Graft rejection causes and prevention

Allorecognition

a. Direct allorecognition

In direct allorecognition T cells recognise intact MHC molecules on donor cells. 7 Because there are enough similarities between the recipient and donor MHC molecules, T cell can determine non-self MHC molecule. Studies have shown that T cell receptors are capable of recognizing foreign MHC molecules with some, but not other, bound peptides suggesting that the type of peptide determines whether or not its binding MHC molecule will be recognized. Most rejection episodes are elicited through direct presentation. Clinical studies have shown that allografts are identified more readily, and responded to with more vigour than normal non-self antigens e. g. bacterial antigen. This increased sensitivity is because most host T-cells are able to recognise a single foreign MHC molecule. Each foreign cell can have up to 105 copies of each MHC molecule with each molecule forming a complex with a distinct peptide. A T-cell only needs to recognize one of those residues for it to act on that MHC molecule. Several different classes of T-cells may therefore recognize a single MHC molecule. This way, a single foreign cell can activate many different T-cells. Graft endothelial cells possibly promote direct allorecognition by their role as antigen presenting cells or as targets for T cell mediated cytotoxicity. Because graft endothelial cells have MHC molecules and can stimulate T cells directly. Experiments on graft endothelial cells in vitro have shown that graft endothelial cells are able to stimulate proliferation and production of cytokines by allogeneic T cells.

b. Indirect allorecognition

Here T cells recognise processed alloantigen presented by the hosts' antigen presenting cells (APCs)7. See Fig. 1 below. This is similar to antigen presentation in all cells and has been implicated in acute rejection. Phagocytosis of alloantigens by host APCs produce peptides which are presented by host MHC to CD4 or CD8 T cells. This " cross priming" is similar to antigen presentation in all cells and has been implicated in acute rejection which I will discuss later on. miHA are usually processed and presented indirectly to host T-cells by host APCs and MHC molecules. The indirect pathway contributes to the progression to chronic rejection making the two pathways not necessarily mutually exclusive.

Intact MHC molecules on donor APCs present graft-derived peptides which are recognised by T cells in direct pathway (left). In the indirect pathway, recipient APCs take up graft proteins and present donor-derived processed peptides/ alloantigens to T cells (right).

The immunology of rejection

In graft rejection, the recipient's immune system attacks the allograft as it is recognized as foreign. The immune response to grafts has both lymphocyte and antibody mediated mechanisms although T cells play a major role. The role of T cells in allograft rejection was shown in an experiment in mice that lack a thymus and therefore functional T cells. These mice are in capable of allograft rejection and accept xenografts. CD4 and CD8 T cells collaborate in rejection. Removal of CD8 T cells in mice had no effect on graft survival and rejection took 15 days, the same as in control mice. Removal of CD4 T cells

increased graft survival by another 15 days. When both CD4 and CD8 T cells were removed, allografts survived up to 60 days. 5

Rejection consists of the sensitization and the effector stages. The sensitization stage involves the recognition of alloantigen on cell surface by CD4 and CD8 T cell receptors. Alloantigen is presented in the context of major histocompatibility complex (MHC). 5, 16 A second signal is provided by the co-stimulatory receptor-ligand interaction of the T cell and the antigen presenting cell. The effector stage is the immunologic response to activation of T cells in the sensitization stage. Expression of adhesion molecules, class II MHC, growth factors, chemokines and cytokines e. g. IL-2 is upregulated, increasing antigen presentation to T cell. 5 Endothelial cells activated by cytokines express class II MHC, adhesion molecules and co-stimulatory molecules. These can present antigen, recruit more T cells and amplify the rejection process. CD4+ T cells initiate macrophage-mediated delayed type hypersensitivity (DTH) responses and help antibody production in B cells. Growth factors e. g. endothelin cause intimal thickening, interstitial fibrosis and smooth muscle proliferation. CD8+ Tcells mediate cell-mediated cytotoxicity by inducing apoptosis and causing tissue necrosis. 16 The graft is thus rejected.

The ability of the immune system to distinguish self from non-self depends largely on the MHC, which functions to present antigenic peptides to T cells. 5, 7 MHC is different from person to person. There are five different polymorphic genes that code for MHC found on the short arm of chromosome 6 in humans. 16 As a result of their polymorphism, the MHC genes have multiple alleles at each locus. A perfect match between donor and recipient

MHC is therefore extremely rare. Often the donor and the recipient have identical MHC molecules but graft rejection still occurs as a result of difference in the minor histocompatibility peptides that are presented by the MHC molecules to the host’s T cells. Transplanted tissue can also be rejected because of differences in minor histocompatibility antigens (miHA). These occur as a result of polymorphisms in proteins not encoded in the MHC. 16 miHA antigens are recognised when presented in the context of self MHC molecules. Rejection induced by differences in miHA is less vigorous than that induced by MHC mismatches. However miHA induced rejection will still cause graft rejection. 5, 7 T cells, recognizing foreign minor histocompatibility peptides mount an immune response against the graft within a few days. The more the mismatched alleles between donor and recipient, the faster and greater is the rejection response. 1

Rejection mechanisms

Acute rejection normally occurs within days to weeks and up to 100 days following transplantation with the highest risk period being the first three months after transplant. This presents a major problem in short-term transplant survival and is associated with high morbidity and mortality rates. A single episode of acute rejection rarely leads to organ failure as long as it is detected and treated quickly. In acute rejection, T-cells differentiate before rejection begins. They cause cells in the transplanted tissue tolyse, or produce cytokines that cause necrosis of the transplanted tissue. Acute transplant rejection can be treated by using chemotherapeutic drugs designed to suppress the immune system.

Hyperacute rejection is acomplement-mediated response in recipients with pre-existingantibodiesto the donor for example, ABOblood typeantibodies. Hyperacute rejection occurs within minutes of transplant. The transplant never vascularises and must be immediately removed to prevent a severesystemic inflammatory response. Antigen –antibody complexes that form activate complement and cause infiltration of neutrophils into the graft tissue resulting in inflammation. This is a particular risk inkidneytransplants. A prospective cytotoxic crossmatch is performed prior tokidney transplantationto ensure that antibodies to the donor are not present. Pregnant women are exposed to paternal alloantigens of the fetus and may develop antibodies to these antigens with repeated pregnancies. Hyperacute rejection is prevented by transplanting only ABO-compatible grafts.

Recurrent episodes of acute rejection are associated with chronic rejection which occurs anytime after day 100of transplantation. It is irreversible. The best treatment is re-transplantation with a new organ. Acute and chronic rejection mechanisms are concerned with differentimmune cellsubsets, cytokineprofiles, host targets, and respond differently to treatment.

Accelerated rejection is a rare form of graft rejection caused by antibodies that are produced immediately after transplantation.

Graft-versus-host disease (GvHD)

GvHD is a form of rejection seen in some bone marrow transplant patients. Here, the patient, usually a leukaemia patient, receives bone marrow from a genetically non-identical donor. The immune cells from that bone marrow start rejecting the body as they recognise it as foreign. The same reaction

can be seen following blood transfusions. For GvHD to occur, the donor graft should possess mature and immunologically competent cells that will react in the host before they are rejected. If abone marrow transplantcan be performed as well as the allograft, the recipient's immune system can be replaced with the donor's immune system, thus enabling the recipient's body to accept the new organ without risk of rejection. The bone marrow has to be from the same organ donor, an identical twin or a clone. There is a risk of GvHD. Graft-versus-host-disease can largely be avoided by performing a T-cell depleted bone marrow transplant. However these types of transplants come at a cost of diminished graft-versus-tumour effect, greater risk of engraftment failure or cancer relapse,[3]and generalimmunodeficiency, resulting in a patient more susceptible toviral, bacterial, andfungalinfection

Preventing graft rejection

a. Donor and recipient screening

Recipients should be given closely matched organs in order to avoid graft rejection. 7 The donor and the recipient are both screened for ABO-blood group compatibility. Any antibodies to the blood group antigens expressed on the donor's endothelial, epithelial and red blood cells will activate complete and cause graft rejection. MHC typing is done by a microcytotoxicity test which indicates the presence or absence of different MHC alleles in donor and recipient. DNA-based tissue typing allows for more precise HLA matching between donors and transplant patients, which have been proven to reduce the incidence and severity of GVHD and to increase long-term survival. 2.

b. Lymphoid irradiation

This method has also been used to avoid allograft rejection. Lymphoid irradiation eliminates lymphocytes in transplant recipient before grafting. Patients are given a total of 3400 rads at 200rads per day to the thymus, spleen and lymph nodes before surgery to achieve immunosuppression.

c. Immunosuppressive drugs

Transplant patients are given immunosuppressive drugs to minimize graft rejection, this is essential even when donor and recipients are a complete match. 11In their absence, transplanted organs are always rejected. However, even with the use of these drugs, transplants are always eventually rejected. Table 1. below shows some of the common immunosuppressive drugs used and their method of action.

Table1. Immunosuppressive drugs used to prevent graft rejection. 11

Class of drug	Example	Mechanism of action
Inhibitors of DNA synthesis	Azathioprine	Stops T cells from proliferating in response to alloantigen. A mitotic inhibitor that targets cells in the

		S-phase of the cell cycle and block synthesis of inosic acid which is needed for the production of purines, adenylic and guanylic acid. B cell proliferation is also stopped by Azathioprine.
Inhibitors of cytokine production	Cyclosporine	Binds to cyclophilin and blocks calcineurin thereby

		preventing activation of NFAT a transcriptio n factor in the production of cytokines.
Inhibitors of cytokine binding	IL-2 receptor-specific monoclonal antibody	Binds the IL-2 receptor α chain preventing IL-2 from binding to its receptor.
Fungal metabolites-inhibitors of cytokine receptor signal transduction	Rapamycin, Cyclosporine A	Rapamycin blocks the mammalian target of rapamycin (mTOR)

pathway.

Cyclosporin e A blocks activation of resting T cells by inhibiting the transcriptio n of genes encoding IL-2 receptor α

Blocking co-stimulatory signals for naive T cell activation by using monoclonal antibodies prolongs allograft survival. 9, 20 The most crucial T cell co-stimulatory receptors are CD28 (which has ligands on APCs) and CD154. 15 Specific antibodies to CD154 will block the CD154-CD40 pathway. 9 Fig2. below shows how co-stimulatory blockade can be achieved.

Immunosuppressive drugs do not solve the problems of drug toxicity and immune deficiency which they invariably cause. 20A disadvantage of mitotic inhibitors is that they are not specific to cells involved in graft rejection so they target all other cells undergoing division which may have serious effects. Corticosteroidssuppress the T-cell mediated immune onslaught on the host tissues. In high doses this immune-suppression raises the risk of

infections and cancer relapse. 11 CSA causes acute nephrotoxicity in kidney transplants. The biggest challenge in transplant medicine is to come up with a strategy that will see the termination of immunosuppressive therapy as this has many undesired side-effects. This can be achieved by inducing graft tolerance.

d. Transplantation Tolerance

This is " a state in which a donor organ is indefinitely accepted without chronic immunosuppressive therapy while the rest of the immune system is left intact." 11, 20Tolerance can be induced via chimerism. This is based on Medawar's studies in the 1950s which showed that exposing eight-week old pups to allogeneic skin grafts was tolerated and no rejection occurred. This is because at eight weeks, the immune system is still learning to how to recognise self from non-self. This tolerance was not possible in adult mice as their potent immune cells quickly recognised foreign cells and rejected the graft. This experiment had less success in humans. Most transplant patients are aged 50-64; chimerism is therefore very difficult to induce in them. Transplants have a higher chance of survival when donor and recipient immune cells can co-exist: mixed chimerism. A portion of host marrow is replaced by donor marrow thereby supplying precursor T cells to the thymus for the rest of the patients' life. Host T cells that would attack the graft and donor T cells that would attack the recipient, are removed by specific monoclonal antibodies, avoiding GvHD. 20 there is a concern however that following induction of mixed chimerism, reconstitution of T cells is poor.

Sachs et al. achieved mixed chimerism in 6 patients who had multiple myeloma and renal failure as a result. They were each given bone marrow

and a kidney from a sibling. 4 patients achieved mixed chimerism and were put off immunosuppressive drugs. 2 patients developed full chimerism but this caused GvHD. 3 patients; 2 mixed chimeras and 1 full, were treated of the myeloma and renal failure. The results suggest a novel way achieving transplant tolerance without the need of immunosuppressive drugs. However, there is only a very small number of cancer patients who can get a closely matched kidney as those in this experiment. More studies will have to be done with cancer patients that will not be able to get a closely matched organ and also in patients without cancer where marrow may or may not be replaced or partly replaced.

Less than 1% of transplant patients achieve microchimerism, a state where less than 1% of the cells in their blood are donor cells. These patients are able to come off immunosuppressive drugs after some time. Microchimeric cells migrate from the graft into the lymph nodes and other organs e. g. the heart where they sustain a weakened anti-donor immune response preventing graft rejection. Chimerism is therefore necessary for graft tolerance. Starzl et al. have shown that microchimerism can be developed by killing some of the recipient’s immune cells and replacing them with donors’ immune cells before organ transplantation. His results on this study are yet to be published but show that some patients are able to take reduced doses of immunosuppressive drugs and may stop taking them after a few months.

T regulatory cells (Tregs) have been shown to assist in graft tolerance. This has been shown in studies using rapamycin which helps Tregs to tamp down T cell response to graft.

Dendritic cells induce tolerance due to their immunostimulatory capacity. They present exogenous antigens on MHC molecules to CD8 T cells. These antigens are rejected. When dendritic cells were inhibited in mice, graft acceptance was improved. Pre-treatment of recipient with donor dendritic cells has been shown to prolong heart and pancreas transplants in mice.

Recent studies have suggested that direct recognition might inhibit graft rejection after the early stages of immune respone. 7 This was seen in studies where skin grafts from donors who lacked expression of some MHC antigens were rapidly rejected but in some cases graft survival was longer when compared to grafts from normal MHC-disparate grafts. 7 More studies on this are needed but if this case were true, methods of upregulating direct allorecognition may be implemented to promote graft survival.

Although transplant tolerance may be achieved at some point in the future and transplant patients abandon immunosuppressive drugs, there is still one major problem: the availability of compatible organs.

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