Summary

- Cancers share the characteristics of uncontrolled growth and the ability to invade and metastasize.
- A series of intracellular changes are required.
- Alteration to a particular gene may arise in a variety of ways.
- Mutation to an oncogene causes a gain of function. Only one allele need be affected.
- Mutation of tumour suppressor genes results in a loss of function. Both alleles must be affected.
- A mutation is inherited through germ cells in familial cancers
- Mutations to some of these genes are also found in sporadic cancers.
- The effects of genetic mutations are to increase the potential for growth or to make the cell resistant to inhibitory signals and apoptosis.
- Signals affecting the growth of a cell are received by a membranous receptor, relayed inside the cell, and transmitted through a signalling cascade into the nucleus, where they affect gene transcription. Any of these steps may be affected in carcinogenesis.
- There are many intracellular signalling pathways, with extensive 'crosstalk' between them.

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acid results in increased stability and concentration of p53. These high concentrations lead to apoptosis or arrest of the cell cycle, allowing the cell time to repair damaged deoxyribonucleic acid, thereby protecting it from accumulating mutations. p53 stimulates production of the protein p21, which prevents the cell from progressing from G1-S phase. p21 is not made available and abnormal cell growth can occur if p53 is mutated. p53 mutation is found in 50% of all human cancers. Mutant p53 is more stable than wild type, and therefore accumulates in cells, hence overexpression of p53 is observed in many tumours.

Summary (Figure 11)

Cancers result from an accumulation of errors within the machinery of a cell. Making generalizations about the mechanisms of carcinogenesis is difficult because a vast array of diverse molecular factors combine to produce a malignant phenotype. Even different tumours of the same tissue type will show differing combinations of genetic alterations, and malignant cells are genetically unstable and continue to change their characteristics. The end result is that malignant cells share similar patterns of behaviour i.e. the ability to avoid normal regulatory mechanisms governing the balance between cell division and death. ◆

Transplant immunology I: immunological mechanisms of graft injury

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Solid-organ transplantation has enjoyed great success over the last twenty years. Graft survival after kidney, liver or heart transplantation exceeds 90% at one year and is about 50% at 10 years in most centres in the UK. Better immunosuppressive drugs and more thorough understanding of the nature of the immune response against the graft are the reasons for this success.

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

Basic principles

Graft rejection is mediated by antigen-independent (i.e. innate immune system) and antigen-dependent (i.e. acquired immune response) mechanisms.

The innate immune system (i.e. monocytes, natural killer cells, neutrophils) comprises cells that lack specific antigen-recognizing mechanisms, but which recognize a broad spectrum of antigens consisting of conserved motifs on pathogens. These motifs are termed 'pathogen-associated molecular patterns'. Pathogen recognition by monocytes (unlike lymphocytes, see below) does not require clonal expansion. There is strong evidence that components of the innate immune system are responsible for mediating graft damage at the earliest stages of the transplant procedure (e.g. during brainstem death of the donor, ischaemia reperfusion injury). Thus, grafts are infiltrated with monocytes and neutrophils very early after implantation, resulting in expression of a range of new molecules (including adhesion molecules, proinflammatory cytokines and chemokines) which promote graft infiltration by lymphocytes. Complement (although usually associated with antibody activation) can also become activated in the absence of antibody, and is therefore also part of the innate immune system. Complement activation occurs during ischaemic reperfusion injury and causes tissue damage and amplifies the immune response (e.g. C3, a split product of complement, is highly chemotactic for leukocytes).

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The acquired immune response is the response by antigen-specific lymphocytes to antigen. The cardinal feature of the acquired immune response is its ability to respond rapidly to antigens that have been encountered previously and is achieved through clonal selection and expansion of antigen-specific lymphocytes. Individual lymphocyte clones have specificity for distinct antigenic motifs with the large diversity of the antigen receptors generated through somatic gene rearrangements. The large diversity of the T-cell receptor and B-cell receptor means that lymphocyte clones with specificity for a given antigen are always present. The clonal expansion of antigen-specific lymphocytes takes up to five days, leading to the production of effector cells, most of which undergo programmed cell death (apoptosis) on removal of antigen. The remaining cells form 'memory cells' which persist in the absence of antigen, but which can rapidly respond on re-exposure to the same antigenic stimulus.

Rejection is mediated by lymphocytes (cell-mediated) and antibody. Graft damage may occur at different times after transplantation, that is:

- minutes to hours ('hyperacute' rejection)
- days to weeks ('acute' rejection)
- months to years ('chronic' rejection).[1]

These terms relate to the time of clinical presentation of symptoms of rejection, but they also tend to reflect somewhat different mechanisms regarding contribution made by cells and antibody. Rejection is described in terms of hyperacute, acute and chronic rejection.

Hyperacute rejection

Hyperacute rejection is caused by preformed antibodies to graft antigens in the recipient. The endothelial cells lining the blood vessels of the new organ are the principal targets. Binding of antibodies to endothelial cells may have catastrophic consequences for the graft, causing complement-dependent cell lysis or activation of endothelial cells, leading to intravascular thrombosis and coagulation. The blockage of the blood supply to the graft is dramatic. A newly anastomosed graft turns pink as the blood flow is restored, but then the graft rapidly darkens in colour and begins to swell after a few minutes.

Preformed antibodies in allotransplantation: there are three reasons why patients may have preformed antibodies to donor antigens: blood transfusions, previous transplants or pregnancy. About 20% of transfused patients make antibodies against human leukocyte antigens. Pregnant women (particularly multiparous women) may have very high concentrations of antibodies directed at the paternal antigens of fetal origin. (Histocompatibility laboratories use pregnancy sera as tissue typing reagents.) Antibodies against blood group antigens can cause hyperacute rejection. Until recently, it was a contraindication to transplant across an ABO incompatibility. There are two exceptions to this rule:

- transplantation of ABO-mismatched hearts into neonatal human recipients[2]
- renal transplants from living donors into conditioned recipients (i.e. patients who have been treated to reduce circulating concentrations of anti-blood group antigen antibody before transplantation).

Avoidance of hyperacute rejection of allografts: clinical hyperacute rejection is exceedingly rare because tissue typing laboratories identify and characterize antibodies in potential transplant recipients that could bind to donor antigens. The basic test is known as the 'leukocyte crossmatch test'.

Leukocyte crossmatch test – at the time of harvesting of the donor organs, recipient serum is mixed with donor blood leukocytes in complement. The crossmatch test is positive and the transplant cannot proceed if the recipient serum contains anti-donor antibodies and kills the donor cells. A limitation of the test is that it uses leukocytes rather than endothelial cells, hence rare positive reactions against endothelium may be missed.

The panel reactivity antibody test is a variation of the crossmatch test and is used before transplantation. A panel of e.g. 60–100 different leukocyte samples that express a wide range of antigens is tested with the recipient serum. The serum may kill 40%, 60% or even 100% of the cell samples in the panel. A 100% reactivity virtually excludes the possibility of transplantation, while lesser degrees of reactivity on the panel can be analysed to identify the specificity of antibodies present to improve the chances of finding a crossmatch-negative donor. This search for antibodies may be done using more sensitive methodology, for example:

- a flow cytometer ('flow crossmatch test')
- beads coated with purified major histocompatibility complex (MHC) antigens rather than cells.

Greater sensitivity detects more crossmatch-positive patients and may exclude them from transplantation, so the appropriate level of sensitivity must be determined.

Acute rejection

Of the 5×10^{11} T-cells present in the average human, up to 1 in 1000 can directly recognize a transplanted organ.[3] Furthermore, each cell has the capacity to divide very rapidly, doubling in numbers every 8 to 18 hours, so that each T-cell can (in theory) produce between one thousand and one million daughter cells specific for graft antigens. Therefore:

- large numbers of T-cells can recognize and respond to a transplant
- the immune system has an enormous capacity for expansion.

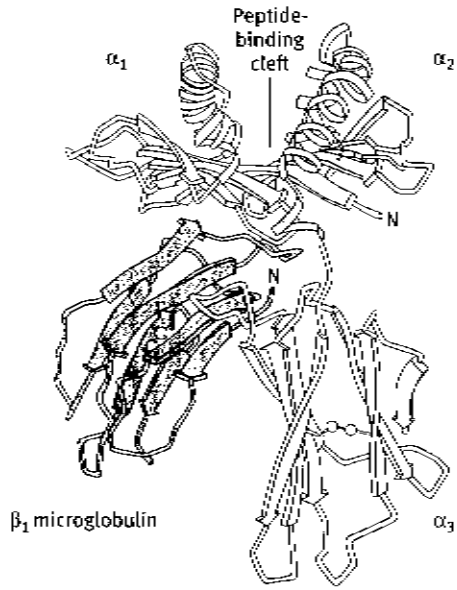
Normal function of MHC-complex molecules

MHC molecules are central to the normal physiology and function of T-cells. The latter have receptors that bind to peptide fragments of foreign antigens held in a binding groove of MHC molecules. T-cell receptors bind strongly to the combined peptide and MHC molecule, but very weakly to peptide or MHC molecule alone.

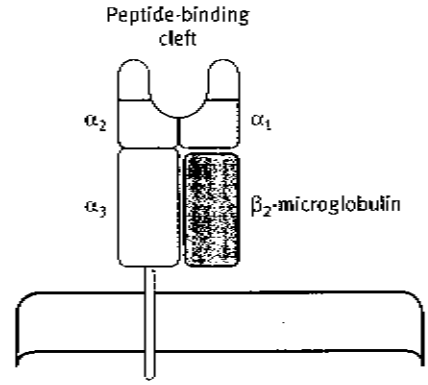
The peptide-binding grooves of MHC molecules on healthy tissues are occupied by peptides derived from the proteins of the cells on which the MHC molecules are expressed (Figure 1).

Recognition of self-peptide antigens presented by MHC molecules is usually avoided because potentially self-reactive T-cells have been deleted in the thymus ('negative selection'). Simultaneously, the thymus positively selects T-cells that bind weakly to MHC molecules so that they can recognize self-MHC-presenting foreign peptide complexes. In a virus infection, the self peptides in self-MHC molecules are replaced by viral peptides. The T-cells 'see' the self-MHC–foreign peptide complexes and respond to destroy the virus-infected cells.

The major histocompatibility complex class-I molecule



a The major histocompatibility complex class-I molecule showing the peptide-binding cleft in a groove formed by the alpha-helix conformation of the α_1 and α_2 domains.



b Shown schematically, the MHC class-I molecule is a heterodimer of a membrane spanning α-chain (43,000 kDa) non-covalently linked to β_2-microglobulin (12,000 kDa).

1

Antigen-presenting cells (APCs)

Recognition of MHC molecules is not sufficient to activate T-cells for graft destruction; the antigen must be presented on the surface of leukocytes or endothelial cells within MHC class-I or MHC class-II molecules. One set of leukocytes in the body is particularly good at activating T-cells: 'professional APCs'.[4]

In a sense, virtually all nucleated cells in the body are APCs because they express MHC class-I antigens and, if they become infected with a virus, are capable of displaying arrays of MHC class-I–virus peptide complexes. Professional APCs acquire foreign antigens, process them (into peptide fragments) and create MHC–peptide complexes that are displayed on the cell surface. In the context of transplantation, cells expressing MHC class-II antigens are the most important APCs because they can cause direct stimulation of resting cluster of differentiation-4 + (CD4 +) T-cells. To do this, they carry other cell-surface molecules called 'costimulation molecules' that interact with corresponding molecules on the surface of the T-cell and are necessary for the activation of T-cells (Figure 2).

The most potent professional APCs are dendritic cells, but macrophages and B-cells also have professional-APC activity.

The most potent immunogenic cell in an organ transplant is the 'passenger leukocyte' (dendritic cells derived from bone marrow that are temporarily resident in every organ).[5] These cells detect antigen exposure in the tissues and activate the protective immune response. Removal of passenger dendritic cells from rat transplants can reduce the immune response so that the organs are not rejected acutely, although they undergo chronic rejection later.[5] Clinical attempts to remove passenger dendritic cells from human organs by perfusion with monoclonal antibodies to leukocytes during the period of storage did not have a measurable outcome on graft rejection.

Allorecognition

There are two ways in which transplanted tissues are recognized by T-cells: direct and indirect allorecognition.[6]

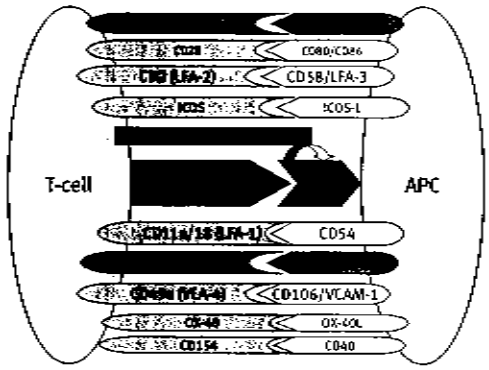
Direct allorecognition is the inherent capacity of the immune system to recognize and respond to transplanted tissues. However, why should there be so many cells in a person capable of recognizing the antigens of someone else?[3]

When a transplant is introduced, unmatched MHC molecules on the graft are recognized by the T-cells of the recipient because of one of two reasons.

- All T-cells are programmed in the thymus to recognize MHC antigens and it is only those that have receptors for self-MHC–self-peptide complexes that are eliminated. Thus, the vast numbers remaining contain T-cells capable of binding with sufficient affinity to the foreign-MHC–foreign-peptide complexes to respond. Also, the MHC antigens are expressed densely on most cells, providing plenty of antigen for avid binding and recognition; this is termed the 'high determinant density' concept of allorecognition.
- The peptide-binding grooves of the unmatched MHC molecules of the donor and the recipient differ and therefore display a different array of normal tissue peptides. To the T-cells of the recipient, such foreign-MHC–foreign-peptide complexes resemble self-MHC molecules presenting, for example, virally-derived foreign peptides; T-cells recognizing virus-infected self cells respond to foreign MHC-antigens. This has been termed the 'cross-reactivity' concept of allorecognition, as well as the 'multiple binary complex' hypothesis (because there are many possible donor-derived peptides, so many T-cells may respond).

These two possibilities to explain the high numbers of alloreactive cells are not mutually exclusive and overlap to a large extent.

Costimulatory molecules are needed for the activation of T-cells



Diagrammatic representation to show interactions between APC (e.g. dendritic cell, B-cell) and T-cells that result in activation of T-cells. Some costimulatory interactions with T-cells result in activation of T-cells (e.g. CD86/CD28, ICOS-L/ICOS), while others result in inhibition of activation of T-cells (e.g. CD86/CTLA-4, PD-L1/PD-1).

APC: Antigen-presenting cell; CTLA: Cytotoxic T-lymphocyte-associated protein; CD: Cluster of differentiation; LFA: Lymphocyte function-antigen; ICOS: Inducible costimulator protein; ICOS-L: Inducible costimulator protein-ligand; TCR: T-cell antigen receptor; MHC: Major histocompatibility complex; AG: Antigen; PD: Programmed death-1; Programmed death-1 ligand; VLA: Very late activation; VCAM: Vascular cell adhesion molecule; OX-40: Tumour necrosis factor receptor superfamily member-4; OX-40L: Tumour necrosis factor receptor superfamily member-4 ligand.

2

Minor histocompatibility antigens are peptides, albeit presented by MHC molecules. As outlined above, normal cells bear, on their surface, MHC molecules complexed with peptides derived from normal cellular proteins. Some normal proteins vary from person to person (allelic variants, e.g. allele-1 and allele-2). Thus, the same MHC molecule on cells from two people having different alleles of a normal protein express MHC–peptide complexes that can be distinguished by T-cells (i.e. MHC plus peptide from allele-1 or MHC plus peptide from allele-2). These minor histocompatibility antigens (MHC molecules with allelic peptides) can contribute to allorecognition so that, even when the donor and recipient are very closely matched for their MHC antigens, minor histocompatibility differences can trigger rejection responses.

Indirect allorecognition: direct allorecognition is relevant in only the non-physiological context of transplantation. By contrast, indirect allorecognition occurs according to the normal physiological pathways of antigen recognition by T-cells. As alluded to previously, peptides from a pathogen (such as virus peptides described above) are taken up and presented in the grooves of MHC molecules to the receptors of T-cells. In this case, the T-cells and the APC presenting the MHC–peptide complex are identical, so the T-cells see self-MHC plus foreign (viral) peptides. Essentially the same process occurs when donor antigens are shed from a graft and are taken up and presented by recipient APC, so the recipient T-cells see self-MHC plus foreign (donor) peptide (Figure 3). Hence, indirect allorecognition is identical to the physiologically normal recognition of foreign antigens by T-cells.

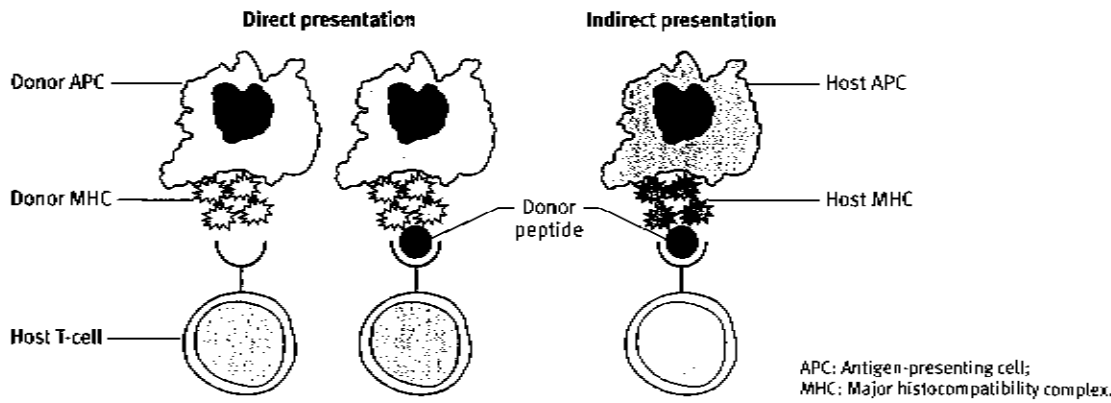
The relative capacity for direct and indirect allorecognition: one of the major differences between direct and indirect allorecognition is the number of T-cells with receptors that can recognize the graft. The frequency of reactive cells is 1 in 1000 to 1 in 10,000 in the case of direct allorecognition. By contrast, the frequency of indirectly alloreactive T-cells is 1 in 100,000 to 1 in 1,000,000.

Effector responses in acute rejection

The response to clinical organ transplants is dependent on T-cells. Rodents depleted or deficient of T-cells are unable to reject allografts. These T-cells recognize the graft as foreign because they have antigen-specific receptors, but the mechanism by which they destroy the graft may be specific (antibodies or cytotoxic T-cells) or non-specific (inflammation or delayed-type hypersensitivity).

Role of CD4 + T-cells: studies in which subpopulations of T-cells have been transferred into T-cell-deficient animals (to determine which are capable of rejecting a transplant) have shown that CD4 + T-cells play a central role in graft destruction (Figure 4).[7]

Pathways of antigen stimulation



Diagrammatic representation of mechanisms whereby recipient T-cells recognize allo-class-II determinants. Recipient T-cells recognize donor MHC determinants on donor APC (direct presentation) or they recognize donor MHC peptides which have been released from donor cells and processed and presented by host APC within self-MHC molecules (indirect presentation).

3

CD4 + T-cells may cause graft damage in four ways.

Cytotoxic – CD4 + T-cells can act as cytotoxic (killer) T-cells. However, they are unlikely to cause extensive parenchymal damage because they directly recognize MHC class-II antigens that are present on activated endothelium or epithelium (but virtually nowhere else in transplanted organs). Such cells may be responsible for endothelialitis.

Helper – CD4 + T-cells may act as 'helper' T-cells in the activation of B-cells and their differentiation into antibody-producing plasma cells. They can act in this manner only if they have been activated by the indirect allorecognition pathway because they must interact with MHC class-II molecules on recipient B-cells. Help for activation of B-cells is provided by cytokines released by the T-helper cells. CD4 + T-helper cells are divided into two subsets according to the cytokines they produce. It is the T-helper-2 subset of cells that make the cytokines necessary to promote antibody production; these cytokines include interleukin-4, -5 and -6.

Interferon – the CD4 + T-helper cells may be of the T-helper-1 subset that makes interferon-γ and interleukin-2. Such cells may be activated by the direct or the indirect pathways of allorecognition. Interferon-γ has many effects in the transplantation. It increases the expression of adhesion molecules of the graft endothelium, facilitating the adhesion of circulating leukocytes to the walls of blood vessels and their transmigration into the graft. Graft infiltration is one of the hallmarks of acute rejection. Interferon-γ activates macrophages and induces them to release enzymes, free radicals and other noxious agents (intended to clear infection) as well as inflammatory cytokines (e.g. interleukin-1, tumour necrosis factor-α). This pathway, graft-specific T-cells activating macrophages (in a manner analogous to type IV or delayed-type hypersensitivity) seems to be very important in graft damage. In particular, recipients of kidney, heart or liver transplants who have been shown to be producers of high concentrations of tumour necrosis factor-α *in vitro* or who have a tumour necrosis factor-α genotype associated with higher production of tumour necrosis factor-α are more likely to lose their graft to acute rejection. Interferon-γ also increases the expression of MHC class-II molecules on the graft parenchyma and endothelial cells, and increases the ability of APCs to activate T-cells by increasing their ability to process and present antigen and to express costimulatory molecules.

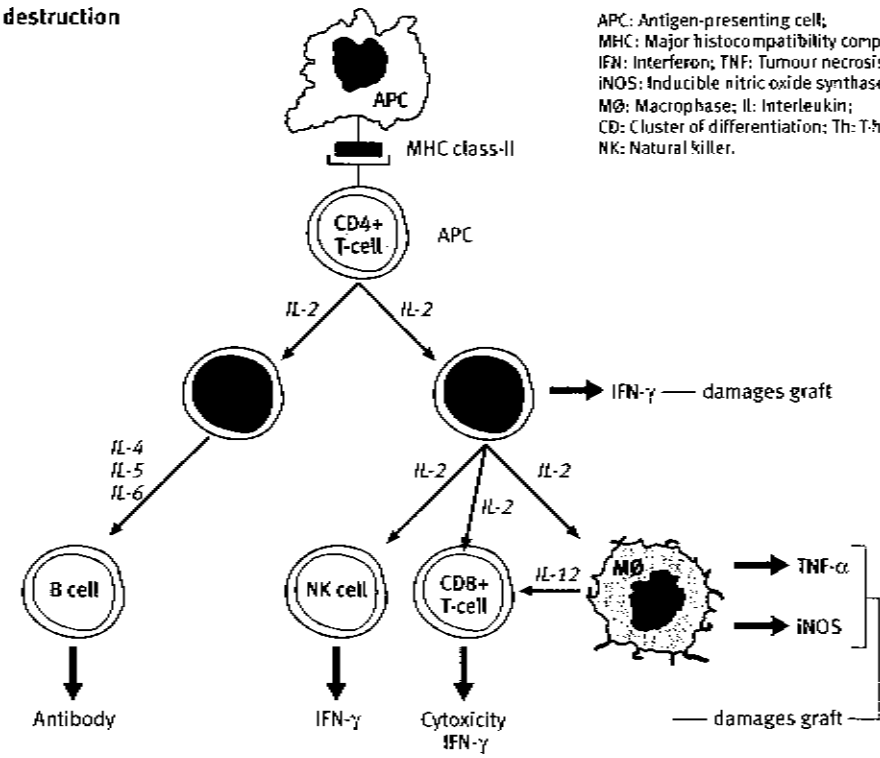
Interleukin-2 has a major influence on graft rejection. It is a growth factor for T- and B-cells, dramatically increasing their proliferation and survival. Graft rejection can be suppressed by blocking:

- production of interleukin-2 with the calcineurin inhibitors cyclosporine or tacrolimus
- uptake of interleukin-2 by interleukin-2 receptors using monoclonal antibodies to CD25 (one of the chains of the interleukin-2 receptor)
- intracellular signalling through the interleukin-2 receptor with rapamycin.

One of the cell types dependent on interleukin-2 for activation is the cluster of differentiation-8 + (CD8 +) cytotoxic T-lymphocyte.

The role of CD8 + T-cells in graft rejection is controversial.[7] Cytotoxic T-lymphocytes must be activated by the direct pathway of allorecognition to damage the graft. Highly activated cytotoxic T-lymphocytes can be recovered from grafts undergoing rejection, and activated cytotoxic T-lymphocytes injected into tissues cause cell death and necrosis at the injection site.[8] On this basis (i.e. cytotoxic T-lymphocytes in rejection and their potential to damage tissues), cytotoxic T-lymphocytes probably play a role in acute

Central role of CD4+ T-cells in graft destruction



APC: Antigen-presenting cell; MHC: Major histocompatibility complex; IFN: Interferon; TNF: Tumour necrosis factor; iNOS: Inducible nitric oxide synthase; MØ: Macrophase; IL: Interleukin; CD: Cluster of differentiation; Th: T-helper; NK: Natural killer.

Activation of CD4+ T-cells results in production of Th1 or Th2 cells, production of their characteristic cytokine profiles and maturation of effector mechanisms.

graft rejection. However, rejection can occur in the absence of histological evidence of graft cell lysis. When naïve CD8+ T-cells are transferred into a transplant recipient with no other T-cells, they do not become activated and bring about graft rejection.[9] This merely suggests that they are not able to grow and differentiate in the absence of T-helper-1 cells making interleukin-2. The intravenous transfer of preactivated CD8+ T-cells into graft recipients does not cause graft damage either. This may be because they do not 'home' into the graft or they die very quickly. The latter is supported by an experiment in which activated CD8+ T-cells were transferred into recipients given regular doses of interleukin-2 to maintain the viability of the CD8+ cytotoxic T-lymphocytes, a protocol that led to the rejection of the organ graft. Thus, it appears that CD8+ cytotoxic T-lymphocytes, recognizing the MHC class-I molecules expressed on most nucleated cells can, and probably do, contribute to graft damage.

Cytotoxic T-lymphocytes cause damage to target cells by inducing lytic or apoptotic cell death. Both types of killing are thought to play a role in graft rejection and the apoptotic pathway may explain how cytotoxic T-lymphocytes can damage grafts without histological evidence of lysis of tissue cells.

Lytic cell death is caused by the directed release of two molecules onto the target cell surface:

- perforin (which permeates the target cell membrane)
- granzyme (which enters the cell and initiates the lytic programme).

The expression of perforin and granzyme in transplant biopsies and in peripheral blood cells is being studied as a molecular marker of acute rejection. Lytic cell death is a pathological process and is not part of the turnover of cells in normal tissues.

Apoptotic cell death is a process in which the nucleus fragments (chromatin) are degraded and the cell is broken up into small pieces that are rapidly phagocytosed by macrophages and destroyed without the release of any of the target cell contents. This is a physiologically normal process that regulates the overall size of cell populations, and is important in the remodelling of tissues (e.g. embryogenesis). The cytotoxic T-cell induces apoptosis in target cells by interaction with the target cell using Fas–Fas ligand interaction.

Role of antibody: in experimental models, acute rejection is usually accompanied by production of alloantibody, but this antibody may not be damaging to the graft. Recent studies using mice that lack antibodies but have an otherwise intact immune system show that T-cells are the main effectors of acute graft rejection. However, when T-cells are compromised, antibodies become important as effectors of graft rejection.[10] Consistent with a role for antibodies in acute rejection, experiments have shown that treatment to suppress rejection reduces the binding of antibodies in the graft. Also, evidence suggests that some types of T-helper cell bring about graft rejection in adoptive transfer models by helping B-cells to make antibodies.

Until recently, post-transplant monitoring of antibody production in human recipients was done in few centres, and it is likely that antibody-mediated rejection was underestimated.[11] This is because of the difficulty in diagnosing antibody-mediated acute rejection. Antibody-mediated rejection was a diagnosis made after the exclusion of other possibilities. For example, a heart graft may begin to fail in the absence of histological evidence of cell-mediated acute rejection. In such cases, plasmaphoresis and additional immunosuppression with cyclophosphamide or methotrexate have been attempted, not always with success. This situation changed with the discovery of C4d (a split component of complement) as an immunohistological feature of humoral or antibody-mediated rejection. About 10% of protocol biopsies removed from cardiac and renal transplant recipients contain capillary deposition of C4d. Diagnostic criteria of humoral rejection after heart and renal transplantation now include C4d deposition within the graft, along with serological evidence of anti-HLA antibodies and clinical deterioration. Using these criteria, more centres are diagnosing humoral rejection and treating patients with plasmapharesis, immunoglobulin (i.v.) and antibodies to B-cells.

Interpretation of antibody responses after transplantation are not always straightforward. Some studies suggest that antibodies can protect allografts (organs and tumours) from acute rejection. For example, the passive transfer of anti-donor antibodies into rats given organ grafts protects them from rejection ('passive immunological enhancement') and caused damage only when rabbit complement was also injected. Similarly, *in vitro* experiments show that very low doses of antibody protect cells from cytokine-mediated upregulation of adhesion molecules. Thus, very low levels of circulating anti-graft antibodies are not necessarily detrimental and they may even be beneficial to the graft. ◆

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