Transplant immunology II: overcoming acute and chronic rejection

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The major strategies in overcoming acute and chronic rejection are to minimize immune activation, to reduce the number of T-cells in the recipient, to prevent activation and proliferation of T-cells, and to suppress inflammation. The ultimate achievement is long-term acceptance of the graft without the need for continuous immunosuppression i.e. immunological tolerance of the graft.

This contribution should be read with Rose and Hutchinson and King and Wills (see CROSS REFERENCES).

Human leukocyte antigen (HLA) matching

Immune activation of the recipient can be minimized by matching the major histocompatibility (MHC) antigens of the donor with those of the recipient. Of the MHC class-I and class-II loci, it is most important to match for HLA-A, HLA-B and HLA-DR antigens. An individual is diploid, so two HLA-A antigens, two HLA-B antigens and two HLA-DR antigens must be matched. Hence, a full match is a 'six-antigen match' according to standard tissue typing practices. This is the optimal situation, when a donor and recipient share all six antigens; a 'zero match' or 'six-antigen mismatch' is the worst situation. The statistics of graft survival according to HLA mismatch show that graft outcome gets worse as the degree of mismatch increases. It is most important to match for the HLA-DR antigens, with acute rejection being minimal in recipients of fully HLA-DR-matched kidneys and hearts.[1]

Immunosuppressive agents

Corticosteroids were the first immunosuppressive agents to be used clinically and have two major uses: low maintenance doses given over a long period after transplantation or large bolus doses given as a very short course. Low-dose corticosteroids:

- suppress the activation of macrophages
- reduce the expression of adhesion molecules on endothelium
- suppress the expression of MHC antigens on the graft
- interfere with the antigen-presenting function of dendritic cells
- inhibit the proliferation of lymphocytes by interrupting the intracellular signalling pathways necessary for cell activation.

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High-dose bolus corticosteroids are used to treat rejection episodes because activated lymphocytes are susceptible to corticosteroid-induced apoptosis. Hence, bolus corticosteroids are used to eliminate large numbers of activated B- and T-cells from patients experiencing rejection episodes.

Antiproliferative agents: corticosteroids alone do not completely prevent activation of T-cells, partly because they are too toxic to be given at sufficient doses. The next sort of immunosuppressive agent introduced was antiproliferative, acting to prevent the vast expansion in the numbers of activated lymphocytes. Azathioprine is an antiproliferative agent and, for many years, the standard immunosuppressive protocol was dual immunosuppression with azathioprine and corticosteroids.

Another approach is to inhibit biochemical pathways upon which lymphocytes rely for their proliferation and activation. Mycophenolate inhibits an enzyme, inosine monophosphate dehydrogenase, involved in the *de novo* pathway of DNA synthesis.[2] Proliferating lymphocytes are almost uniquely dependent on the *de novo* pathway of synthesis of deoxyribonucleic acid (DNA), so mycophenolate has a remarkably lymphocyte-specific effect. It inhibits the expansion in numbers of T-cells and B-cells, thereby preventing antibody production as well as the cellular mechanisms of graft rejection. Another advantage of mycophenolate over azathioprine is that it is a non-competitive inhibitor of DNA synthesis (it inhibits a cofactor necessary for the enzymatic activity of inosine monophosphate dehydrogenase), unlike azathioprine which is incorporated as a purine analogue into the DNA of dividing cells, causing DNA damage and therefore having mutagenic activity.

Calcineurin inhibitors: in the 1980s, an agent that inhibited the synthesis of interleukin-2 (and other cytokines such as tumour necrosis factor-α and interferon-α) was discovered: cyclosporine. Once the mode of action of cyclosporine was known, another agent (FK506, tacrolimus) with the same properties (i.e. inhibition of production of interleukin-2) was identified. Cyclosporine and tacrolimus inhibit an enzyme called calcineurin, a phosphatase important in activating the gene for interleukin-2.[3]

The clinical introduction of cyclosporine revolutionized transplantation, dramatically improving results and widening the applicability of transplantation to previously untransplantable patients, including children. For 15 years, the 'gold standard' for immunosuppression was triple therapy using corticosteroids, cyclosporine and azathioprine, but the calcineurin inhibitors have toxic side effects, in particular nephrotoxicity.

Anti-lymphocyte sera: antibodies raised against human thymocytes have been used widely to decrease circulating T-cells. These have been used particularly in cardiothoracic transplantation, as pretreatment of transplant patients (induction therapy) or to treat rejection episodes; these consist of antilymphocyte serum, antilymphocyte globulin and antithymocyte globulin; these antibodies are raised in rabbits, goats or horses and are polyclonal. The advent of the monoclonal antibodies led to attempts to make monoclonal antilymphocyte preparations. The virtue of monoclonal antibodies is that endless supplies of a reliably active product can be made and the precise antigen specificity is known. With polyclonal antibodies, it is not known which antibodies in a crude antilymphocyte serum are active; many of the antibodies

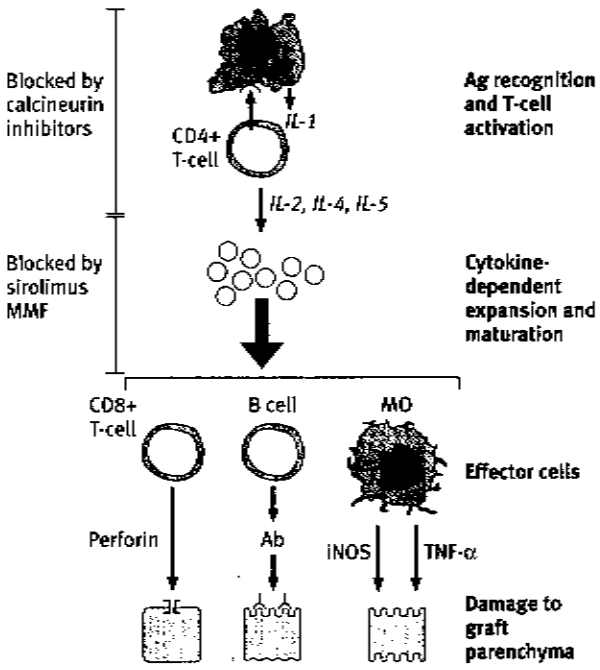
are not directed to T-cells. Those antibodies directed at molecules associated with the T-cell receptor (particularly the CD3 molecules) are immunosuppressive and a monoclonal agent, OKT3 (specific for CD3) achieved widespread use. In some respects it is too effective, causing over-immunosuppression in some patients, leading to viral infections and, in some cases, to post-transplant lymphoproliferative disorders.

Target of rapamycin (TOR) inhibitors: the most recent drug to be approved for clinical use is rapamycin.[4] This agent inhibits the activating signals into lymphocytes delivered by the interleukin-2 receptor. Its action is still being elucidated, but it binds to a cytosolic receptor and interferes with progression of lymphocytes through the cell cycle in response to interleukin-2. It is an anti-proliferative agent that interferes with the interleukin-2 pathway of lymphocyte activation. Immunosuppressive drugs act on different pathways of the immune activation cascade (Figure 1).

Induction of graft acceptance and tolerance

Immunosuppressive agents have potential side effects, and it would be better to care for patients without them. In animals (particularly rodents), it is quite easy to induce long-term acceptance of the graft by manipulation before or after transplantation. (Note that long-term graft survival in this context means 100 days, hardly an acceptable clinical definition.) There are some human transplant recipients who stop taking their immunosuppression agents for whatever reason, or who are on very low maintenance doses of drugs. Given that graft acceptance and, perhaps, immunological tolerance of the graft is possible, the mechanisms thought to underlie this acceptance have been studied extensively.

Effect of immunosuppressive drugs on pathways of immune activation



APC: Antigen-presenting cell; Ag: Antigen; MMF: Mycophenolate; IL: Interleukin; Ab: Antibody; iNOS: Inducible nitric oxide synthase; MO: Macrophage; TNF: Tumour necrosis factor.

Early activation of T-cells results in production of IL-2, which is inhibited by the calcineurin inhibitors. Calcineurin inhibitors have no effect on cytokine-dependent proliferation and maturation of T-cells, but this stage is blocked by rapamycin and MMF. None of the existing drugs inhibit damage mediated directly by effector cells.

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Manipulation of the graft

The first and so far unsuccessful approach to manipulate the graft is to deplete it of passenger leukocytes by perfusion of antibodies or by a storage technique that does not favour leukocyte survival. Instead of antibodies, the graft might be perfused with cytokines to change the characteristics of the dendritic cells into those necessary for the induction of tolerance.

Grafts from donors that have been stressed (and have increased expression of heat shock proteins in the tissues) appear to survive relatively well compared with unstressed tissues. Xenografts placed into animals depleted of natural antibodies are not rejected when the antibody concentrations return because of 'graft accommodation'. The mechanism of graft protection is unknown, but one theory is that 'protective genes' are activated; this may be related to the stress response.[5] A better understanding of graft accommodation may enable this state to be induced in allografts and xenografts at the time of transplantation.

The graft is accessible, so it can be transfected with various genes that may influence the immune response before transplantation. Attempts have been made to transfer the genes for regulatory cytokines and other molecules (e.g. CTLA4Ig) in the hope that local production of these cytokines will protect the graft.[6]

Manipulation of the recipient

The recipient, rather than the graft, may be manipulated by treatment with antigens, antibodies or drugs. The aim is to induce a state of immunological unresponsiveness against donor tissue. Such unresponsiveness is due to largely active immune responses against donor antigens but, instead of damaging the graft, they modify or suppress the rejection responses. Some authors suggest that clinical transplant recipients do not usually become tolerant of their transplants (an outcome readily achieved in many animal models) because the clinically used immunosuppressive agents interfere with the immune responses of graft acceptance.

Specific unresponsiveness implies that only the T-cells able to recognize the graft are affected, so the mechanisms of graft acceptance could be deletion, anergy or suppression of donor-specific T-cells.

Deletion of self-reactive T-cells occurs during the natural maturation of T- cell precursors in the thymus. Self-reactive T-cells escaping that process are inactivated in the periphery, after they have left the thymus (hence the terms 'thymic' and 'peripheral' tolerance). One approach to tolerance induction is to introduce donor antigen into the thymus to delete the donor-reactive T-cells as they undergo maturation. Direct injection of donor antigen into the thymus induces graft acceptance, particularly if this protocol is combined with a treatment to reduce the number of mature T-cells in the circulation.[7] This is also likely to be the mechanism of neonatal tolerance in rats and mice. Rodents are born before an escape of T-cells from the thymus into the periphery is possible. Injection of donor bone marrow in the neonatal period introduces antigen into the thymus that deletes donor reactive T-cells and skin

grafts are accepted.[8] Tolerance of the skin graft is not induced if the bone marrow injection is delayed until seeding from the thymus.

Acceptance of organ transplants can be induced in adults by antigen pretreatment. An injection (i.v.) of donor cells a minimum of 5–7 days before kidney or heart transplantation induces long-term (100 days) acceptance of the graft. Pretreatment allows time for the active development of unresponsiveness (as discussed above) and graft acceptance can be undermined by drug treatment. The same effect has been observed clinically. Individuals who have had many blood transfusions from random donors (to expose them by chance to the antigens of their organ donor) or fewer transfusions from their prospective donor, are less likely to reject their grafts.[9] This is known as the 'blood transfusion effect', and is paradoxical because one might anticipate that prior exposure to antigen would sensitize rather than suppress. In rodent models, intravenous antigen prolongs graft survival, but subcutaneous antigen accelerates rejection. Thus, the route of immunization, directing antigen to the antigen-presenting cells (macrophages) of the liver and the spleen, is important for the induction of unresponsiveness. By contrast, antigen injected into a subcutaneous site drains to the local lymph node where the major antigen-presenting cell is the dendritic cell which is very good at activating rejection responses. To maximize the uptake of donor antigen by the antigen-presenting cells in the liver, antigen can be introduced by the portal vein (or the dorsal penile vein that drains into the portal system) and this route is particularly efficient in inducing unresponsiveness.

Arranging pretreatment with donor antigens is fraught with difficulties even in renal transplantation, and is impracticable for cardiothoracic transplantation. However, the graft itself can be seen a source of donor antigen capable of inducing graft acceptance. In animal models of organ transplantation, a short course (7–10 days postoperatively) of cyclosporine or tacrolimus induces long-term unresponsiveness. This probably delays the activation of the graft rejection response without inhibiting the mechanisms of unresponsiveness. For example, interleukin-2 is important in the activation of graft-damaging cells. Interleukin-2 deprivation of T-cells during the activation process causes them to undergo apoptosis or to become anergic, not dying but unable to respond to antigen. Antibodies to the interleukin-2 receptor, CD25, and rapamycin, that inhibits signalling through the interleukin-2 receptor, should also work in this manner. The early protocols proposed to induce clinical transplantation tolerance under the auspices of the Immune Tolerance Network were based on giving rapamycin. Giving other drugs may inhibit tolerance induction, although it takes courage initially to abandon some of the mainstays of clinical immunosuppression!

Other approaches have been tried e.g. blocking the interaction between antigen-presenting cells and T-cells during the initial stages of activation. T-cells require antigen and costimulation to become activated. Without costimulation, the T-cells undergo apoptosis or become anergic. Antibodies to CD40 or to CTLA4-Ig that block the interactions between CD40 and CD40L or between B7 (CD80 and 86) and CD29, respectively inhibit graft rejection in rodents and monkeys. Alas, the early clinical trials have run into some unforeseen problems.

One common feature of these treatments is that the frequency of donor-reactive T-cells in the recipient is diminished, and cells capable of suppressing the activation of the rejection responses appear. The latter cells were called 'suppressor T-cells', but are now called 'regulatory T-cells'. Recent evidence suggests that anergic cells have the properties of regulatory cells, so that agents suppressing the interleukin-2 pathway or costimulation favour the induction of unresponsiveness.[10] The regulatory cells probably act on the antigen-presenting cells, bathing them in cytokines (e.g. transforming growth factor), inhibiting their ability to activate rejection, and changing them so that they activate only regulatory cells ('infectious tolerance'). This leads to an accumulation of regulatory cells and increasingly solid acceptance of the graft.

Dendritic cells can be divided into two functional subsets: one of which activates the cells involved in rejection while the other activates the cells involved in graft acceptance. This has led to attempts to manipulate the maturation of dendritic cells. For example, dendritic cells exposed to transforming growth factor-β activate regulatory cells (production of transforming growth factor-β regulatory cells could explain infectious tolerance) and favour the induction of graft acceptance. The transfer of genes into dendritic cells to alter their function is under investigation.

Chronic rejection

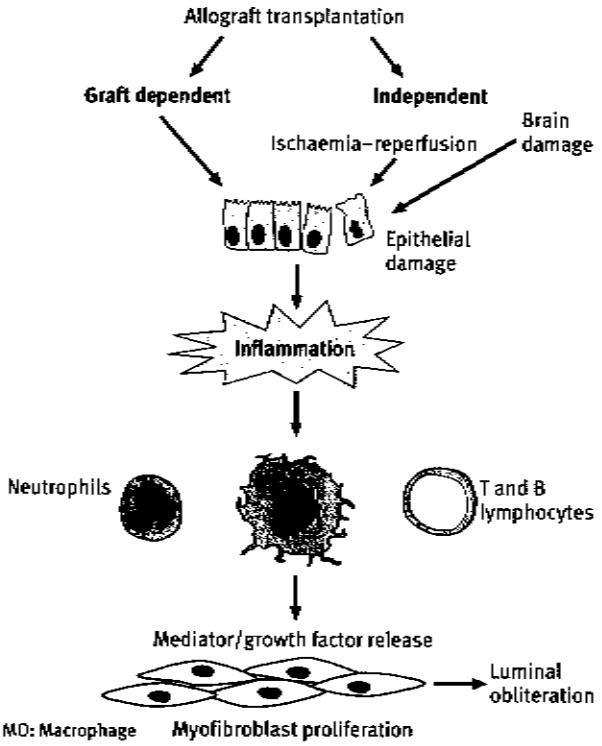
Immunosuppressive drugs prevent or limit parenchymal damage caused by infiltrating mononuclear cells in the first few months after transplantation. As time progresses, grafts become damaged by an insidious process, resulting in obliteration of the main conduits of the graft. In heart transplantation, this presents as a gradual occlusion of the coronary arteries and small blood vessels of graft origin, called 'transplant-associated coronary artery disease' or 'graft vasculopathy'. After lung transplantation, the main disease is occlusion of the small and large airways by proliferating myofibroblasts and deposition of collagen and extracellular matrix; this is known as 'obliterative bronchiolitis' or 'bronchiolitis obliterans syndrome'. Chronic graft rejection is not prevented for most experimental models where acute rejection is prevented by tolerance-inducing regimens.

Such disease progression must be explained in the face of strong immunosuppressive agents that strongly inhibit activation of CD4+ T-cells. Chronic rejection has antigen-dependent and antigen-independent components. The endothelial response to injury hypothesis (proposed by Ross in 1993 to explain non-transplant atherosclerosis) is equally applicable to transplant-associated coronary artery disease and bronchiolitis obliterans syndrome. Ross proposes that an initial insult results in endothelial cell activation and upregulation of cytokines, chemokines and adhesion molecules. This leads to transendothelial migration of monocytes which may be further modified in the vessel wall by modified low-density lipoproteins, where they subsequently release cytokines and growth factors leading to proliferation and migration of smooth muscle cells.

In lung transplantation, Ross *et al* propose that an initial insult leads to damaged epithelial cells, resulting in upregulation of cytokines, chemokines and adhesion molecules. Damaged lungs contain many neutrophils, as well as macrophages and T-cells. The three phases of the disease process (Figure 2) are:

- an antigen-independent phase consisting primarily of damage by neutrophils and free radicals
- an alloimmune phase with lymphocytic infiltration of bronchiolar structures

Stages leading to obliterative bronchiolitis after lung transplantation



This figure illustrates the three stages of damage after lung transplantation i.e. antigen-independent damage to epithelial cells accompanied by neutrophil infiltration; antigen-dependent infiltration of effector lymphocytes; and chronic fibroproliferative phase leading to obliteration of the airways.

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- a chronic fibroproliferative phase leading to partial or total occlusion of the airway lumen.

Alloimmune response in chronic rejection

Role of CD4+ T-cells: CD4+ T-cells have been widely implicated in experimental episodes of parenchymal and vascular rejection. As in acute rejection, they can contribute to chronic rejection in three ways, by:

- provision of signals that promote the generation of CD8+ cytotoxic T-cells
- provision of signals that promote differentiation and activation of alloantibody-producing B-cells
- activating antigen-independent effector leukocytes which damage the tissue. (The best known of these effector leukocytes are activated macrophages which damage tissue through release of mediators such as reactive oxygen intermediates, nitric oxide and degradative enzymes.)

Cytokines released by macrophages (e.g. tumour necrosis factor-α) or T-cells (interferon-γ) can directly damage graft parenchymal cells. Interferon-γ is of particular interest as an effector mechanism of chronic rejection. Injection of human interferon-γ into SCID mice bearing human aortic grafts causes proliferation of aortic smooth muscle cells and vessel remodelling.

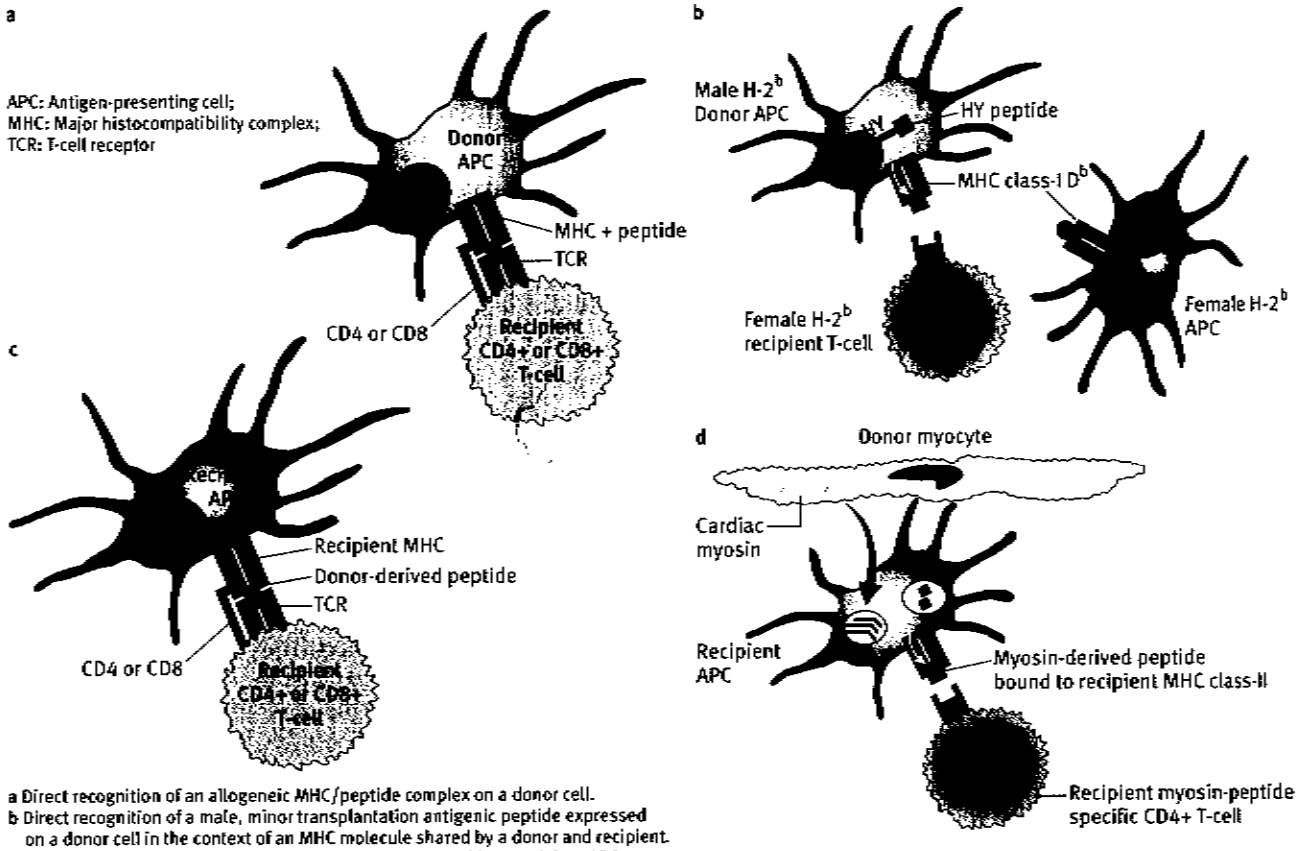
In the non-transplant setting, these host responses serve a protective function by eradicating foreign microbes. If the same effector mechanism is activated by an effector mechanism that is not associated with a pathogenic microbe, the resultant tissue injury is known as 'delayed-type hypersensitivity'.[11] Acute delayed-type hypersensitivity has been invoked as a possible effector mechanism in acute allograft rejection. One can hypothesize that, if the antigen that evokes delayed-type hypersensitivity is not eliminated, the CD4+ T-cells and macrophages remain persistently activated and release cytokines and growth factors that act on mesenchymal cells to promote stromal cell growth and fibrosis. It is likely that chronic delayed-type hypersensitivity is one of the contributing factors of chronic rejection.

Pathways of antigen presentation: an antigen-presenting cell can present antigen and cause activation of resting T-cells. Only specialized cells can cause activation of resting T-cells (e.g. dendritic cells). In the non-transplant setting, T-cells recognize nominal antigen in the context of self-MHC. An important step in the understanding of alloreactivity came with the discovery that T-cells can engage and respond to allogeneic molecules directly. This form of antigen recognition ('direct pathway') is responsible for the strong proliferative response of alloreactive CD4+ T-cells seen *in vitro* (the mixed lymphocyte response) and quite possibly for the vigorous acute rejection seen in certain combinations of experimental strains. T-cells can also recognize peptides that have been processed and presented within self-MHC molecules by host antigen-presenting cells in the same manner that T-cells recognize normal antigen. These peptides may derive from MHC molecules of donor origin, or they may be minor antigens or autoantigens derived from parenchymal tissue (Figure 3).

There is much evidence that chronic rejection is driven by indirect presentation. Hence, as time progresses, donor-derived parenchymal cells shed antigens that are captured by dendritic cells in secondary lymphoid tissues. Studies of kidney and heart transplant recipients show that T-cells from long-term patients are hyporesponsive to donor HLA presented directly, even though the patients may have chronic rejection.[12] In contrast, a study of seven cardiac transplant patients with chronic rejection showed that five of them were hyperresponsive to donor antigens presented via the indirect pathway; none of the four patients without chronic rejection were hyperresponsive to indirectly presented donor antigens. There have been three studies of lung transplant patients to date; these results are similar to those for heart patients i.e. patients with bronchiolitis obliterans syndrome are hyperresponsive for donor antigens presented in the indirect pathway (Figure 3).

Role of CD8+ T-cells: the measurement of T-cell responses described above relates to CD4+ T-cell responses. Activation of CD4+ T-cells leads to maturation of other effector mechanisms of immune damage, including maturation of antigen-specific cytotoxic CD8+ T-cells. If frequencies of CD4+ T-helper cells were reduced during chronic rejection, this would also apply to frequencies of cytotoxic T-cells. This is true after heart and kidney transplantation, where long-term patients become hyporesponsive for direct recognition of targe T-cells by cytotoxic CD8+ lymphocytes.[12] This confirms that patients become tolerant or anergic in the direct pathway, but the indirect pathway of antigen recognition is not inhibited by long-term immunosuppressive agents. No author has investigated whether the direct pathway of antigen recognition is rendered hyporesponsive after lung transplantation; this could be

Recognition of different types of antigens via the direct and indirect pathway



a Direct recognition of an allogeneic MHC/peptide complex on a donor cell.
b Direct recognition of a male, minor transplantation antigenic peptide expressed on a donor cell in the context of an MHC molecule shared by a donor and recipient.
c Indirect recognition of a donor-derived peptide expressed by a recipient APC.
d Indirect processing and presentation of a donor, cardiac myosin-derived peptide by recipient MHC on a recipient APC.

Source: Heeger P S. T-cell allorecognition and transplant rejection: a summary and update. *Am J Transplant* 2003; **3**: 525–33. Reproduced with permission from Blackwell.

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done by measuring the extent of donor-specific CD4+ T-helper responses compared to a third party. Alternatively, cytotoxic CD8+ T-cell responses could be measured. In view of the heightened indirect response in lung transplant patients, one cannot assume that lung recipients become hyporesponsive in the direct pathway, as do recipients of heart and kidneys allografts.

There may be an argument for tissue-specific damage mediated by cytotoxic T-cells after lung transplantation. A study of mucosal epithelia (including gut and lungs) identified a characteristic population of CD8+ T-cells that express the adhesion molecule αEβ7—only 2% of peripheral lymphocytes express this integrin. The integrin is identified by monoclonal antibodies to the CD103 antigen on the αE subunit. The only molecule to which the αEβ7 integrin binds with high affinity is E-cadherin, a molecule constitutively expressed by most epithelial cells. It was shown that CD103+ CD8+ T-cells accumulate within the tubular epithelial layer of renal biopsies showing acute rejection. The authors suggested that transforming growth factor-β (expressed in renal tubules during acute rejection) promotes differentiation of local infiltrating CD8+ T-cells to become CD103-positive.

This raises the possibility of tissue-specific T-cell damage to epithelial cells, which may apply to epithelial cells lining the bronchi. Donor epithelial cells are destroyed early after experimental tracheal transplantation and are replaced by recipient epithelial cells. Researchers suggest that an intact epithelium is crucial to prevent development of bronchiolitis obliterans syndrome. For example, investigators showed that if rat tracheal syngeneic graft is transplanted into the omentum, and the epithelial cells have been removed by protease digestion before grafting, the syngeneic grafts developed luminal occlusion, an effect that was inhibited by reseeding with epithelial cells. The same research group also compared different immunosuppressive drugs on the development of bronchiolitis obliterans syndrome and showed a direct relationship between drugs that preserve the integrity of epithelial cells and those that prevent airway obliteration. This group suggested that an intact epithelium discourages or inhibits mesenchymal proliferation. How epithelial cells exert such an inhibitory effect is not known.

Role of antibody: many clinical and experimental studies have shown an association between chronic production of antibody and development of chronic rejection or transplant vasculopathy after cardiac transplantation. Calcineurin inhibitors (cyclosporine, tacrolimus) inhibit T-cell-dependent antibody responses, but have no effect on a secondary immune response involving production of antibody. After cardiac transplantation, chronic production of antibodies to HLA class-I antigens is associated with transplant vasculopathy. After lung transplantation, a multivariate analysis

of risk factors associated with bronchiolitis obliterans syndrome showed HLA mismatches at the class-I locus (in this case, the HLA-A locus) and antibodies to HLA-A antigens were significantly independent predictors of disease development.

Antibodies to MHC class-I antigens probably contribute to chronic rejection by their ability to cause activation of parenchymal cells within the graft. Monoclonal and anti-HLA class-I antibodies derived from patients cause activation of nuclear factor-kappa beta in human macrovascular and microvascular endothelial cells, resulting in expression of fibroblast growth factor receptors and cell proliferation *in vitro*. Ligation of MHC class-I antigens on smooth muscle cells induces tyrosine phosphorylation, production of fibroblast growth factor and cell proliferation. Of direct relevance to lung transplantation, activation of an airway epithelial cell line with anti-MHC class-I antibodies results in production of fibrogenic factors, allowing proliferation of adjacent fibroblasts. It is therefore likely that chronic production of antibodies exacerbates the fibroproliferative lesions of bronchiolitis obliterans syndrome.

Breakdown of tolerance of self-antigens

Chronic rejection is driven by indirect recognition of HLA antigens released from the graft and processed by recipient dendritic cells in secondary lymphoid tissues. A variety of antigens are involved in this process and the phenomenon of epitope spreading has been described after transplantation, as in autoimmune disease. Antigens are constantly released from the graft, and the antigen (i.e. graft) is never cleared, contributing to the chronic immune response.

Patients make antibodies to non-HLA antibodies after transplantation. The authors have found production of antibodies to the intermediate filament protein vimentin is a significant independent risk factor for the development of graft vasculopathy after cardiac transplantation. Such antibodies react with donor and recipient vimentin, and are therefore autoantibodies. Other autoantibodies after heart transplantation include anti-myosin and anti-phospholipid antibodies. Anti-epithelial and T-cell responses to epithelial cells have been described in renal transplant patients. There are numerous reports describing anti-endothelial cell antibodies after cardiac and renal transplantation.

Such antibodies are probably produced in an organ-specific manner as a response to autoantigens being exposed or released from the damaged graft. Thus, one can predict that anti-cardiac myosin antibodies would not be found after renal or lung transplantation. Few studies have investigated if autoantibodies are made after lung transplantation. Studies by the authors showed that some lung patients had cytotoxic antibodies to an epithelial cell line (A549) before transplantation, and such patients had a significantly worse graft survival at one year. This study suggested an organ- or cell-specific response contributes to rejection, but it did not investigate *de novo* antibody formation after transplantation. *De novo* production of antibodies to non-HLA antigens in 30% of patients with bronchiolitis obliterans syndrome has been described. In some cases, these antibodies, bound to a 60 KDa antigen on epithelial cells, caused cell activation.

Collagen-V has been detected in specimens of bronchoalveolar lavage of lung transplant patients, and a small number of these patients were shown to have a delayed-type hypersensitivity response against collagen-V. The same research group showed that feeding collagen-V to rats (inducing oral tolerance) downregulated the cellular response to lung allografts. Collagen-V is a major component of the extracellular matrix in fibrotic lungs and the results suggest that it is a candidate autoantigen in the pathogenesis of bronchiolitis obliterans syndrome.

Allotransplantation breaks tolerance to self-antigens in experimental models. Thus, after cardiac allotransplantation in mice, *de novo* T- and B-cell responses to cardiac myosin were elicited, but this response was not elicited after syngeneic heart transplantation. The same peptide of cardiac myosin that causes autoimmune myocarditis in mice was found to be the target of the autoimmune response after cardiac transplantation. One wonders if such autoantibody responses are damaging. Pre-immunization of mice with cardiac myosin caused accelerated rejection of allogeneic hearts and also caused rejection of syngeneic hearts, showing the relevance of the autoimmune response to graft rejection. ◆

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