Major Histocompatibility Complex :genetics and role in transplantation

The major histocompatibility complex (MHC) was discovered from the studies conducted on transplant immunology. It was discovered from the fact that tissues exchanges between non-identical animal are rejected while from identical twins are accepted. **George Snell** and colleagues identified the single genetic region responsible for this rejection in chromosome 17 of mice and named it major histocompatibility complex. Similarly the gene responsible for graft rejection in humans was identified as **human leukocyte antigen** (HLA).

MHC antigens of man (first detected on leukocytes); **H-2 antigens:** MHC antigens of mouse; **Xenograft:** Grafts between members of different species (also known as heterologous or xenogeneic grafts or hetero-grafts); **Allograft:** Grafts between two members of the same species (also known as allogeneic grafts or homo-grafts); **Isograft/syngeneic :** Grafts between members of the same species with identical genetic makeup (identical twins or inbred animals); **Haplotype:** a group of genes on a single chromosome.

PRINCIPLES OF TRANSPLANTATION:

An immunocompetent host recognizes the foreign antigens on grafted tissues (cells), and mounts an immune response, which results in rejection (host -vs- graft reaction). On the other hand if an immunocompromised host is grafted with foreign immunocompetent lymphoid cells, the immunoreactive T-cells present in the graft recognize the foreign antigens on the host tissue and cause their damage (graft -vs- host reaction).

Host-versus-graft-reaction:

The duration of graft survival follows the order, xeno- <allo- <iso- = auto- graft. The time of rejection also depends on the antigenic disparity between the donors and recipient. While the MHC antigens are the major contributors in rejection, the **minor histocompatibility antigens** also play a significant role. Rejection due to disparity in **several** minor histocompatibility

Human MHC:

The human MHC is located on chromosome 6.

Class I MHC:

The class I gene complex contains <u>three major loci of highest significance</u>, **B**, **C** and **A** and some undefined <u>loci of less significance </u>(Figure 1). Each these loci codes for a polypeptide, α-chain that contains antigenic determinants that are **polymorphic** (has many alleles). Each α-chain associates with a β-2 microglobulin molecule (β-chain), encoded by a gene outside the MHC complex. The α-β-chain complex is expressed on the cell surface as the class-I MHC antigen. Without a functional β-2 microglobulin chain, the class I antigen will not be expressed on the cells surface. Individuals with defective a β-2 microglobulin gene do not express any class I antigen and hence they have a deficiency of cytotoxic T cells. antigens may be as quick or quicker than rejection mediated by an MHC antigen. Like in other immune responses, there is immunological memory and secondary response in graft rejection. <u>Thus, once a graft is rejected by a recipient, a second graft from the same donor, or a donor with the same histocompatibility antigens, will be rejected in a much shorter time</u>.

Graft-versus-host (GVH) reaction:

Histoincompatible lymphoid cells when injected into an immunosuppressed host are readily accepted. However, the immunocompetent T lymphocytes found in the grafted cells recognize the alloantigens found on the host and proliferate and progressively cause damage to the host tissues and cells. This condition is known as <u>graft-versus-host (GVH) </u>disease and is often fatal. Common manifestations of GVH reaction are diarrhea, erythema, weight loss, malaise, fever, joint pains, etc. and ultimately death.

The MHC GENE COMPLEX:

The MHC complex contains a number of genes, which control several antigens, most of which influence allograft rejection. These antigens (and their genes) can be divided into three major classes: **class I**, **class II** and **class III**. The <u>class I and class II antigens are expressed on cells and tissues whereas as class III antigens are associated with proteins in serum and other body-fluids (*e.g.*C4, C2, factor B, TNF).</u> **While antigens from class I** and **class II gene products play a critical role in transplantation, those from class III gene products have no direct role in immune responses that determine graft survival**
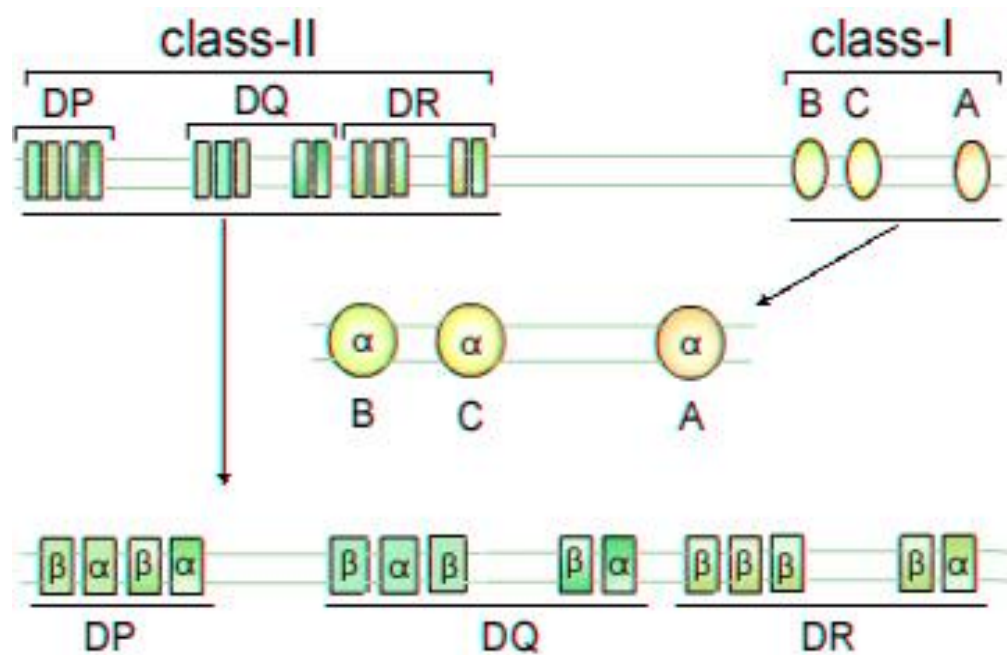

Figure 1. The human MHC gene complex

Human MHC:
The human MHC is located on chromosome 6.

Class I MHC:
The class I gene complex contains <u>three major loci of highest significance</u>, **B**, **C** and **A** and some undefined <u>loci of less significance </u>(Figure 1). Each these loci codes for a polypeptide, α-chain that contains antigenic determinants that are **polymorphic** (has many alleles). Each α-chain associates with a β-2 microglobulin molecule (β-chain), encoded by a gene outside the MHC complex. The α-β-chain complex is expressed on the cell surface as the class-I MHC antigen. Without a functional β-2 microglobulin chain, the class I antigen will not be expressed on the cells surface. Individuals with defective a β-2 microglobulin gene do not express any class I antigen and hence they have a deficiency of cytotoxic T cells.

Class II MHC:

The class II gene complex also contains at least <u>three loci</u>, **DP**, **DQ** and **DR**; <u>each of these loci codes for one α- and a variable number of ß-chain polypeptides which associate together to form the class II antigens</u>. Like the class I antigens, the class II antigens are also polymorphic. The DR locus contains more than one, possibly 4, functional β-chain genes.

MHC Polymorphism: MHC complex is the most polymorphic in the genome. This means that there is an astonishing allelic diversity found within MHC. In humans, the most conspicuously-diverse loci, HLA-A, HLA-B, and HLA-DRB1, have roughly 250, 500, and 300 known alleles respectively. This helps protect the species from extinction that could result from infections and other diseases. However, it is for this very reason, it is extremely difficult to match the donor and the recipient.

Mouse MHC:
The mouse MHC is located on chromosome 17.

Class I MHC:
It consists of <u>two major loci</u>, **K** and **D**. Unlike the human MHC, the mouse class I gene complex loci are not together but they are separated by class II and class III genes (Figure 2).

Class II MHC:
The class II gene complex of mouse contains <u>two loci</u>, **A** and **E** <u>each of which code for one α- and one ß- chain polypeptide, which form one class II molecule</u>. The mouse class II gene complex is also known as the **I-region** and the genes in this complex are referred to as **Ir (immune response) genes** since they determine the magnitude of immune responsiveness of different mouse strains to certain antigens. Products of A and E loci are also termed <u>IA and IE antigens</u>, collectively known as <u>Ia antigens</u>.

MHC ANTIGENS:
Nomenclature:
HLA specificities are identified by a letter for locus and a number (A1, B5, *etc.*), and the haplotypes are identified by individual specificities (e.g., A1, B7, Cw4, DP5, DQ10 DR8). Specificities which are defined by genomic analysis (PCR), are named with a letter for the locus and a four digit number (e.g. A0101, B0701, C0401, etc.)
Specificities of Mouse MHC (H-2) are identified by a number. Since laboratory mice are inbred,
Each strain is homozygous and has a unique haplotype. The MHC haplotype in these strains is designated by a 'small' letter (a, b, d, k, q, s, *etc.*). For example, the MHC haplotype of Balb/c, an inbred strain of mouse, is $H2^d$.
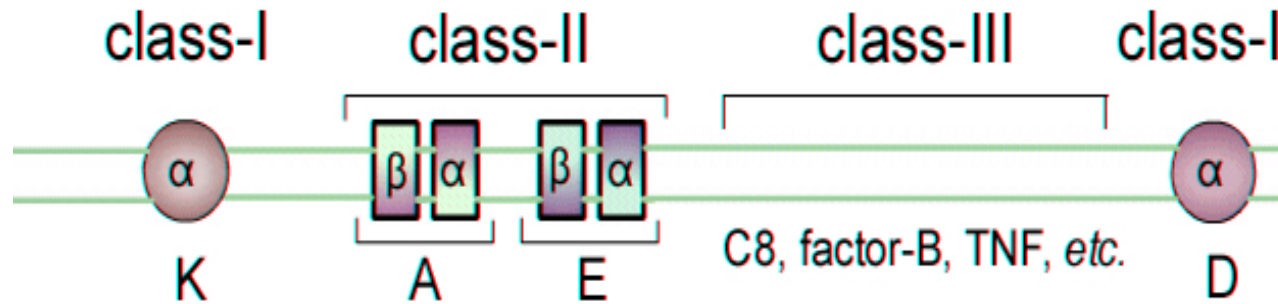
Figure 2. The mouse MHC complex

Inheritance:
MHC genes are inherited as a group (**haplotype**), one from each parent. <u>Thus, a heterozygous human inherits one paternal and one maternal haplotype, each containing three class-I (B, C and A) and three class II (DP, DQ and DR) loci</u>. A heterozygous individual will therefore inherit a maximum of 6 class I specificities (Figure 3: top). Similarly, the individual will also inherit DP and DQ genes and express both parental antigens. Since the class II MHC molecule consists of two chains (α and β), with some antigenic determinants (specificities) on each chain, and DR α- and β-chains can associate in *cis*(both from the same parent) or *trans*(one from each parent) combination, an individual can have additional DR specificities (Figure 3: bottom). Also, there are more than one functional DR β-chain genes (not shown in the figure). Hence, many DR specificities can be found in any one individual.
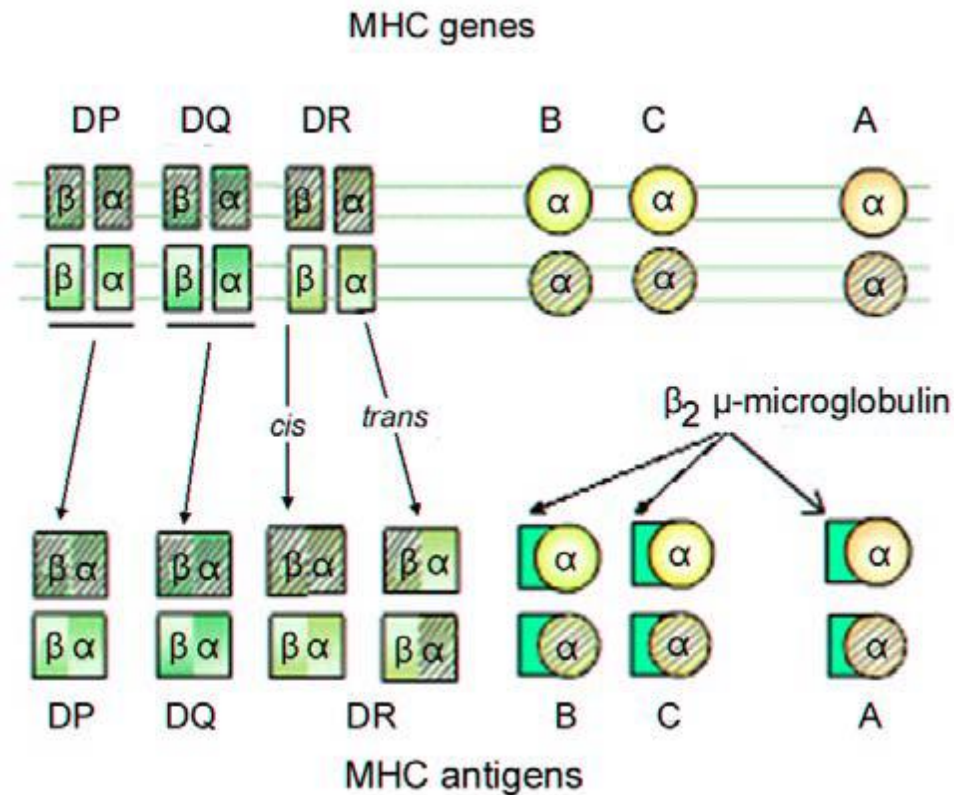
Figure 3. Co-dominant expression of MHC antigens**.**

MHC antigen expression on cells:

MHC antigens are expressed on the cell surface in a **co-dominant** manner: products of both parental genes are found on the same cells. However, not all cells express both class I and class II antigens. While class I antigens are expressed on all nucleated cells and platelets (and red blood cells in the mouse), the expression of class II antigens is more selective. It is expressed on B lymphocytes, a proportion of macrophages and monocytes, skin associated (Langerhans) cells, dendritic cells and occasionally on other cells.

MHC detection by serological test:

The MHC class I antigens are detected by serological assays (Ab and C). Tissue typing sera for the HLA were obtained, in the past, from **multiparous** women who were exposed to the child=s paternal antigens during the parturition and subsequently developed antibodies to these antigens. More recently they are being produced by the monoclonal antibody technology. With most laboratories switching to PCR for tissue typing, the use of serology is rapidly diminishing.

MHC detection by mixed leukocyte reaction (MLR):

It has been observed that lymphocytes from one donor, when cultured with lymphocytes from an unrelated donor, are stimulated to proliferate. It has been established that this proliferation is primarily due to a disparity in the class II MHC (DR) antigens and T cells of one individual interact with allogeneic class-II MHC antigen bearing cells (B cells, dendritic cells, langerhans cells, etc.). This reactivity was termed **mixed leukocyte reaction** (**MLR**) and has been used for studying the degree of histocompatibility. In this test, the test lymphocytes (responder cells)are mixed with irradiated or mitomycin-C treated leukocytes from the recipient, containing B-lymphocytes and monocytes (stimulator cells). The cells are cultured for 4-6 days. The responder T-cells will recognize the foreign class II antigens found on the donor and undergo transformation (DNA synthesis and enlargement: blastogenesis) and proliferation (mitogenesis). The T cells that respond to foreign class II antigens are typically CD4+ TH-1 type cells. These changes are recorded by the addition of radioactive (tritiated, ^{3}H) thymidine into the culture and monitoring its incorporation into DNA.
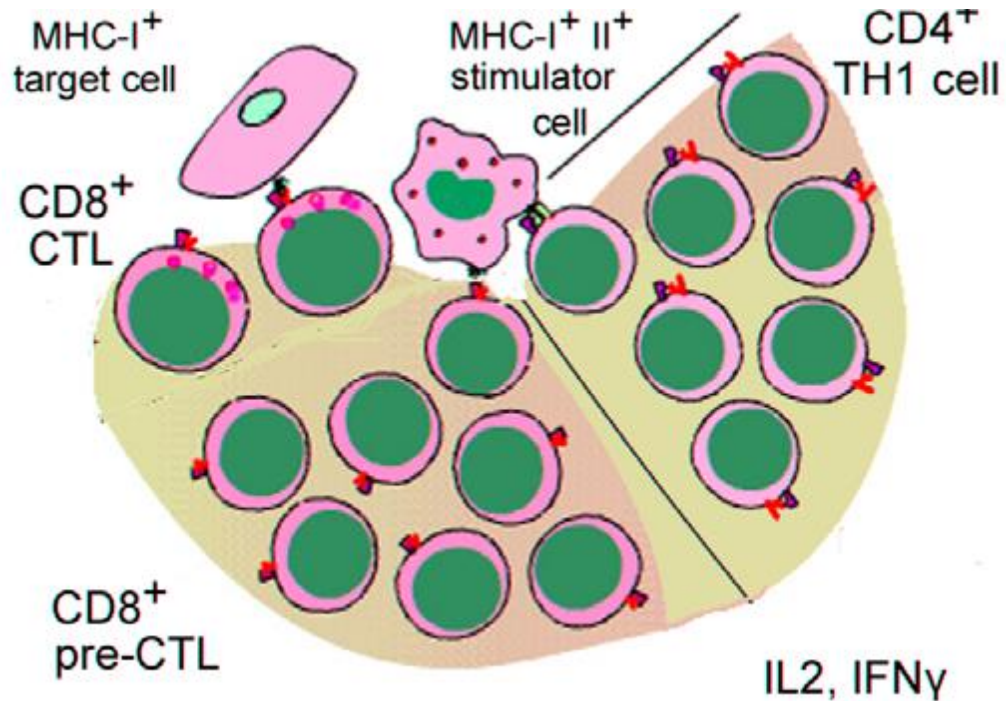
Figure 4. Activation of CTL during MLR

Generation of cytotoxic T lymphocytes

Another consequence of the MHC antigen and T cell interaction is the induction of cytotoxic T-lymphocytes. <u>When T-lymphocytes are cultured in the presence of allogeneic lymphocytes, in addition to undergoing mitosis (MLR), they also become cytotoxic to cells of the type that stimulated MLR</u> (Figure 4). Thus, T-lymphocytes of 'x' haplotype cultured over 5-7 days with B lymphocytes of 'y' haplotype will undergo mitosis and the surviving T-lymphocytes become cytotoxic to cells of the 'y' haplotype. The cytotoxic T-lymphocytes (CTL) primarily recognize class-I antigens and are CD8+.

ALLOGRAFT REJECTION

The clinical significance of the MHC is realized in organ transplantation. Cells and tissues are routinely transplanted as a treatment for a number of diseases. However, reaction of the host against alloantigens of the graft (HVG) results in its rejection and is the major obstacle in organ transplantation. The rejection time of a graft may vary with the antigenic nature of the graft and the immune status of the host and is determined by the immune mechanisms involved (Figure 5 and Table 1).

Hyper-acute rejection:

This occurs in instances when the recipient has preformed high titer antibodies. A graft may show signs of rejection within minutes to hours due to immediate reaction of antibodies and complement.
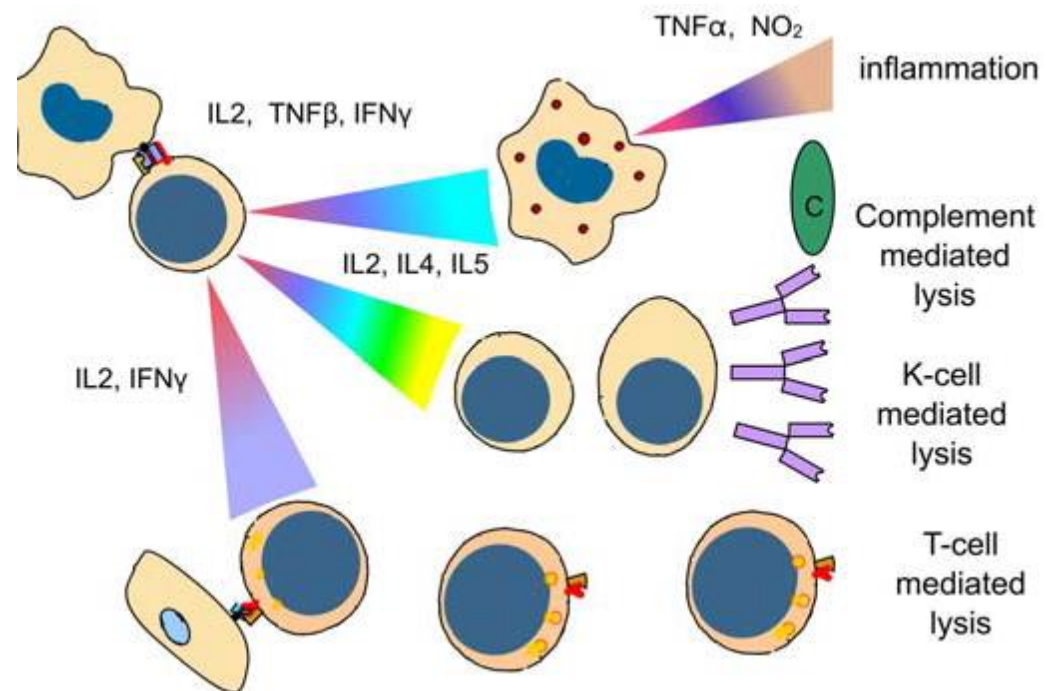

Figure 5. Mechanisms of allograft destruction

Table 1. Different patterns of graft rejection

Type of rejection	Time taken	Cause
Hyper-acute	Minutes-hours	Preformed anti-donor antibodies and complement.
Accelerated	Days	Reactivation of sensitized T cells
Acute	Days - weeks	Primary activation of T cells
Chronic	Months - years	Causes unclear: cross-reactive antibodies, immune complexes, slow cellular reactions, loss of tolerance, recurrence of disease have been suggested.

Accelerated (2nd set; secondary) rejection:

Transplantation of a second graft, which shares a significant number of antigenic determinants with the first one, results in a rapid (2-5 days) rejection. This is due to presence of <u>T-lymphocytes sensitized</u> during the 1st graft rejection. Accelerated rejection is mediated by immediate production of lymphokines, activation of monocytes and macrophages and induction of cytotoxic lymphocytes.

Acute (1st set; primary) rejection:

The normal reaction, which follows the first grafting of a foreign transplant, takes 1-3 weeks. This is known as acute rejection and is mediated by T lymphocytes sensitized to class-I and class-II antigens of the allograft, lymphokines, activated of monocytes and macrophages.

Chronic rejection:

Some grafts may survive for months or even years, but suddenly exhibit symptoms of rejection. This is referred to as chronic rejection, the mechanism of which is not entirely clear. The hypotheses are that this may be due infection, causes which led to failure of the first organ, loss of tolerance induced by the graft, etc.

Fetus as an Allograft: The fetus in an out-bred mammalian species bears antigens derived from both the father and the mother. Thus, truly, the fetus is an allograft and the mother should normally recognize the fetus as foreign and reject the fetus. Nonetheless, such rejections seldom occur. Thus, mammals have adapted in a way that allows implantation of their embryos in the mother's womb and their subsequent survival. There are multiple mechanisms that play a role, of which the most important being the unique structure and function of placenta.

Immunologically privileged sites and tissues: There are certain locations in the body in which allografts are not readily rejected. These include the brain, anterior chamber of the eye, testis, renal tubule, uterus, etc. This stems from the fact that such sites may lack of good lymphatic drainage. Also, such tissues may express molecules such as Fas ligand that kills any immune cell that may come in contact with these tissues. Additionally, such tissues, may have other immune suppressor mechanisms. Similarly, there are some tissues that can be transplanted without matching and without being rejected. Such tissues are called immunologically privileged tissues. Corneal graft is an excellent example that enjoys the highest success rate of any form of organ transplantation. The low incidence of graft rejection is impressive despite the fact that HLA antigen matching of donor and recipient is not normally performed. There are many explanations as to why such grafts are accepted. The avascularity of the graft bed prevents corneal alloantigens from reaching the regional lymphoid tissues. Also, the corneal antigens may be masked. Together, such mechanisms fail to activate the immune system of the recipient.

PROCEDURES TO ENHANCE GRAFT SURVIVAL

In clinical practice, the most successful transplantation programs have been with kidneys and corneas. However, other organs are being transplanted with increasing frequency and success. The success in these programs has been due to a better understanding of immunological mechanisms, definition of MHC antigens and development of more effective immunosuppressive agents.

Donor selection:

Based on extensive experiences with renal transplants certain guidelines can be followed in donor selection and recipient preparation for most organ transplants. The most important in donor selection is the MHC identity with the recipient; an identical twin is the ideal donor. Grafts from HLA-matched sibling have 95-100% chance of success. One haplotype-identical parent or sibling must match at HLA D region. A two-haplotype-distinct donor with reasonable match for D-region antigen can also be used. Organ from two or one DR matched cadaver has been used also with some success. In every case, an ABO-compatibility is essential.

Recipient preparation:

The recipient must be screened for donor-specific anti-HLA antibodies and be negative, must be infection free and must not be hypertensive. One to five transfusions of 100-200 ml whole blood from the donor at 1-2 week intervals improves the graft survival and is practiced when possible.

Immunosuppression:

Immunosuppressive therapy is most essential part of allo-transplantation. The most recent and effective family of agents is cyclosporine, tacrolimus (formerly, FK-506; Prograft®) and rapamycin (Rapmune®). Cyclosporine and tacrolimus inhibits IL-2 synthesis following Ag-receptor binding whereas rapamycin interferes with signal transduction following IL2-IL2R interaction. Thus, all these three agents block T cell proliferation in response to antigen. Other chemical agents used to prevent graft rejection and their modes of action have been listed in Table 2. Whole body irradiation is used in leukemia patients before bone marrow transplantation. Antisera against T cells (anti-thymocyte globulin: ATG) or their surface antigens (CD3, CD4, CD45on activated T-cells, CD25: IL-2 receptors) are being used also to achieve immunosuppression (Table 2).

Strategies for bone marrow transplantation:

In bone marrow transplantation, the most crucial factor in donor selection is class II MHC compatibility. Once again, an identical twin is the ideal donor. From poorly matched grafts, T lymphocytes can be removed using monoclonal antibodies. The recipient must be immunosuppressed. Malignant cells must be eliminated from the recipient blood (in case of blood-borne malignancies). Methotrexate, cyclosporin and prednisone are often used to control GVH disease.

Table 2. Examples of selected immunosuppressive agents.

agent	possible mode of action	application(s)
corticosteroid, prednisone	anti-inflammatory,	organ transplant,
cyclosporine, tacrolimus	altering T-cell and PMN	hypersensitivity,
rapamycin	traffic	autoimmune diseases
azathioprine, 6-MP	inhibition of IL-2	organ transplant
methotrexate	synthesis	organ transplant
cyclophosphamide, melphalan	blocking of IL2-IL2R	organ transplant,
Antibodies: (T-cells, CD-markers,	signal	autoimmuniy
IL- receptors	purine metabolism	organ transplant,
	folate metabolism	autoimmuniy
	alkylation of DNA,	organ transplant,
	RNA and proteins	autoimmuniy
	Eliminating cells and/or	organ transplant,
	blocking receptor	autoimmuniy
	functions	

Other grafts:

Corneal grafts <u>do not contain </u>D region antigens and consequently survival is frequent. Small grafts are better and their survival is improved by corticosteroid use.

Skin allograft have very <u>poor success </u>rate and immunosuppressive therapy is relatively ineffective. Nevertheless, they are often used to provide a <u>temporary covering </u>to promote healing in severe skin damage. Indeed, there will be no rejection, if the host and donor are perfectly matched (identical twins) or the recipient is tolerant to the donor MHC antigens (bone marrow chimeras).