing mature T cells) and bone marrow (BM) transplantation (to restore haemopoiesis) produces 'ATx.BM recipients' which have no T cells and cannot reject grafts.

In any of these animals (nudes, neonatally thymectomized or ATx.MB), the ability to rejects grafts is restored by the injection of T cells from a normal animal of the same strain. Thus T cells are necessary for rejection. This does not imply that antibodies, B cells or other cells play no part. Indeed, antibodies cause graft damage and macrophages may be involved in inflammatory reactions in grafted tissue.

Rejection responses have a molecular basis in the TCR-MHC interaction

Via their T-cell receptors (TCRs), the T cells involved in rejection recognize donor-derived peptides in association with the MHC antigens expressed on the graft. As we already know, the structure of the T cell receptor (TCR) (see **Figure-13**) is such that T cell can only 'see' peptide antigens when they are associated with MHC molecules, and this MHC restriction is imposed by positive selection in the thymus (see **Figure-8**). So, to understand the involvement of T cells in rejection, we need to examine the difference between recipient and graft MHC molecules and how such differences effect the range of antigens presented to the recipient's TCR.

Different MHC molecules have similar structures but different peptide-binding grooves

The structure of different MHC molecules are almost identical, with the overall shape consisting of two α helices lying on a β-pleated sheet stop two immunologlobulin-like

domains which sit on the cell membrane. Between the α helices is a deep groove into which peptides can be bound. The part of the MHC molecule that is important in T cell recognition is the outer surface of these α helices, which is highly conserved between different MHC molecules.

The significant amino-acid sequence differences between two MHC molecules – comparing, for example, A2 and Aw68 allelic variants of the HLA-A antigen – lie deep in the groove between the α helices, not on the outer surface contacted by the TCR (see **Figure-7**). Hence, for T cell recognition, the principal difference between MHC molecules is in the shape and charge of the peptide-binding groove, and this governs which peptides can be bound and in what orientation they are presented to TCRs (see **Figure-11 and 12**).

Graft and host MHC molecules present different peptides

In the normal physiological situation, the MHC groove is occupied by peptides derived from normal cellular constituents by intracellular degradative pathways. Thymic tolerance mechanisms ensure that T cell recognition of these self peptide-self MHC complexes, which would lead to autoimmunity, does not occur. However, when cells are infected (with virus, for example), the normal cell-derived peptides are replaced by peptides of foreign origin, as is the case of 'professional' APCs. T cells then respond to these foreign peptides in association with self MHC molecules.

However in the case of a genetically distinct transplanted tissue a third situation arises. A different array of peptides is presented on the cell surface because of the different shape and charge of the peptide-binding sites of the graft MHC molecules. This allows binding, not only of peptides derived from the foreign MHC and minor allelic histocompatibility antigens, but also peptides of host molecules which do not bind to self MHC and which therefore have not induced tolerance (**Figure-8**). This leads to the expression on transplanted APCs of a very large number of novel antigens which can be recognized by the recipient's T cells. This so-called 'direct' mode of antigen presentation is supplemented by the direct recognition of graft peptides bound by self MHC. It is not surprising therefore that up to 10% of an individual's T cells may respond to these antigens originating from the engrafted tissue.

T-helper (T_H) cells and lymphokines are involved in rejection

The role of T-helper (T_H) cells in rejection

Injecting T cells of the $CD4^+$ subpopulation (TH cells) into nude or ATx.BM recipients leads to acute skin-graft rejection. Naïve, unsensitized $CD8^+$ T cells (Tc cells) are unable to do this, but when $CD8^+$ T cells are mixed with a very low number of $CD4^+$ T cells, or are presensitized to graft antigens (i.e. taken from animals which have already rejected a graft), rapid graft destruction is then seen. Treating recipients with monoclonal anti-$CD4^+$ antibodies (**Figure-9**) confirms the importance of TH cells in rejection.

TH cells are activated by APCs derived from bone marrow and carrying MHC class II molecules. The APCs activating rejection can come from either the donor or the recipient. Those of donor origin are present in the graft as 'passenger leucocytes' (interstitial dendritic cells) and they cause 'direct' activation of the recipient's TH cells. Those of recipient origin are located in draining lymphoid tissues and acquire antigen that is shed from the transplant, and present it to the recipient's TH cells to cause 'indirect' activation. Direct activation is a more powerful stimulus to rejection than the so-called indirect route (**Figure-10**). Thus passenger cells may have a strong influence on graft survival (**Figure-11**).

The role of lymphokines in rejection

In addition to the role of $CD4^+$ TH cells, a multiplicity of immunological mechanisms including lymphokines are involved in the process of rejection. The overall picture is show in **Figure-12**.

The most important lymphokines in cellular rejection are interleukin-2 (IL-2), which is required for activation of Tc cells, and IFNγ, which induces MHC expression, increases APC activity, activates large granular lymphocytes and, in concert with lymphotoxin, activates macrophages.Macrophages, in turn, release TNFα, an important mediator of graft damage. (Note: the mixture of INFγ and lymphotoxin was formerly known as macrophage activating factor or MAF.).

Lymphokines (IL-4, -5 and -6) are also required for B-cell activation, leading to the production of anti-graft antibodies. These antibodies fix complement and cause damage to the vascular endothelium, resulting in haemorrhage, platelet aggregation within the vessels, graft thrombosis, lytic damage to cells of the transplant, and the release of the pro-inflammatory complement components, C3a and C5a.

Not all parts of the graft need to be attacked for rejection to occur. The critical targets are the vascular endothelium of the microvasculature and the specialized parenchymal cells of the organ, such as renal tubules, pancreatic islets of Langerhans or cardiac myocytes.

IFNγ can cause vascular endothelial cells to express high levels of class II MHC molecules, and can induce the expression of class I and II molecules on parenchymal cells, which usually express little or none of these. This upregulation of MHC expression on cells of the graft can provoke greater stimulation of the rejection response and provide a greater number of target molecules within the graft for antibodies and activated cells.

Lymphotoxin and IFNγ also upregulate the expression of adhesion molecules on vascular endothelium, these are required for the adhesion of blood-borne leucocytes to the walls of blood vessels prior to their migration across the endothelium into the tissues.

THE TEMPO OF REJECTION

The rate of rejection depends in part on the underlying effector mechanisms (**Figure-13**).

Hyperacute rejection – This occurs very rapidly in patients who already have antibodies against a graft. Anti-HLA antibodies are indeed by prior blood transformations, multiple pregnancies or the rejection of a previous transplant. In addition, antibodies against the ABO blood group system can cause hyperacute rejection. Preformed antibodies fix complement, damaging the endothelial cell lining of the blood vessels. This damage allows the leakage of cells and fluids and causes aggregation of platelets which then block the microvasculature, depriving the graft of a blood supply (**Figure-14**). Hyperacute rejection can be avoided by ABO matching and by performing cross-matching, in which serum from a prospective recipient is gtested for the presence of cytotoxic anti-donor antibodies.

Because humans have preformed IgM and IgG natural antibodies to animal cells, hyperacute rejection prevents transplantation of animal organs to man. Various approaches to overcoming this – by removing the antibodies, depleting complement or genetically engineering donor animals that have tissues less susceptible to hyperacute rejection – are under active investigation.

Acute rejection – This takes days or weeks to become manifest and is due to the primary activation of T cells and the consequent triggering of various effector mechanisms (**Figure-15-17**). If a transplant is given to someone who has been presensitization to antigens on the graft, a secondary reactivation of T cells occurs, leading to an accelerated cell-mediated rejection response. Accelerated or 'second-set' rejection of skin grafts is particularly dramatic – so-called "white graft rejection" in which the graft is rejected before it has time to heal (**Figure-18**).

Chronic rejection – Depending on the genetic disparity between donor and recipient and the use of immunosuppressive treatment, graft rejection can be a slow process taking months or years. The walls of the blood vessels in the graft thicken and eventually become blocked. This is called chronic rejection and may be due to several different cases, such as a low-grade cell-mediated rejection or the deposition of antibodies or antigen-antibody complexes in the grafted tissue, which damage or activate the endothelial cells lining the vessel and trigger inappropriate repair responses.

The cardinal features of chronic rejection are luminal obliteration (blocking of the blood vessels of the graft by proliferating smooth muscle cells which have migrated from the vessel wall and deposited matrix proteins) and interstitial fibrosis (formation of scar tissue throughout the grafted organ). These processes are controlled by various growth factors, such as TGFβ, released as a consequence of immune or other injury to the transplant (**Figure-19**). The half-life of a kidney transplant is still only 7-8 years, and has not improved in the last 10 years despite the introduction of cyclosporine A to control acute rejection. This strongly suggests that we need to find new immunosuppressive drugs to control the chronic rejection process.

Graft may also be damaged by the recurrence of the original disease process that necessitated the transplanat.

GENETIC PREDISPOSITION OF GRAFT REJECTION

The amount of many of the cytokines that an individual makes is under genetic control. For example, a person may be a high or a low producer of IL-10. Indeed, there may be a ten-fold difference in the amount of IL-10 made by different people. This is related to small differences polymorphisms) in the DNA sequences flanking the IL-10 gene, in the gene promoter region. Most other cytokine genes are polymorphic too, so that each individual inherits a pattern of higher and lower cytokine responses. By using simple genetic tests it is possible to determine whether someone is predisposed to make higher or lower amounts of each cytokine. A person who is genetically pre-programmed to be a high producer of TNFα (an inflammatory cytokine) and a low producer of IL-10 (an anti-inflammatory cytokine) is more susceptible to inflammatory conditions. Likewise, these inherited differences may contribute to susceptibility to infections, allergies and autoimmunity.

Cytokine gene polymorphisms have been shown to influence transplant rejection in humans. The high TNFα producer genotype is associated with the acute rejection of kidney, heart and liver transplants, so that 805 of recurrent acute rejection episodes occur in the 205 of recipients who are high TNFα producers. In addition, such people are more likely to suffer irreversible rejection, a fatal outcome in heart transplant recipients. Similarly because TGFβ plays a major role in chronic rejection, recipients of high TGFβ producer genotype are more likely to suffer chronic rejection of heart and lung transplants.

This recent research will be important in many ways. For example, the drugs that are given to prevent rejection are toxic and can produce sever side-effects. Thus we would aim to give the lowest effective doses. Knowing that someone is unlikely to fiercely reject their graft may allow the use of minimal immunosuppression while, at the opposite extreme, closer monitoring for rejection and higher drug doses may be used. This is a concept called ‘tailoring immunosuppression to the individual needs of the patient’ and contrasts with giving the same treatment to everyone regardless of their requirement.

Another outcome of cytokine genotyping is that it identifies the cytokines that play a pivotal role in transplant rejection. The importance of TNFα antibody or an engineered soluble TNFα receptor, that will neutralize TNFα in the patient may work to suppress acute rejection. Anti-TNFα antibody does suppress acute rejection in rats. Furthermore, anti- TNFα agents are already used clinically to treat patients with rheumatoid arthritis. At present there are no agents that can be used to reduce TGFβ production and influence chronic rejection.

PREVENTION OF REJECTION

The rejection response can be reduced by tissue matching

The perfectly matched donor and recipient would be isogeneic, for example monozygotic twins. However, this situation is rare, and in all other cases there will be major and/or minor histocompatibility differences between the donor and recipient. Only the major (MHC, i.e.HLA) antigens can be practicably matched. This can be done by serology (**Figure-20**), which takes only a few hours and can therefore be performed while the donor organ is preserved on ice. Recently, sensitive and accurate typing has been achieved using the polymerase chain reaction (PCR) to identify HLA genes in the DNA of donors and recipients.

Matching for all known HLA antigens is practically impossible, but good organ graft survival is obtained when the donor and recipient share only the same MHC class II antigens, especially HLA-DR (**Figure-21**), because these are the antigens that directly activate the recipient's TH cells.

The lists of known class I (HLA-A, HLA-B and HL-C) and class II (HLA-DP, HLA-DQ and HLA-DR) antigens are long, and the chances of completely matching two individuals at random are extremely remote.

The mixed lymphocyte reaction (MLR) can also be used to test the responsiveness of recipient lymphocytes to antigens expressed on donor cells (**Figure-22**). Low recipient anti-donor MLR responses are associated with excellent transplant survival. However, the 4-5 days required for the MLR test precludes its use in most clinical organ transplantation, because organs from dead or brain-dead donors cannot be preserved for more than 24-48 hours. In those for when living donors (e.g. relatives) are to be used, MLR can be used. It is especially important in bone marrow transplantation, to assess whether the donor bone marrow cells can respond to recipient antigens and because GVHD DNA typing has now largely superseded these older methods.

Non-specific immunsuppression can control rejection reactions

There are two main categories of immunosuppressive treatment: antigen-non-specific and antigen-specific. Non-specific immunosupression blunts or abolishes the activity of the immune system regardless of the antigen. This can leave a graft recipient very vulnerable to infections. For instance, a large dose of X-ray prevents rejection but also has man deleterious effects, as well as abolishing antimicrobial immunity. Most non-specific treatments used today are selective for the immune system, or are used in a way which creates some selectivity. The very best treatment would take this further and inactivate only those clones of lymphocytes with specificity for donor antigens, leaving other clones intact, so that the patient does not suffer infections or side-effects. Such highly specific immunosupression remain the 'Holy Grail' of transplantation immunobiology and is described later.

The three non-specific agents that are most widely used in current clinical practice are steroids, cyclosporine and azathioprine (**Figure-23**)

Steroids have anti-inflammatory properties and suppress activated macrophages, interfere with APC function and reduce the expression of MHC antigens. In effect, steroids reverse many of the actions of IFNγ on macrophages and transplanted tissues.

Cyclosporin is a fungal macrolide produced by soil organisms, and has interesting and potent immunosuppressive properties. Its principal action is to suppress lymphoine production by TH cells by interfering with the activation of lymphoine genes and, directly or indirectly, to reduce the expression of the receptors for IL-2 on lymphocytes undergoing activation. Other macrolides such as FK506 suppresses lymphokine production by TH cdells in a way similar to cyclosporine. Rapamycin interferes with the intracellular signaling pathways of the IL-2 receptor and therefore preventsIL-2-dependent lymphocyte activation. The comparative structures of cyclosporine, FK506 and rapamycin are shown in **Figure-24**.

The rejection response involves the rapid division and differentiation – proliferation – of lymphocytes. Azathioprine is an antiproliferative drug, an analogue of 6-mercaptopurine. Its incorporation into the DNA of dividing cells prevents further proliferation. New antiproliferative drugs, such as mycophenolic acid derivatives, are under investigation.

These agents can be effective used alone, although high doses are usually required and the likelihood of adverse toxic effects is increased. Used together in various combinations, they work in synergy because they interfere with different stages of the same immune pathway. The doses of individual agents can thus be reduced and the adverse effects minimized. The clinical results obtained since the introduction of cyclosporine are very good (85-90% graft acceptance at 1 year for kidneys, hearts and livers). However, the expected half-life of a kidney transplant is 7-8 years because of the problem of chronic rejection, and long-term use of drugs is still associated with adverse effects. Further improvements might be obtained with the introduction of new drugs.

New non-specific but more selective agents are under development (**Figure-25**). Monoclonal antibodies against lymphocyte surface molecules, especially CD3, CD4, CD8 and the IL-2 receptor, can be used to eliminate cells or to block their function. Cytotoxic drugs can be attached to these antibodies to increase their effectiveness. A related approach is to attach a toxin to IL-2 so that cells undergoing activation in response to graft antigens and expressing receptors for IL-2 take up the IL-2-toxin conjugate and are selectively poisoned.

Specific immunosuppression reduces anti-graft responses without increasing susceptibility to infection

The immune system is regulated various feedback mechanisms that control the magnitude, type and specificity to immunological reactions. It is possible in experimental models to harness these feedback systems to prevent transplant rejection. There are three classical procedures which can be used: neonatally induced tolerance, active enhancement and passive enhancement.

Neonatal exposure to donor antigen can induce unresponsiveness to transplants in animals

Neonatal rodents (unlike humans) are born just before mature T cells are first exported from the thymus (the equivalent stage of human development is 16-20 week's gestation). If a persistent source of antigen, for instance viable cells with potential for growth or repeated injections of antigen, is given to the neonate rodents, the development of mature T cells that react with that antigen is suppressed. Classically, bone marrow cells from an (A x B) F_1 mouse are injected into B-stained neonate. (The donor cells used are (A x B) F_1 to obviate the A-strain anti-B GVH reaction that occurs if A donor cells are used). The bone marrow inoculum produces cells that provide a continuous source of antigen. When the B-strain mouse grows a adulthood it is unresponsive to the A antigens to which it has been exposed neonatally and is tolerant of the A antigens on skin grafts and other tissues from A or (A x B) F_1 strain donors.

Antigen may selectively activate certain subpopulations of lymphocytes. It is currently proposed that there are two major types of TH cell, known as TH1 and TH2 cells. Neonatally tolerized mice can have a deficit of donor-specific TH1 cells and an increased number of donor specific TH2 cells. TH1 cells make IFNγ and IL-2 and are the TH cells illustrated in **Figure-23**, which are involved in rejection. By contrast, TH2 cells make other cytokines including IL-10 or cytokine synthesis inhibitory factor (CSIF), which interferes with the synthesis of lymphokines by TH1 cells. For neonatally tolerized mice, fewer donor-specific TH1 cells and more donor-specific TH2 cells mean a shift in the balance between rejection and acceptance, leading to tolerance of the graft. This form of tolerance is not strictly unresponsiveness *per se* but, rather, a deviated response. Interestingly, cyclosporine may have a preferential effect on TH1 cells and spare TH2 cells.

Finally, antigen can activate suppressor T cells (Ts cells). The precise identity of these T cells is still shrouded in mystery. What is known is that, when transferred to another animal, T cells from an animal tolerant of a graft from donor A can prevent rejection of a graft carrying A antigens. This is referred to as the adoptive transfer of suppression and the cells responsible can be TH or Ts cells. Much controversy still exists concerning Ts cells and their mode of action, but experimental data provide a clear indication that functional Ts cells do exist. They are resistant to cyclosporin and may contribute to this agent's mode of action and mediate tolerance by active suppression.

Equivalents in humans – A direct equivalent of neonatally indeed tolerance is not possible in humans. However, procedures such as total lymphoid irradiation (TL1), in

which mature lymphocytes are severely depleted by radiation while the bone marrow is shielded and therefore remains intact, may create in adults a situation analogous to that in the neonatal rodent. Indeed, TLI followed by antigen exposure induces profound tolerance. However, TLI is rather hazardous for routine clinical use. Antilymphocyte serum (ALS), made by immunizing animals with human lymphocytes, is widely used in heart transplant recipients to deplete circulating T cells. The use of monoclonal antibodies to mature T cells may achieve their depletion in a much safer but equally effective way, and anti-CD3 antibodies are in clinical use.

Unresponsiveness to transplants can be induced in humans by blood transfusios

In some cases, prior exposure to donor antigens can cause prolonged or indefinite graft survival (**Figure-26**). This is, of course, contrary to expectation, as one might expect accelerated or hyperacute rejection. The phenomenon is called active enhancement of graft survival. The route of exposure of antigen is important, possibly because it impinges on particular lymphoid tissues. It has been shown in a rat kidney-graft model that a transfusion of donor blood given intravenously to the recipient 1 week before kidney transplantation leads to long-term organ-graft acceptance, while the same close of blood given subcutaneously causes accelerated rejection. The effect is immunologically specific, so the blood donor and the kidney donor must share at least some antigens.

An active enhancement effect has been employed clinically using donor-specific transfusions (DST). For example, if a parent is about to donate a kidney to a child, the recipient can be treated with blood transfusions from the parent before transplantation. Unfortunately, about 20% of patients receiving DST develop and anti-donor antibodies and cannot then receive the kidney as planned, for fear of hyperacute rejection. However, of the remaining 80% the transplant success rate is 95-100%

The beneficial effect of pretransplant blood transfusion, known as the blood transfusion effect, has also been documented in patients receiving random transfusions, perhaps because of the enhance exposure to antigens which happen to be on their transplant **Figure-27**). Indeed, the blood transfusion effect increases with the number of random transfusions, and for a time most transplant centers adopted the policy of deliberately transfusing prospective recipients. However, there is always a risk of sensitization of the patient, as well as the transmission of AIDS, and improvements in the availability and use of immunosuppressive drugs have largely made this practice redundant.

Active enhancement requires an active response b the recipient to the injected donor antigen. The mechanism could be induction of anergy, selective activation of TH2 cells, or activation of Ts cells by the blood transfusion, as described for neonatally induced tolerance. Alternatively, the mechanism might involve the production of ‘enhancing antibodies’ which block recognition of specific donor antigens, thus interfering with the graft rejection process, or by destroying highly immunogenetics passenger leucocytes within the graft. Enhancing antibodies may also be formed to antigen receptors, thus

eliminating donor reactive cells of affecting antigen presentation so that, for instance, TH2 and Ts cells are selectively activated after transplantation.

Antibody can have a feedback role in transplanted individuals. Injection of anti-donor antibody (passive enhancement) into a rat kidney-graft recipient at the time of grafting can cause long-term graft acceptance (**Figure-26**).

Clinical transplantation

Organ transplanted	Exaples of disease
Kidney	End-stage renal failure
Heart	Terminal cardiac failure
Lung or heat/lung	Pulmonary hypertension, cystic fibrosis
Liver	Cirrhosis, cancer, biliary atresia
Cornea	Dystrophy, keratitis
Pancreas or islets	Diabetes
Bone marrow	Immunodeficiency, leukaemia
Small bowel	Cancer
Skin	Burns

Figure-1 Organs and tissues are transplanted to treat various conditions. Each type of transplant has its own particular medical ad surgical difficulties.

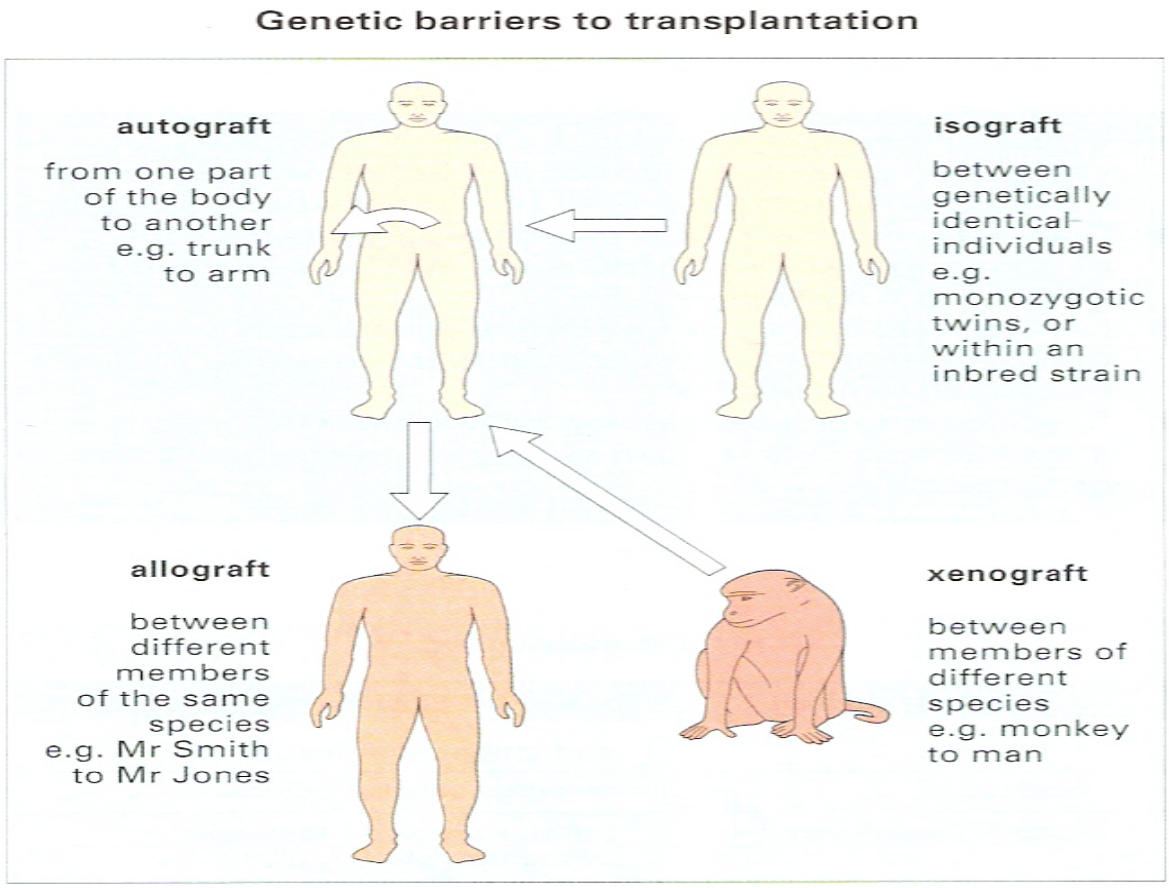

Figure-2 The genetic relationship between the donor and recipient determines whether or not rejection will occur. Autografts or isografts are usually accepted, while allografts and xenografts are not.

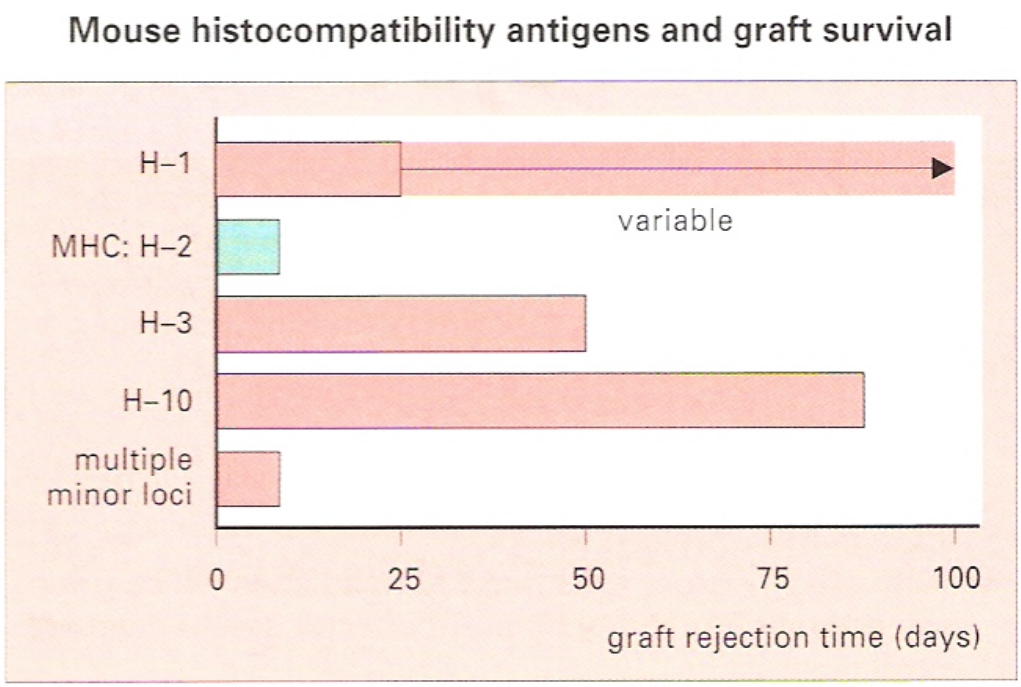

Figure-3 This chart gives the rejection times for skin grafts between mice differing at the minor histocompatibility loci (red) or at the major histocompatibility H-2 locus (green). Grafts which differ at multiple minor loci are rejected as quickly as those that differ at H-2.

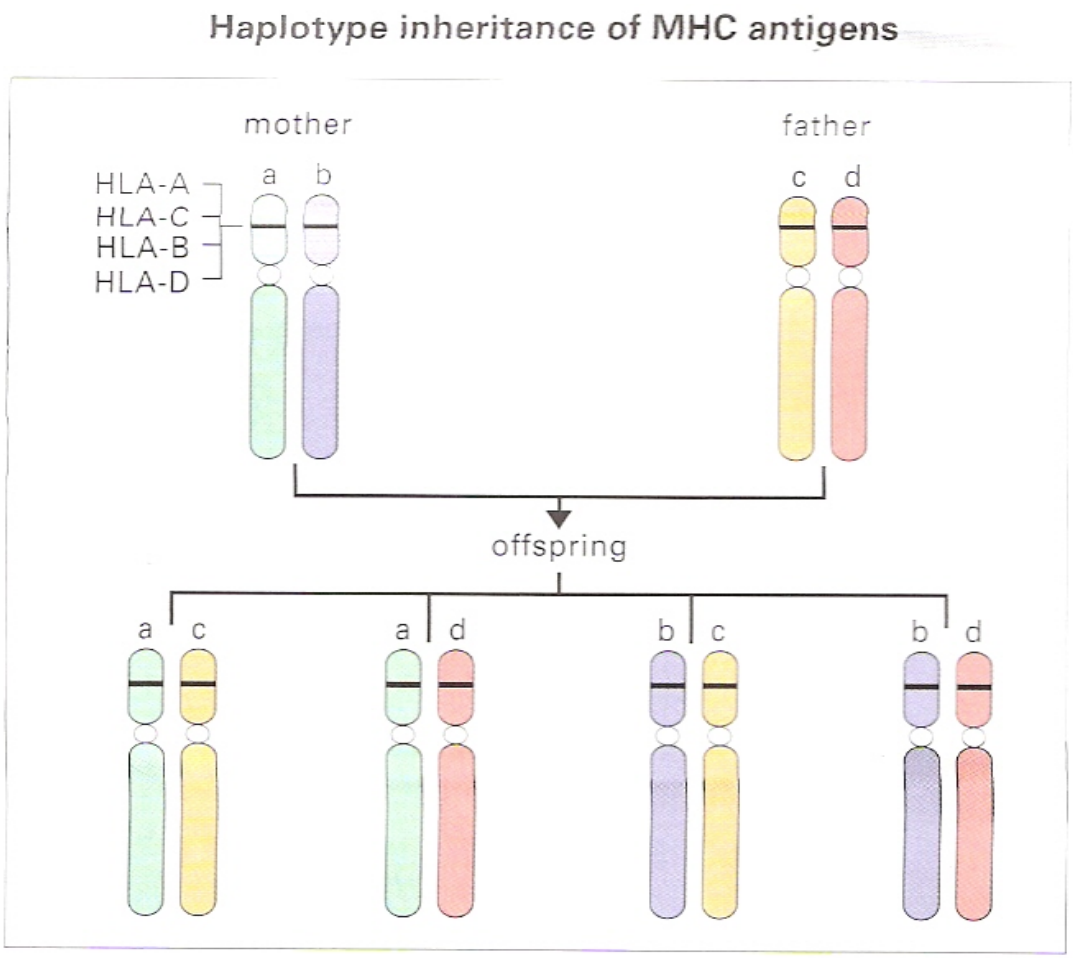

Figure-4 The human MHC (HLA) is located on the short arm of chromosome 6. One set (haplotype) of the MHC class I (HLA-A, -B and -C) and class II (HLA-D) antigens are inherited en bloc from each parent according to simple Mendelian inheritance.

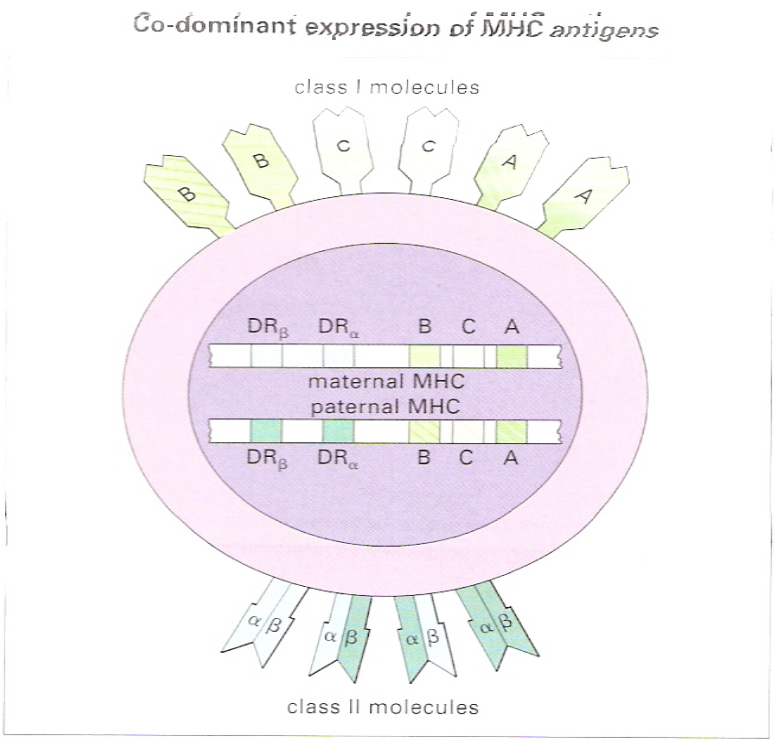

Figure-5 Inherited MHC genes are all expressed on the cell surface. For each maternal and paternal class I gene there are class I molecules o the membrane. For each class II α and β gene there are α and β chains on the cell surface, but these can associate to form four different molecules. Note that there are other class II α and β genes coding for DP and DQ antigens as well B cells have 23 x 10^5 class I molecules and the same number of class II molecules per cell.

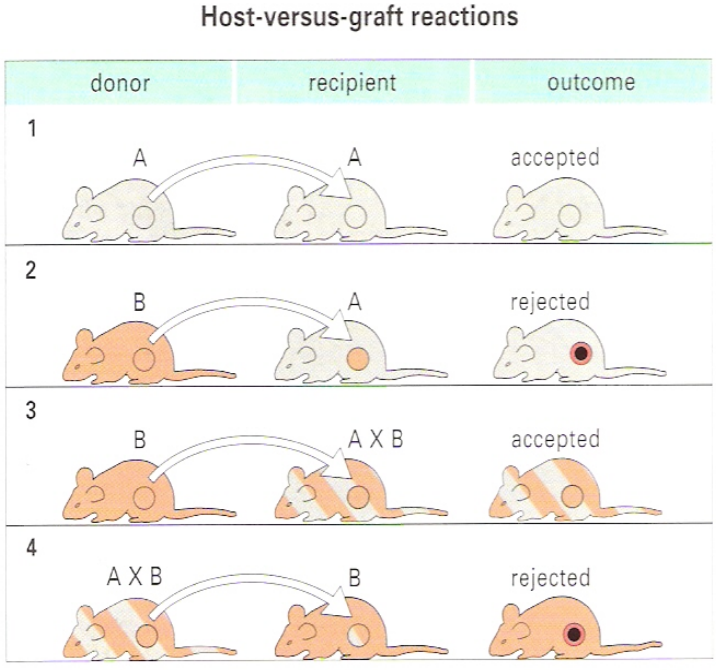

Figure-6 Grafts between genetically identical animals are accepted. Grafts between genetically non-identical animals are rejected with a speed which is dependent on where the genetic differences lie. For example syngeneic animals, which are identical at the MHC locus, accept grafts from each other (1). Animals that differ at the MHC locus reject grafts from each other (2). The ability to accept a graft is dependent on the recipient sharing all the donor's histocompatibility genes: this is illustrated by the difference between grafting from parental to (A x B) F_1 animals (3) and vice versa (4). Animals that differ at loci other than the MHC reject graft from each other, but much more slowly.

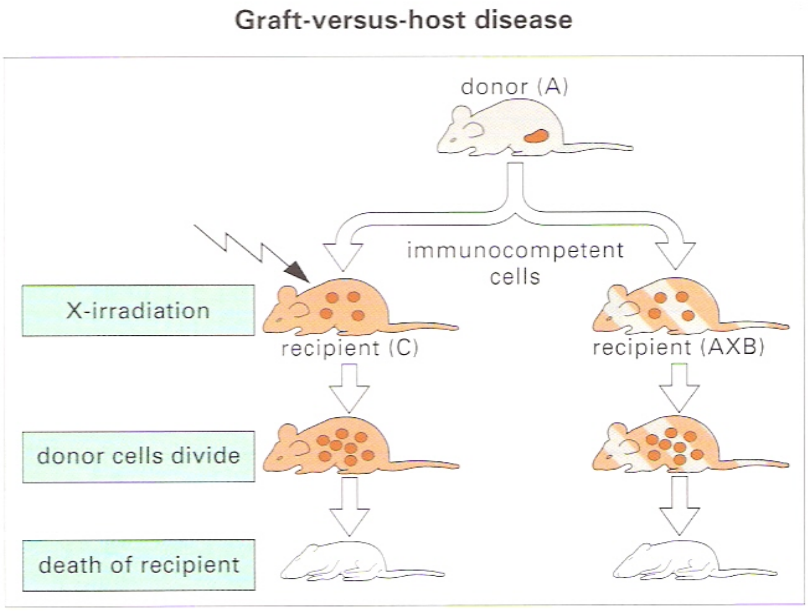

Figure-7 Immunocompetent cells from a donor of Type A are injected into an immunosuppressed (X-irradiated) host of Type C, or a normal (A x A) F_1 recipient. The immunosuppressed individual is unable to reject the cells and the F_1 animal is fully tolerated to parental Type A cells. In both cases the donor cells recognize the foreign tissue Types B or C of the recipient. They divide the react against the recipient tissue cells and recruit large numbers of host cells to inflammatory sites. Very often the process leads to the death of the recipient.

Presentation of graft antigens

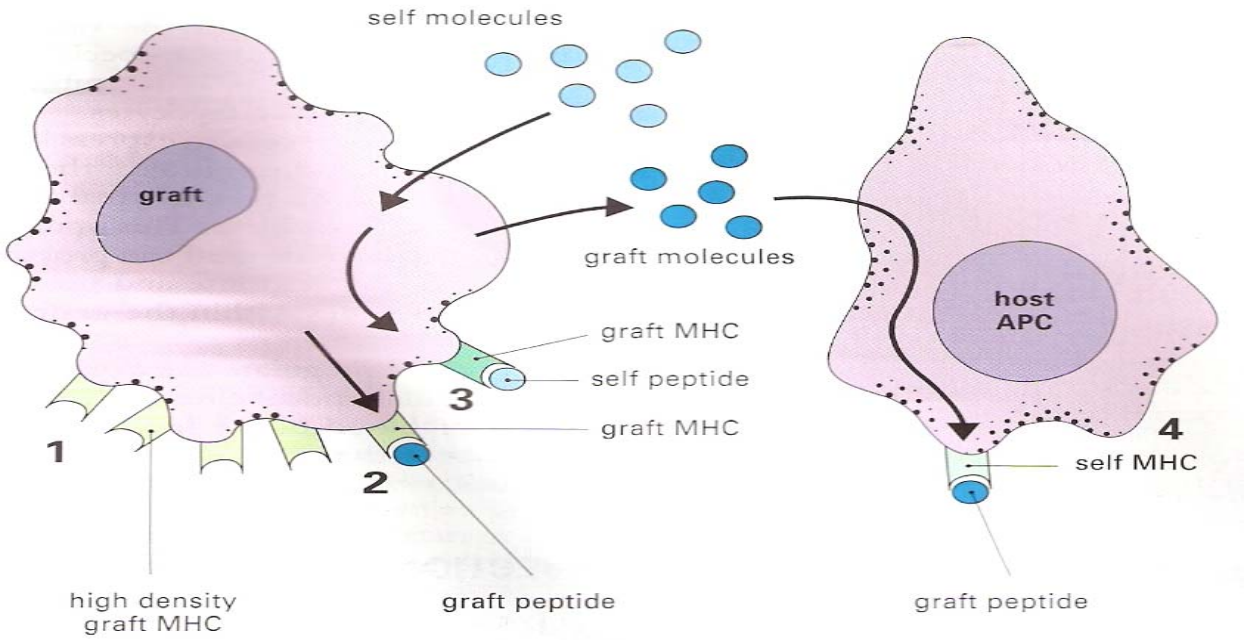

Figure-8 There are several ways in which grafts and graft antigens can be recognized by T cells in the host. This may account for the relatively high proportion of host T cells which is capable of responding to engrafted tissue. 1. A high density of graft MHC molecules, which individually react weakly with the TCR may generate a sufficient signal for T cell activation. 2. Graft MHC molecules can present the graft's own peptides including molecules from both major and minor histocompatibility antigens. 3. Graft MHC molecules can present processed antigens of host molecules. Because the graft MHC is different to self MHC it will present a different set of peptides to the host, and the host will not be tolerant of this MHC/antigen combination. 4. Allotypically different graft molecules, including histocompatibility antigens can be taken up by host antigen presenting cells, and be processed and presented on self MHC molecules.

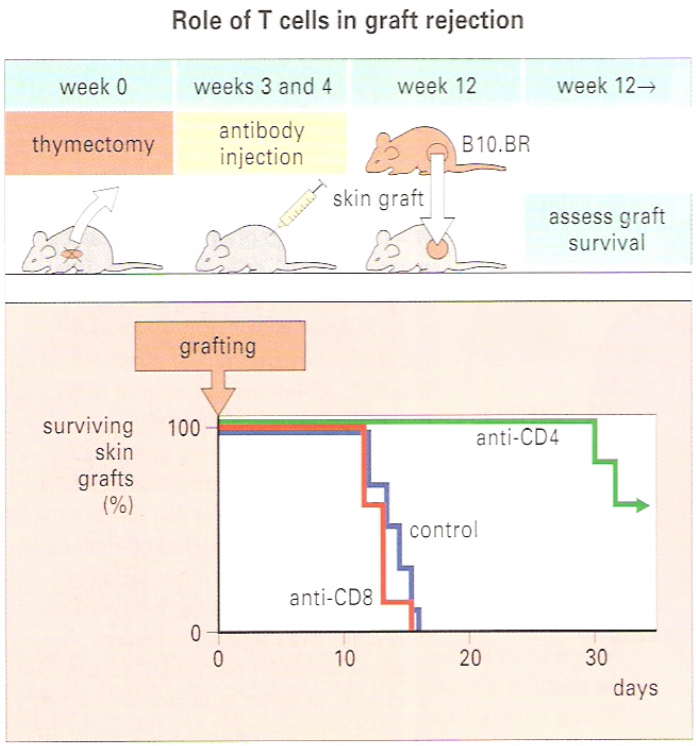

Figure-9 Thymectomized CBA mice were treated with cytotoxic monoclonal antibodies to CD4 or CD8, to selectively deplete TH and Tc cell populations, respectively. They were then grafted with skin from B10.BR mice, which differ at minor histocompatibility loci. The survival of the grafts was assessed. Animal treated with anti-CD4 had greatly extended graft survival by comparison with untreated animals (control) or those treated with anti-CD8. This emphasizes the importance of the $CD4^+$ (TH) population in graft rejection.

A comparison of direct and indirect antigen presentation

	Direct antigen presentation	Indirect antigen presentation
General comment	Abnormal situation restricted to recognition of transplanted tissues	Normal physiological route of antigen processing and presentation
Origin of antigen presenting cells	Donor	Recipient
Antigen recognised by recipient T cells	Donor MHC plus donor and 'neo-self' peptides	Recipient MHC plus donor peptide
Frequency of the activated T cells	1/1000 to 1/10 000	1/100 000 to 1/1000 000
Function of the activated T cells		
TH cells	Produce cytokines No cognate interaction with B cells	Produce cytokines Provide cognate help for B-cell activation and antibody formation
Tc cells	Can kill cells in the graft because they recognize donor MHC + peptide in the transplant	Cannot kill cells in the graft because they do not recognize antigens expressed on donor cells
Duration of stimulus	Donor APC (passenger leucocytes) are normally lost from the graft within days, therefore their influence is short-lived*	Continues for as long as the graft (source of donor peptide) survives, and can therefore stimulate chronic rejection

Figure-10 Direct presentation of alloantigens stimulates a very powerful rejection response because of the large number of recipient T cells that may become activated. However, the T cells activated may act in different ways, and the stimulus through the direct route of alloantigen presentation is likely to be of limited duration. *Note there is evidence that donor-derived passenger leucocytes can remain I the recipient for a long time, a state known as chimerism. Some researchers suggest that the presence of chimerism is associated with graft acceptance, and may even play a role in maintaining unresponsiveness to the graft.