1 Immunology of transplantation

Qiquan Sun, Jason M Zimmerer, and Gregg A Hadley

The Ohio State University Medical Center, Columbus, Ohio, USA

The transplantation of internal organs has been feasible since the turn of the nineteenth/twentieth centuries when surgical techniques for construction of vascular anastomoses were first developed. However, the early results of organ transplantation were abysmal. In 1908, Alexis Carrel reported the results of kidney transplantation in a series of nine cats. Despite normal early graft function, all grafts ultimately failed after 1 month. The first human kidney transplantations were performed in the 1950s with similarly disappointing results. There is no technical reason why organ transplants from other individuals should not be compatible with the host's own tissues; indeed, organ transplants exchanged between genetically identical individuals are uniformly successful. Rather, it is now recognized that rejection of a graft is a manifestation of a complex immune mechanism that serves to recognize and eliminate foreign (non-self) antigens, and which evolved to protect the host against pathogens. With the availability of immunosuppressive drugs that efficiently blunt the recipient's immune system, clinical organ transplantation is now routine, and indeed is the preferred treatment for end-stage failure of the kidneys, heart, liver, and lungs. In the USA, over 25 000 such transplantations are now performed each year. However, currently available immunosuppressive drugs do not completely prevent immune injury to a graft. For all organ types,

All authors contributed equally to the preparation of this chapter.

graft loss rates approach 50% after 10 years. The purpose of this chapter is to detail the immunologic basis of this important clinical problem.

Terminology

Here is a list of key terms used to communicate ideas in the field of transplantation immunology:

• *Allografts:* grafts transplanted between individuals of the same species

• *Isografts:* grafts transplanted between genetically identical (*syngeneic*) individuals

• *Autografts:* grafts transplanted in the same individual, i.e., the donor is the recipient

• *Xenografts:* grafts transplanted between individuals of different species

• *Graft rejection:* immunologic destruction of transplanted tissues

• *Histocompatibility (H) antigens:* antigens that evoke graft rejection; a graft is histocompatible if all of its H antigens are expressed by the recipient (i.e., none is foreign), histoincompatible if they are not

• *First-set graft:* a first graft from a given individual or inbred strain

• *Second set graft:* a second graft of the same type from the donor of the first, or from a donor genetically identical to the first; the second set is also used to describe the accelerated rejection of a graft by a specifically sensitized recipient

• *Orthotopic:* graft placed at the normal anatomical site

• *Heterotopic:* graft placed at site distinct from the normal anatomical site.

Immunologic nature of allograft rejection

The immune system is composed of two components referred to as innate and adoptive immunity. The innate system involves cells such as macrophages and natural killer (NK) cells that constitutively express a limited set of receptors recognizing common elements of a broad range of pathogens. The innate system is capable of a rapid response. The adaptive system involves T and B cells that express a very broad range of receptors, each cell's receptor having very a narrow specificity. As the frequency of cells expressing any one receptor is low, the cells recognizing a particular antigen must replicate before they can mount an effective response. Once this expansion has occurred, however, the adaptive system is capable of a rapid memory response if the antigen is encountered a second time. Although both components of the immune system contribute to graft rejection, the adaptive system is more important in transplant-related immune responses, and therefore is the primary target of most immunosuppressive therapy.

That allograft rejection has an immunologic basis was established initially through the studies of Sir Peter Medawar. As a physician treating burn patients during World War II, Medawar noted that skin allografts were rejected in an accelerated fashion if the recipient had previously received an allograft from the same donor. He followed these observations with an elegant series of skin grafting experiments in mice and rabbits. Through these studies, Medawar conclusively demonstrated that allograft rejection encompasses both memory and specificity, the classic features of adaptive immunity.

Memory

This feature is well illustrated by the behavior of skin allografts (Table 1.1). First-set skin allografts exchanged between different mouse strains survive for approximately 10 days. During the first few days after transplantation, first set skin allografts are indistinguishable from isografts or autografts by either gross inspection or histological criteria. However, second set skin allografts transplanted onto specifically immunized hosts are rejected in approximately 3 days, and there is little or no latent period because immunity is acquired. This memory response is analo-

Table 1.1 Behavior of skin allografts

Donor strain	Recipient strain	Treatment	Rejection
A	B	None	Slow (10 days)
A	B	Sensitized with strain A graft	Rapid (3 days); exhibits *memory*
C	B	Sensitized with strain A graft	Slow (10 days) exhibits *specificity*

gous to the primary versus secondary response to conventional antigens (e.g., measles virus), and is likely mediated by memory T cells. There is now compelling evidence that memory T cells play an important role in immune responses to transplanted organs.

Specificity

Allograft rejection is exquisitely specific. For example, accelerated rejection occurs only when the second-set graft shares mismatched H antigens with the first set graft. Moreover, the immune system easily distinguishes between histocompatible and histoincompatible grafts even when they are contiguous, e.g., a skin autograft placed on the same bed or even interspersed with an allograft is accepted despite vigorous rejection of the adjacent allograft.

Histocompatibility antigens

Historical perspective

Studies of tumor and skin grafts exchanged between inbred strains of mice led to formulation of the "laws of transplantation." These observational studies led to the recognition that H antigens are encoded by polymorphic loci (i.e., loci that differ between individuals of the same species). In addition, the expression of H antigens is co-dominant (i.e., both alleles are expressed). Subsequent studies estimate the total number of independently segregating H antigen loci at >100, although, as discussed below, some H-antigen loci are more immunogenic than others.

Key points 1.1 Laws of transplantation

Transplantations within inbred strains will succeed

Transplantations between different inbred strains will fail

Transplantations from an inbred parental strain to an F1 offspring will succeed, but those in the reverse direction will fail

Three sets of antigens play dominant roles in stimulating graft rejection: major histocompatibility complex (MHC) antigens, minor histocompatibility antigens (mHAs), and blood group antigens.

Major histocompatibility complex molecules
MHC molecules, as their name implies, are the most important antigens responsible for graft rejection. Every vertebrate species has MHC molecules. In humans, they are called HLA (human leukocyte antigens). There are two basic forms of MHC molecules: class I and class II. Class I MHC molecules are expressed on almost every type of cell whereas MHC class II molecules are expressed primarily on a subset set of cells that have "antigen-presenting" capacity, including dendritic cells, B cells, and macrophages. Class I HLA molecules are encoded by three separate genetic loci referred to as A, B, and C in humans. There are also three separate loci (called DR, DQ, and DP) that encode class II molecules. Both of the alleles

inherited at all six of these loci are expressed on the cell surface, a phenomenon referred to as co-dominant expression. One of the striking features of MHC molecules is their extraordinary polymorphism. There are dozens of different alleles in the human population that can be expressed in each of the MHC loci, making MHC-encoded genes the most polymorphic loci known to humans.

The gene products of six different MHC loci can be expressed on the surface of a given cell, and two alleles are expressed per locus. Thus, an individual's MHC genotype technically should be described by a list of 12 alleles. However, the A, B, and DR loci exert a more powerful effect in transplantation than the C, DP, and DQ loci. Therefore, only HLA antigens encoded by the A, B, and DR loci are typically identified before transplantation. For example, one individual might be A2, A4, B3, B7, DR3, DR4 and another might be A18, A24, B7, B21, DR6, DR9. As there are so many different alleles in humans for each locus, the chances of two unrelated people expressing the same six HLA antigens is very small – of the order of one in a million. However, within families, the mother's and father's MHC alleles tend to be inherited as a group, called haplotypes. As a result, among siblings, a quarter are likely to share no haplotypes (no HLA antigens), a half are likely to share one of the two haplotypes (half of the HLA antigens or "haploidentical"), and a quarter are likely to share both haplotypes, in which case they will be "HLA identical" (Figure 1.1).
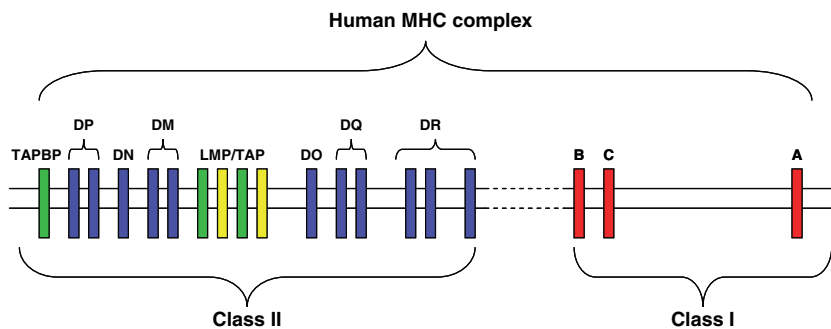

Figure 1.1 Schematic map of human MHC (major histocompatibility complex) loci. Located on chromosome 6, several polymorphic gene loci are clustered together. Note that this genetic region includes a variety of loci encoding proteins involved in antigen processing and presentation (LMP, TAP, DM) in addition to class I (A, B, C) and class II (DR, DP, DQ) MHC molecules. Sizes of genes and intermediate DNA segments are not shown to scale.

Although MHC molecules are important in causing graft rejection, and were originally discovered as a result of transplantation experiments, their intended function in the immune system has nothing to do with transplantation! The molecular structure of MHC molecules is critical to their physiologic function. All MHC alleles share in common the expression of four extracellular domains, the outer two of which are configured to form a groove or "cleft." This cleft has the capacity to carry within it short peptides of 8–22 amino acids in length. The many different MHC alleles in the human population each have clefts that are configured slightly differently, making each of them capable of carrying a different set of peptides. After HLA molecules are first constructed inside a cell they are then transported to the cell surface where they will be expressed. During this transport, the

HLA molecules encounter elaborate intracellular machinery that samples all the proteins within the cell, breaking them down into peptide fragments, and loading those peptides into the clefts of the new HLA molecules if they have a suitable size and structure. Thus, when MHC molecules are expressed on the surface of a cell, they carry with them a sampling of all the proteins that exist within that cell. Most of those proteins are normal components of a healthy cell, but in some cases they may be foreign proteins picked up from the environment, produced endogenously by viruses, or resulting from malignant transformation. Thus, one of the primary functions of MHC molecules is to provide an external display of the internal cellular elements both in health and during disease (Figure 1.2). Generally, class I MHC molecules present peptides derived from cytoplasmic
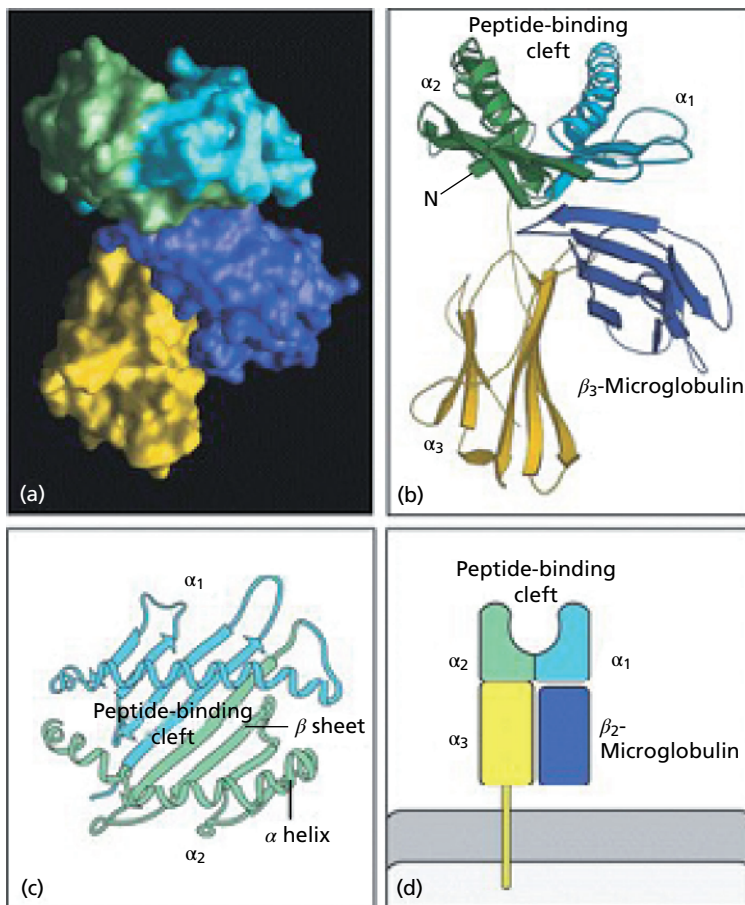

Figure 1.2 Structure of a class I MHC (major histocompatibility complex) molecule. Class I molecules are composed of a polymorphic alpha chain noncovalently bound to the nonpolymorphic β_2-microglobulin. The amino-terminal α_1- and α_2-segments of the α chain interact To to form a cleft large enough to bind peptides that are 8 to 11 amino acids in size. (Reproduced from *Immunology*, 6th edn. New York: Garland Science, 2005.)

proteins whereas all II MHC molecules present peptides derived from extracellular proteins.

Minor histocompatibility antigens

Even in situations where the transplant donor and recipient share all their MHC alleles (i.e., a perfect HLA match), other antigens, referred to as minor histocompatibility antigens (mHAs) can provoke rejection. In contrast to MHC-mismatched allografts, which are generally rejected in a matter of days, allografts exchanged between MHC-identical mouse strains that differ at a single minor H antigen may survive for weeks or months before they are rejected. These antigens are generated by allelic differences for some of the non-MHC proteins within a cell. Slight amino acid differences in a cytoplasmic protein generate peptides when that protein is broken down for display by the MHC molecules of the donor that are distinct from the set of peptides previously encountered by the recipient. These MHC/peptide complexes can therefore be recognized by host T cells that mediate rejection of the transplanted organ.

This is analogous to recognition of viral or tumor antigens, i.e., foreign peptides derived from endogenous proteins enter the class I presentation pathway. Note that recognition of minor H antigens occurs only in situations where donor and recipient are at least partially MHC matched for MHC-encoded antigens. It has been estimated that there are more than 100 loci for mHAs.

Blood group antigens

Red blood cells and vascular endothelial cells express surface glycoproteins, called blood group antigens, that can also cause transplant rejection by serving as targets of anti-donor antibodies. In humans, there are three important forms of these antigens, called blood groups O, A, and B. The blood group O glycoprotein represents a common backbone expressed by all humans. This backbone can be modified by enzymes to produce the A, B, or both (AB) determinants. As the A and B determinants are very similar to glycoprotein determinants expressed by intestinal microbes, humans who do not themselves express either A or B begin producing anti-A or anti-B antibodies relatively soon after birth. If there is a blood group incompatibility, these naturally occurring antibodies can bind to the A and B determinants on the vascular endothelium of a donor organ and cause hyperacute rejection (see below).

Key points 1.2 General course of transplantation

Recognition of mismatched histocompatibility antigens

T-cell activation and the production of cytokines; B-cell activation and production of anti-donor antibodies

Effector mechanisms: delayed-type hypersensitivity (DTH) mediated by host CD4+ T cells, cytotoxicity mediated by host CD8+ T cells, antibody-mediated injury

---

B cells and humoral rejection

B-cell biology

Although allograft rejection was traditionally held to be a T-cell-mediated process, it is now widely recognized that B cells play a key role in promoting destruction of transplanted tissues and organs by the production of anti-donor antibodies that bind to allografts. The antibody response to transplanted tissues and other foreign antigens is referred to as the humoral immune response. Antibodies are polypeptides with the ability to bind foreign antigens, thus tagging them for removal by the immune system. However, before B cells can secrete antibodies, they generally require interaction with other cell populations including CD4+ T cells. The antigen receptor of the B cells is membrane-bound IgM and IgD, which recognizes the native conformation of foreign antigen. When these receptors engage a foreign antigen, with the assistance of CD4+ T cells, the B lymphocyte is stimulated to differentiate into an antibody-secreting plasma cell. Plasma cells represent the final phase of B-cell differentiation and secrete antibodies with the same specificity as the original B lymphocyte. Each B-cell clone expresses a unique receptor and is present in the body at very low frequency. Consequently, B-cell clones recognizing a particular antigen must replicate before they can mount an effective response.

Antibodies

Antibodies comprise four polypeptide chains, two light chains and two heavy chains. According to

5

differences in the heavy chains, there are five different types of antibodies, referred to as isotypes: IgM, IgG, IgA, IgD, and IgE. Once B cells are stimulated by a specific antigen, the secreted antibodies initially are of the IgM isotype, but eventually IgG antibodies are secreted, a process referred to isotype switching, which requires interaction of B cells with helper T cells. After the initial response, memory B cells are preserved and can maintain production of specific antibody for many years. IgG and IgM antibodies both can play important roles in the pathogenesis of transplant rejection.

The most important antibodies that stimulate allograft rejection are those directed to donor MHC (or, in humans, HLA) molecules. Anti-HLA antibodies can be induced by a previous transplant, pregnancy, or blood transfusion. There is increasing evidence that autoantibodies and antibodies to non-MHC proteins also take part in the humoral alloimmune response to transplanted tissues. As discussed above, antibodies to ABO incompatibilities also can trigger hyperacute rejection of organ allografts.

Mechanisms of antibody-mediated graft damage

After antibodies (IgM or IgG) bind to antigens on the allograft endothelium, they can initiate the complement cascade via the classic pathway, leading to production of the membrane attack complex (MAC) from its terminal components. The MAC can directly cause endothelial cell lysis and subsequent graft injury. In addition, chemoattractants such as C3a and C5a liberated by the complement cascade can attract inflammatory cells to the graft and mediate graft injury (Figure 1.3). C4d, a fragment of C4 produced during the classic complement activation pathway, is highly stable and persists at the cell surface well after the time at which antibody is no longer detectable. As C4d is readily detected, and correlates with the existence of donor specific anti-HLA antibodies, it is widely utilized as an *in situ* marker of antibody-mediated rejection. However, it is important to note that C4d deposition in the graft is merely a marker of rejection and that other, more labile, complement components are likely responsible for actual graft injury.

Another important effect of antibody and complement fixation to the graft vasculature is activation of graft endothelial cells. Complement components increase adhesion molecule expression by graft endothelial cells, can trigger proliferation of endothelial cells via release of growth factors and chemokines, and can induce synthesis of tissue factors that regulate the extrinsic clotting system. Thrombotic injury can dominate in severe cases, such as hyperacute rejection mediated by pre-existing anti-donor antibodies. Such injury is characterized by thrombotic microangiopathy with diffuse vascular damage and thrombosis. There is also evidence that antibodies can mediate graft damage through a complement-independent mechanism, which is thought to cause chronic graft injury. Anti-donor antibodies may also lead to destruction of target cells by a process of antibody-dependent cell-mediated cytotoxicity (ADCC).

Pathogenesis of antibody-mediated rejection

Antibody-mediated rejection (AMR) can occur in any time period after transplantation and is manifest of one of several distinct syndromes (Table 1.2), depending on the titer or the pattern of antibody. High-titer, pre-existing, donor-specific antibodies can cause hyperacute rejection, which can destroy a transplanted kidney within minutes. In hyperacute rejection, pre-existing circulating antibodies to donor MHC or blood group antigens lead to rapid binding of large quantities of antibodies to the graft vasculature, resulting in activation of the classic complement cascade and production of the MAC as described above. The MAC, in turn, stimulates endothelial activation that can become manifest within minutes to hours. This type of endothelial activation (type I) causes the cells to retract from each other, and to express procoagulant factors. As a result, leakage of blood into the interstitium produces a swollen blue organ with subsequent intravascular thrombosis. Hyperacute rejection is the most severe type of rejection after organ transplantation. There is no therapy that can reverse this process once it has started. In some cases infarction of the organ occurs before the completion of surgery.

A second form of rejection caused by antibodies is referred to as acute antibody-mediated rejection (AAMR). This clinical entity is thought to represent an important cause of early kidney transplant failure.
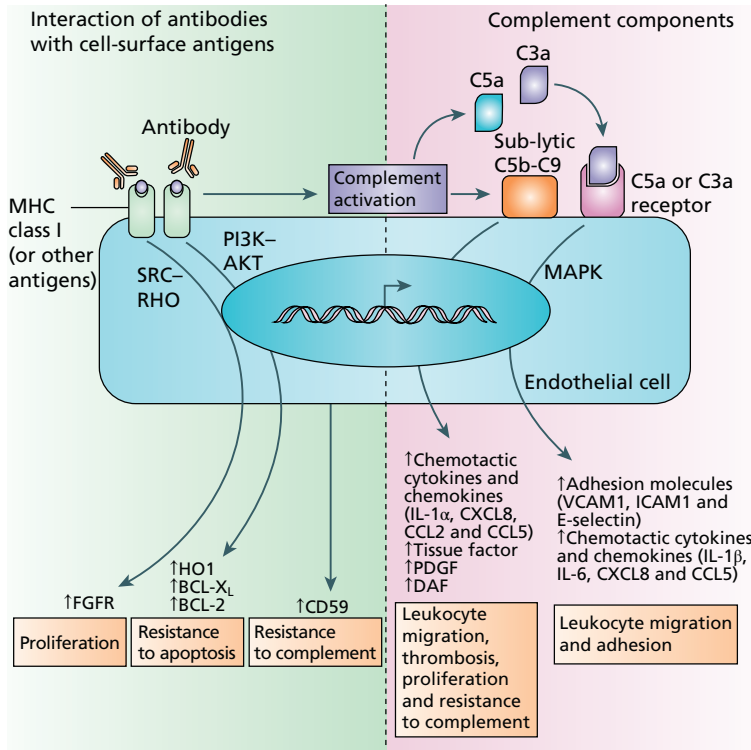
Figure 1.3 Effects of antibody and complement components on human endothelial cells. Effects mediated by the interaction of antibody with antigen at the surface of endothelial cells are shown on the left and the effects caused by complement components on the right. Target antigens may be MHC (major histocompatibility complex) class I and II molecules, ABO blood-group antigens, or other non-MHC antigens. BCL, B-cell lymphoma; CCL, CC-chemokine ligand; CXCL8, CXC-chemokine ligand 8; DAF, decay-accelerating factor; E-selectin, endothelial-cell selectin; FGFR, fibroblast growth-factor receptor; ICAM-1, intercellular adhesion molecule 1; IL, interleukin; MAPK, mitogen-activated protein kinase; PDGF, platelet-derived growth factor; PI$_3$K, phosphatidylinositol 3-kinase; RHO, RAS homolog; VCAM-1, vascular cell-adhesion molecule 1. (Reproduced from Colvin RB, Smith RN. Antibody mediated organ allograft rejection. *Nature Rev Immunol* 2005;**5**:807–17.)

Table 1.2 Clinical syndromes of antibody-mediated rejection (AMR)

Syndromes	Antibody involved	Time course	Clinical manifestations
Hyperacute rejection	Pre-existing antibodies	Immediately after reperfusion, takes minutes to hours	Immediate graft loss, cannot be treated
Acute AMR	Pre-existing or new antibodies	Any time after transplantation, takes days to weeks,	Rapid graft dysfunction, can be reversed
Chronic/late AMR	Mostly new antibodies	Months to years	Slow but progressive loss of graft function, difficult to be controlled

AAMR can occur when antibodies appear in the circulation very early after the transplantation, usually within a few days. The antibodies involved may be pre-existing antibodies present initially at very low concentrations, or antibodies produced anew after transplantation. The binding of these anti-donor antibodies over time causes a second type of endothelial activation (type II), which occurs more slowly than the type I activation responsible for hyperacute rejection. In this case, the two major consequences of the activation are generation of procoagulant factors and the appearance of fibrinoid necrosis of the vessels. The hallmark of this type of rejection is diffuse C4d staining, especially in peritubular capillaries (Figure 1.4). AAMR can sometimes be reversed by some combination of treatment with plasmapheresis, anti-CD20 monoclonal antibody (rituximab), intravenous immune globulin (IVIG) and increased pharmacologic immunosuppression.

A third syndrome induced by antibodies is chronic antibody-mediated rejection (CAMR). In this situation, antibodies develop very slowly, usually over the course of years. The pathologic picture includes myointimal hyperplasia in the vessels and progressive interstitial fibrosis, with additional features that are organ specific (Figure 1.5). In some cases of chronic rejection, anti-donor antibodies can be detected in the transplanted organ or in the circulation, suggesting a pathogenic role. However, there appear to be multiple potential etiologies for chronic rejection and determining the contribution of any one is not practical currently.

Key points 1.3 Antibody-mediated rejection

Can be mediated by antibodies to MHC molecules, ABO blood group antigens, and a variety of non-MHC molecules

Can occur at any time after transplantation

Risk factors include prior transplantation, multiple pregnancies, and a history of blood transfusions

C4d deposition in the graft peritubular capillaries correlates with the presence of circulating anti-donor antibodies and is thus a widely accepted marker of antibody-mediated rejection, but there is no evidence that C4d deposition is causally related to graft injury

Detection of antibodies

Several methods are currently employed to identify and/or monitor the development of anti-donor antibodies in clinical transplant recipients. The cross-match assay is widely used to select recipients for renal transplantation. In the cytotoxic cross-match assay, cells from the potential donor are mixed with serum from the recipient along with an exogenous source of complement. If the recipient serum contains anti-
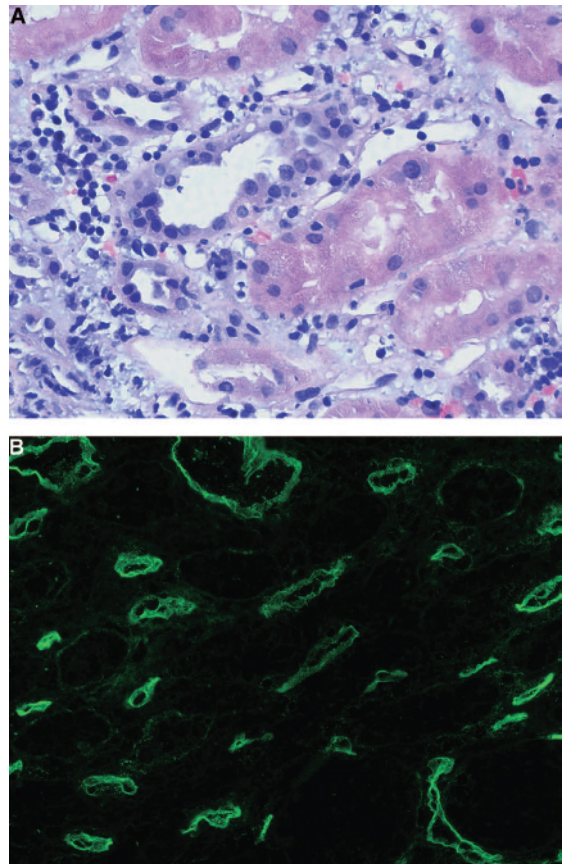

Figure 1.4 Histological features of acute humoral rejection. (a) Light microscopy shows interstitial edema, tubular injury, and infiltration of neutrophils and mononuclear cells into the peritubular capillaries (PTCs). (b) Immunofluorescence (IF) microscopy demonstrates widespread, bright, linear staining of PTCs for C4d. (From Colvin RB. Antibody-mediated renal allograft rejection: diagnosis and pathogenesis. *J Am Soc Nephrol* 2007;**18**:1046–56.)

Figure 1.5 Chronic humoral rejection: (a) transplant glomerulopathy with duplication of the glomerular basement membrane (GBM) and accumulation of mononuclear cells in glomeruli (periodic acid–Schiff stain). (b) Immunofluorescence staining shows patchy distribution of C4d positivity in peritubular capillaries (PTCs). (c) Electron microscopy shows duplication of the GBM and reactive endothelial cells. (d) The PTCs have prominent multilamination of the GBM. (From Colvin RB. Antibody-mediated renal allograft rejection: diagnosis and pathogenesis. *J Am Soc Nephrol* 2007;**18**:1046–56.)
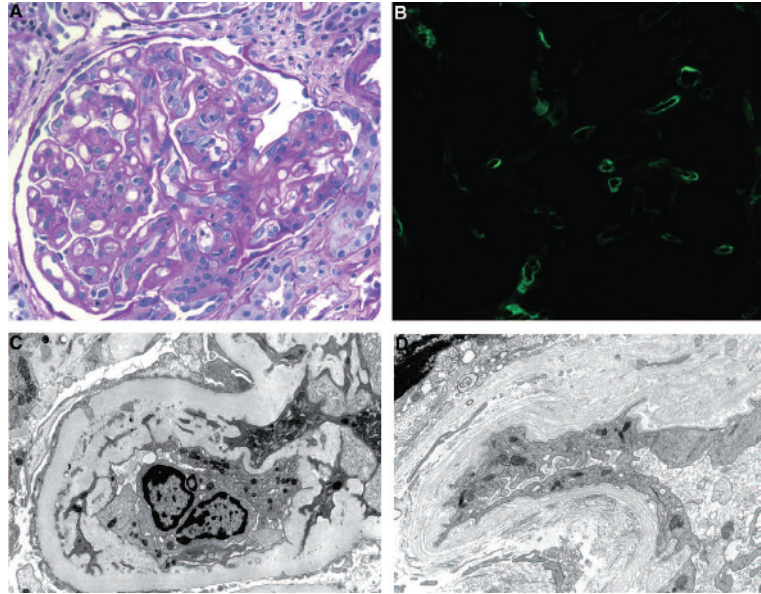
donor antibodies, these will fix the complement and lyse the donor cells. Death of the donor cells signifies a "positive cross-match," in which case organ transplantation is generally not performed because of the high risk of hyperacute rejection. The cross-match assay can also be employed to monitor development of anti-donor antibodies post-transplantation, which is important in diagnosing AMR. Increasingly, more advanced and sensitive techniques are being employed to perform the cross-match assay, such as flow cytometry using either donor cells or antigen-coated beads.

In an effort to predict the likelihood of a positive cross-match, it is common to test recipient sera against a panel of HLA antigens derived from donors expressing a broad range of the human MHC antigens. This method is called panel reactive antibody (PRA) detection. High PRA values are associated with an increased risk of acute rejection after transplantation.

Table 1.3 T-cell subsets and their role in allograft rejection

T-cell subset	Function	Role in allograft rejection
CD8+ (CD4−)	Cytotoxic T cells	Mediate direct killing activity
CD4+ (CD8−)	Orchestrate the overall immune response by facilitating the activation and differentiation of other immune cells	Mediate DTH and provide help for B cell and CD8+ T-cell responses
FoxP3+ (CD4+ or CD8−)	Inhibitor of T-cell activation/function	Instrumental in T-reg induction

T cells and T-cell-mediated rejection

Basic elements of T-cell biology

T cells are a subset of lymphocytes that play a central role in allograft rejection. The abbreviation "T," in T cell, stands for the thymus gland because it is the principal organ responsible for the development of these lymphocytes. In contrast to B cells, which accomplish their function by secreting antibodies into the circulation, T cells accomplish their function by direct cell-to-cell contact or by the secretion of soluble factors (i.e., cytokines) that regulate other cells in the local environment. T cells can be divided into subpopulations, defined primarily by their surface phenotype (Table 1.3). The most characterized subpopulations are CD4+ and CD8+ T cells.

T cells can be distinguished from other lymphocyte types, such as B cells and macrophages, by the presence of a unique receptor on their cell surface, called the T-cell receptor (TCR). Unlike B-cell receptors, which are essentially membrane-bound antibody, TCRs recognize foreign peptides only in the context of self-MHC molecules. CD4+ T cells respond to foreign peptides associated with class II MHC molecules whereas CD8+ T cells respond to foreign peptides presented by class I MHC molecules. Foreign peptides not expressed in association with MHC molecules do not induce a T-cell response.

This mechanism, in which TCRs recognize only foreign proteins when their peptides are presented by self-MHC antigens, is referred to as MHC restriction. T cells can be activated only by contact with other cells, because MHC molecules are expressed primarily on surface of cells. As a result, T cells can function only in relation to other cells, whereas B cells – through production of antibody – can respond to any foreign protein, bound or unbound to another cell.

T cells are educated to recognize foreign peptides in the context of host (self) MHC molecules in the host thymus. When pre-T cells enter the thymus, they randomly recombine TCR gene segments to generate a diverse TCR repertoire. Consequently, the vast majority of the newly generated T cells (referred to as thymocytes) are not restricted by self-MHC molecules. The first step of thymic maturation requires that the T cells express TCRs that effectively bind to self-MHC antigens expressed on the surface of that individual's thymic endothelium. A binding of adequate affinity allows for the T cell to receive survival signals. Developing thymocytes that do not have sufficient affinity for self-MHC cannot serve useful functions in the body and therefore die by apoptosis (programmed cell death) as a result of the lack of the aforementioned survival signals. This process is called positive selection. Whether a thymocyte becomes a CD4+ cell or a CD8+ cell is also determined during positive selection. Double-positive cells (CD4+/CD8+) that are positively selected on MHC class II molecules will become CD4+(CD8−) cells, and cells positively selected on MHC class I molecules will become CD8+(CD4−) cells. Thymocytes that survive positive selection are again presented with self-antigen in complex with MHC molecules on antigen-presenting cells (APCs), such as dendritic cells. Thymocytes that interact too strongly with the self-antigen receive an apoptotic signal that causes cellular death. This process is called negative selection, an important mechanism that prevents the formation of self-reactive T cells capable of generating autoimmune disease. The remaining T cells then exit the thymus as mature naïve T cells.

> ## Key points 1.4 Important properties of T cells
>
> T cells have specific T-cell receptors that recognize foreign proteins
>
> T cells recognize foreign protein only on other cells as a MHC–peptide complex
>
> T cells cross-react at high frequency with MHC alloantigens

T-cell activation through APC interaction

After thymic maturation, naïve T cells migrate into the peripheral lymphoid organs where they are potentially activated in response to foreign antigens. Although the specific mechanisms of activation vary slightly between different types of T cells, the three-signal model in T cells holds true for most. The first signal is provided by binding of the TCR to a short peptide presented by the MHC on another cell. This ensures that only T cells expressing a TCR specific to that specific peptide are activated. The partner cell is usually a professional APC, generally a dendritic cell, although B cells and macrophages also can be important APCs (Table 1.4). As discussed above, CD8+ T cells recognize peptides in the context of MHC class I molecules whereas CD4+ T cells respond to peptides associated with MHC class II molecules.

The second signal comes from co-stimulation, in which surface receptors on the APC are induced and bind to co-stimulatory receptors expressed by naïve T cells. Co-stimulation involves reciprocal and sequential signals between cells. Low constitutive levels of B7.1 and/or B7.2 on the APC activate CD28 on the T cell, inducing upregulation of CD40L on the T cell. CD40L in turn binds to CD40 on the APC, enhancing B7.1/B7.2 expression and reinforcing the CD28/CD40-positive feedback loop. Other co-stimulatory and inhibitory molecules regulated by the initial co-stimulatory signals can further shape the specific outcome of the interaction. The second signal

Table 1.4 Types of antigen-presenting cells

Type	Unique characteristic
Dendritic cell	Constitutively express MHC class I and class II molecules as well as co-stimulatory ligands for optimal activation of host CD4+ and CD8+ T cells
B cell	Can concentrate and present antigen, to which the clonotypic B-cell receptor (surface antibody) is directed, to host CD4+ T cells
Macrophage	Phagocytose cellular debris and present resulting peptides in association with self-MHC class II molecules to host CD4+ T cells

MHC, major histocompatibility complex.

licenses the T cell to respond to an antigen. Without it, the T cell becomes anergic and reactivation in the future becomes more difficult. This mechanism prevents inappropriate responses to self, because self-peptides will not usually be presented with suitable co-stimulation.

The third signal follows the interactions of the T cell with MHC/peptide and co-stimulatory molecules, and involves a cascade of biochemical events in the T cell that subsequently results in the expansion and differentiation of the specific T cell clone. This occurs primarily through an increase in interleukin IL-2 secretion by the T cell and an increase in the density of IL-2 receptors on the T-cell surface. IL-2 is a potent T-cell growth cytokine that acts in an autocrine fashion to promote the growth, proliferation, and differentiation of the T cell recently stimulated by antigen (Figure 1.6).
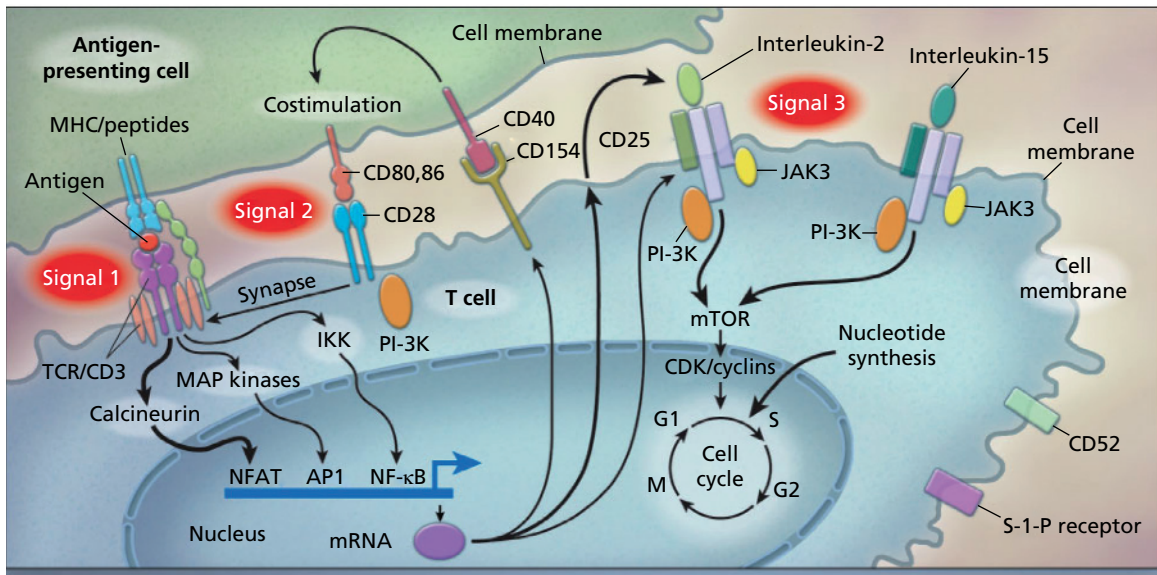

Figure 1.6 Three-signal model of T-cell activation: antigen-presenting cells (APCs) of host or donor origin migrate to T-cell areas of secondary lymphoid organs. These T cells ordinarily circulate between lymphoid tissues where APCs present donor antigen to naive T cells. Antigen triggers T-cell receptor (TCR) signaling (signal 1) and synapse formation. (See https://content.nejm.org/cgi/content/full/351/26/2715–R5#R5.) CD80 (B7–1) and CD86 (B7–2) on the APCs engage CD28 on the T cells to provide signal 2. These signals activate various signal-transduction pathways that activate transcription factors. The result is expression of CD154 (which further activates APCs), interleukin-2 receptor α chain (CD25), and interleukin-2 (IL-2). IL-2 and IL-15 deliver growth signals (signal 3) that initiate the cell cycle. T cells, then, are fully activated and undergo clonal expansion and differentiation to mature T effector cells. (From Halloran PF. Immunosuppressive drugs for kidney transplantation. *N Engl J Med* 2004;**351**:2715–29.)

Direct and indirect allogeneic antigen presentation in transplantation

T-cell recognition of mismatched MHC alleles is a key event in the pathogenesis of allograft rejection. Allogeneic MHC molecules are presented for recognition by host T cells in two fundamentally different ways. The first, called *direct* presentation, involves the recognition of an intact MHC molecule displayed by the donor and is a consequence of the similarity in structure of an intact foreign MHC molecule and self-MHC molecules. As many as 2% of an individual's T cells are capable of directly recognizing and responding to a single foreign MHC molecule, and this high frequency of T cells reactive with allogeneic MHC molecules is one reason that allografts elicit strong immune responses.

The second pathway of T-cell allorecognition, called *indirect* presentation, involves processing of donor MHC molecules by recipient APCs and presentation of peptides derived from allogeneic MHC molecules in association with self-MHC molecules. CD8+ T cells that are generated by the indirect pathway are self-MHC restricted and therefore cannot directly kill the foreign cells in the graft, so when alloreactive T cells are stimulated by the indirect pathway, the principal mechanism of rejection is thought to be mediated by CD4+ T cells recognizing donor alloantigens, thus stimulating other immune cells (Figure 1.7).

Effector mechanisms of T-cell-mediated graft injury

After activation and proliferation, T cells exit the draining lymphoid compartments and circulate through the body to eliminate cells expressing the specific antigen. Once activated T cells come in contact with the mismatched H antigen, the mechanisms of graft destruction depend on the type of T cell responding. CD4+ T cells initiate an indirect response by helping other immune cells, especially B cells and CD8+ T cells, to respond more efficiently to the graft. One important function of CD4+ T cells is to promote the maturation of B cells, which produce anti-donor antibodies. Such responses require the activation of B cells by helper T cells that respond to the same molecule. This is called linked recognition. This means that, before B cells can be induced to
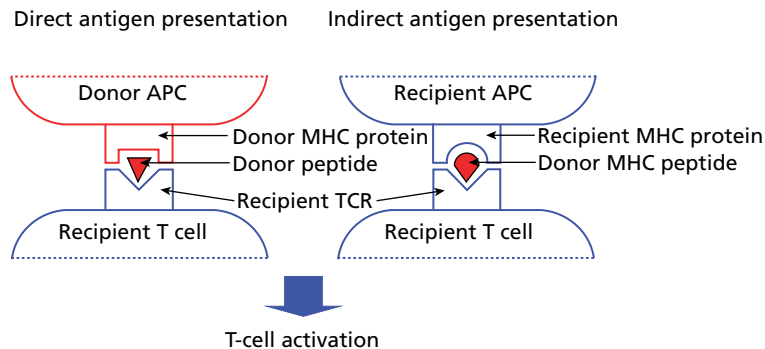

Direct antigen presentation   Indirect antigen presentation

Donor APC   Recipient APC

Donor MHC protein   Recipient MHC protein
Donor peptide   Donor MHC peptide
Recipient TCR
Recipient T cell   Recipient T cell

T-cell activation

Figure 1.7 Direct and indirect pathways of T-cell allorecognition: direct and indirect pathways of T-cell allorecognition are mediated by different antigen-presenting cells (APCs). Direct antigen presentation involves recognition of intact donor MHC (major histocompatibility complex) molecules by host T cells. Indirect antigen presentation involves recognition of processed donor MHC antigen presented in association with self-MHC molecules by host APCs. Both pathways are important mechanisms of allograft rejection. It is thought that the direct pathway is responsible for T-cell-mediated rejection and that the indirect pathway is responsible for humoral rejection. TCR, T-cell receptor.

Table 1.5 Cytokines in transplantation

Cytokine	Function in transplantation
IL-2	Clonal expansion of activated T cells
IL-4	Induces B cells to produce antibody
IL-12	Enhances cytotoxic activity of activated CD8+ T cells
IFN-γ	Upregulates MHC expression on target cells, activates APCs

IFN, interferon; IL, interleukin; MHC, major histocompatibility complex.

make antibody to a foreign protein, a CD4+ T cell specific for peptides from this pathogen must first be activated to produce the appropriate activated helper T cells. Although the epitope recognized by the activated helper T cell must therefore be linked to the same molecule recognized by the B cell, the two cells need not recognize identical epitopes. CD4+ T cells also provide help to promote CD8+ T-cell responses, and can induce delayed-type hypersensitivity (DTH) responses that can damage the graft. DTH is caused via stimulation of lymphocytes and macrophages, resulting in chronic inflammation and cytokine release (Table 1.5). T cells play an important role in this hypersensitivity, because they activate against the stimulus itself and promote the activation of other cells at the graft site, particularly macrophages.

In contrast, CD8+ T cells can respond directly to cells expressing mismatched H antigens, albeit with help provided by CD4+ T cells. When exposed to a foreign antigen-expressing cells, cytotoxic CD8+ T cells (CTLs) release cytotoxins such as perforin and granzymes that induce target cell apoptosis. Perforin serves to form pores in the target cell's plasma membrane either by directly inducing target cell necrosis or by allowing granzymes to enter the cell, thereby inducing apoptosis by activation of cellular enzymes called caspases. A second way that CD8+ T cells induce apoptosis of donor cells is via cell-surface interactions between the CTL and the antigen-expressing cell. For example, activated CD8+ T cells express the surface protein Fas ligand (FasL), which can bind to Fas molecules expressed on the target cell and thereby induce target cell apoptosis.

In clinical organ transplantation, pharmacologic immunosuppression is imperative to protect the graft from the anti-donor immune response. Indeed, a daily and life-long regimen of immunosuppressive drugs is required to prevent clinical organ allograft rejection. A variety of immunosuppressive drugs is regularly used to inhibit T-cell responses including corticosteroids, antimetabolites, antibodies, drugs acting on immunophilins, among other therapies (Table 1.6). Corticosteroids act by inhibiting transcription factors such as nuclear factor-κB (NF-κB), thus markedly inhibiting genes that code for the cytokines ranging from IL-1 through IL-8 and tumor necrosis factor (TNF)-α, some of which are indispensable for T-cell expansion. Antimetabolites such as azathioprine and mycophenolate mofetil function to inhibit lymphocyte proliferation. Azathioprine was formerly a mainstay of transplant immunosuppression but is increasingly being supplanted by mycophenolate mofetil. Corticosteroids and antimetabolites inhibit downstream T-cell responses by preventing the clonal expansion of lymphocytes in the induction phase of the immune response. In addition, polyclonal antibody treatments such as antithymocyte globulin are widely used to further suppress anti-donor immunity.

Alternatively, monoclonal antibodies (mAbs) can be used to block specific pathways, such as an mAb to the IL-2 receptor which is thought to inhibit T-cell expansion. Finally, drugs such as cyclosporine and tacrolimus act to inhibit calcineurin. Calcineurin inhibitors specifically inhibit IL-2 transcription by host T cells, again leading to reduced T-cell function. It is important to note, however, that all such immunosuppressive drugs have serious side effects, and leave the patient vulnerable to opportunistic infection and malignancy. Consequently, the development of more specific strategies to inhibit the anti-graft immune response remains the Holy Grail in the field of transplantation immunology.

Mechanisms of self-tolerance

Since the pioneering studies of Medawar demonstrating that specific tolerance to allogeneic skin grafts could be produced in mice by *in utero* injection of donor hemopoietic cells, the induction of donor-specific unresponsiveness in transplant recipients has been a major goal of modern transplantation immunology. Immunologic tolerance is defined as

Table 1.6 Commonly used immunosuppressant drug classes used in clinical transplantation and their mechanisms

Immunosuppressant	Mechanisms
Corticosteroids	This general class of immunosuppressants inhibits transcription factors such as nuclear factor-κB (NF-κB) activation, thus markedly decreasing cytokine secretion, and thereby inhibiting immune responses in general
Azathioprine	Antimetabolite; inhibits purine synthesis by converting 6-mercaptopurine to tissue inhibitor of metalloproteinase and thereby prevents proliferation of lymphocytes
Mycophenolate mofetil	Antimetabolite; blocks purine synthesis by inhibiting synthesis of guanosine monophosphate nucleotides, and thereby prevents proliferation of lymphocytes
Cyclosporine	Calcineurin inhibitor; binds to cyclophilin, and thereby inhibits calcineurin phosphatase and T-cell activation
Tacrolimus	Calcineurin inhibitor; binds to FKBP12, and thereby inhibits calcineurin phosphatase and T-cell activation
Sirolimus	Binds to FKBP12; inhibits target of rapamycin, and thereby inhibits IL-2-driven T-cell proliferation
Basiliximab	Anti-CD25 monoclonal antibody; binds to CD25 antigen (IL-2R) expressed on activated T cells, leading to T-cell depletion and inhibition of IL-2-induced T-cell activation
Rituximab	Anti-CD20 monoclonal antibody; binds to CD20 expressed on B cells and thereby mediates B-cell depletion
Muromonab-CD3	Anti-CD3 monoclonal antibody; binds to CD3 expressed on T cells, and thereby blocks T-cell function and/or induces T-cell depletion

IL, interleukin; IL-SR, interleukin-2 receptor.

unresponsiveness to an antigen that is induced by a previous exposure to that antigen. When specific lymphocytes encounter antigens, the lymphocytes may be activated, leading to an immune response, or the cells may be inactivated or eliminated leading to tolerance. There are several mechanisms of tolerance (Table 1.7).

One mode of tolerance induction is *central* tolerance, also known as negative selection. As described above, immature T cells that recognize antigens with high avidity within the thymus are deleted. The two main factors that determine whether a particular self-antigen will induce negative selection of self-reactive thymocytes are the concentration of that antigen and the affinity of the TCRs that recognize the antigen. T-cell recognition of an antigen that is abundantly expressed in the thymus and has a strong association with TCRs will result in apoptosis of the attached T cell. Studies in mouse models indicate that adoptive transfer of donor bone marrow to recipients condi-

Table 1.7 Strategies for tolerance induction

Type of tolerance	Current strategies
Central tolerance	Introduction of donor APCs into the recipient thymus; induction of mixed hemopoietic chimerism
Peripheral tolerance	Administration of drugs that block T-cell co-stimulatory pathways; donor-specific blood transfusions to trigger antigen-induced cell death of alloreactive T cells; induction of regulatory T cells to counter the anti-donor immune response

APC, antigen-presenting cell.

tioned by irradiation or immunotherapy harnesses the phenomenon of negative selection to eliminate donor-reactive T cells by central deletion, after interaction

with donor APCs that have accessed the recipient's thymus.

Another mode of tolerance is *peripheral* tolerance. Peripheral tolerance is the mechanism by which mature T cells that recognize self-antigens in peripheral tissues become incapable of subsequent response to the antigen. This mechanism is responsible for T-cell tolerance to self-antigens that are not abundant in the thymus. The same mechanisms may induce unresponsiveness to foreign antigens. Peripheral tolerance is due to anergy, deletion, or suppression of T cells. If T cells recognize peptide antigens presented by APCs in the absence of co-stimulatory molecules, the T cells survive but are rendered anergic, or incapable of responding to the antigen even if it is later presented by competent APCs. Repeated stimulation of T lymphocytes, by persistent antigen, results in the death of activated cells by apoptosis. This form of regulation is called activation-induced cell death. Many current immunotherapies are thought to promote peripheral tolerance to the donor either by blocking the transduction of co-stimulatory signals at the cell surface molecules or via the downstream intracellular signaling events.

Peripheral tolerance can also be mediated through regulatory T cells. Regulatory T cells are a specialized subpopulation of T cells that act to suppress activation of the immune system and thereby maintain immune homeostasis and tolerance to self-antigens. Regulatory T cells actively suppress activation of the immune system and prevent self-reactivity. Interest in regulatory T cells has been heightened by evidence from experimental mouse models demonstrating that the immunosuppressive potential of these cells can be harnessed therapeutically to treat autoimmune disease and facilitate transplantation tolerance.

It is increasingly clear that the immune response that distinguishes self from non-self is regulated at multiple levels. Although it is clear that all individuals have the genetic potential to mount anti-self immune responses at both the T- and B-cell levels, regulatory mechanisms usually prevent such autoreactivity, leading to self-tolerance. It is therefore logical that most methods for inducing tolerance to allogeneic transplants in some way make use of mechanisms that are utilized normally to prevent autoreactivity. A better understanding of the regulation of normal immune responses is thus crucial to understanding the mechanisms for induction of non-responsiveness to transplanted tissues and organs.

Xenotransplantation

An urgent problem in clinical organ transplantation is the shortage of donor organs. This shortage is only expected to worsen in the future, despite improvements in immunosuppressive therapies and/or advances in inducing donor-specific tolerance. A potential solution to this problem is the use of animals as the source of donor organs. Unfortunately, the current barriers to xenotransplantation are formidable. The main barriers to clinical xenotransplantation are summarized in Table 1.8. For one, humans produce natural antibodies to most species that cause hyperacute rejection of xenografts in minutes to hours. The dominant antibodies mediating hyperacute xenograft rejection are directed against a single carbohydrate epitope, Galα1–3-Galβ1–4GlcNAc-R epitope (αGal). The dominant antibody response to

Table 1.8 Main barriers to clinical xenotransplantation

Barrier	Pathogenesis/reasons
Hyperacute rejection	Caused by natural antibodies directed to the αGal carbohydrate moiety; antibody binding to the αGal expressed on the xenograft endothelium results in type I endothelial activation and hyperacute rejection of the xenograft
Acute humoral xenograft rejection	Caused by αGal via type II endothelial activation, may occur within 24 hours after the transplantation, and can lead to xenograft failure within days or weeks
Cellular mechanisms of xenograft rejection	CD4+ T-cell response via the indirect pathway, innate immune responses
Ethical concerns	Mainly focused on the risk of transmission of severe infectious agents from animal to humans

αGal occurs because humans and other Old World primates lack the enzyme (α1–3-galactosyltransferase) that produces the αGal moiety whereas all other vertebrates possess this enzyme. Once αGal-specific antibodies bind to the endothelium of the xenografts they trigger activation and/or necrosis of the graft endothelium, leading to hyperacute rejection of the xenograft. In humans, up to 75% of natural IgM present in normal human serum binds to αGal, with approximately 1% of all B cells spontaneously producing anti-αGal antibodies. Thus, anti-αGal antibodies are a major barrier to clinical xenotransplantation. Strategies that remove anti-αGal antibodies and reduce complement activity effectively prevent hyperacute xenograft rejection in experimental models, so there is hope that these problems can be overcome in the future.

The indirect pathway for activation of host CD4+ T cells directed to processed xenoantigens is also very strong. The extent to which direct recognition of xenogeneic MHC antigens occurs is poorly defined. Xenografts also stimulate the innate immune response of the recipient; natural killer (NK) cells, macrophages, and neutrophils can be activated, leading to lysis and/or phagocytosis of xenogeneic cells and eventual graft loss.

One of the great promises of xenotransplantation is the potential to alter the animal donors genetically so that the tissues elicit weaker immune responses and/or are resistant to immune attack. There is hope in the transplant community that transgenic herds of pigs can be developed for use as organ transplant donors, though the feasibility of this goal remains to be determined, e.g., scientists have developed genetically engineered pigs with targeted disruption of the αGal gene. Survival of xenografts from such donors is significantly prolonged in non-human primates. Although such grafts are still subject to rejection, it is expected that these problems may be solved by further gene modification.

Tolerance induction is another potential strategy to overcome the barriers to xenotransplantation: several such strategies are in development in animal models, such as blocking of co-stimulatory signals, donor-specific transfusions, co-transplantation of the donor thymus, and induction of mixed chimerism. However, the utility of such approaches in the human system remains to be established. An additional factor that may limit xenotransplantation is public concern (both real and imagined) that new infectious agents, especially endogenous viruses, may be transmitted from the source animal into the human population.

Bone marrow transplantation

This chapter focused on the immunology of organ transplantation. However, it is important to note that bone marrow transplantations (BMTs) are essentially stem cell transplants that differ from organ transplants in several key respects. For one, BMTs require complete ablation of the recipient's immune system to create "space" and to prevent graft rejection because marrow grafts are much more susceptible to rejection than organ grafts, with the innate arm of the immune system playing a critical role in rejection of bone marrow grafts. For another, graft-versus-host disease (GVHD) directed to minor H antigens – and not graft rejection – is currently the major limitation to broader application of BMTs to treat malignancy and genetic disorders. GVHD occurs when an immunologically competent graft is transplanted into an immunologically compromised host; mature donor T cells present within the marrow inoculum respond to the mismatched histocompatibility antigens (usually mHAs) and subsequently attack the host. The primary sites of attack are the skin, liver, and gut, leading to symptoms such as skin lesions, diarrhea, and wasting, respectively, with the potential for death. Thus, GVHD following BMT is essentially the reverse of organ allograft rejection; consequently, the treatment options are fundamentally similar to those used in organ transplantation.

Further reading

Abbas AK, Lichtman AH. *Cellular and Molecular Immunology*, 5th edn. Philadelphia, PA: Saunders, 2003.

Collins AB, Schneeberger EE, Pascual MA, et al. Complement activation in acute humoral renal allograft rejection: Diagnostic significance of C4d deposits in peritubular capillaries. *J Am Soc Nephrol* 1999;**10**:2208–14.

Colvin RB, Smith RN. Antibody mediated organ allograft rejection. *Nature Rev Immunol* 2005;**5**:807–17.

Cooper DK, Dorling A, Pierson RN, et al. α1,3-Galactosyltransferase gene-knockout pigs for xenotransplantation: Where do we go from here? *Transplantation* 2007;**84**:1–7.

Davila E, Byrne GW, Labreche PT, et al. T cell responses during pig-to-primate xenotransplantation. *Xenotransplantation* 2006;**13**:31–40.

Davis MC, Distelhorst CW. Live free or die: an immature T cell decision encoded in distinct. Bcl-2. sensitive and insensitive Ca^{2+} signals. *Cell Cycle* 2006;**5**:1171–4.

Hadley GA. Role of the integrin, CD103, in destruction of renal allografts by CD8+ T cells. *Am J Transpl* 2004; **4**:1026–32.

Halloran PF. Immunosuppressive drugs for kidney transplantation. *N Engl J Med* 2004;**351**:2715–29.

Janeway CA, Travers P, Walport M, Shlomchik M. *Immunobiology*, 5th edn. New York: Garland Publishing, 2001.

Jiang H, Chess L. An integrated view of suppressor T cell subsets in immunoregulation. *J Clin Invest* 2004;**114**: 1198–208.

Krensky AM, Weiss A, Crabtree G, at al. Mechanisms of disease: T lymphocyte-antigen interactions in transplant rejection. *N Engl J Med* 1990;**322**:510–7.

Kuwaki K, Tseng YL, Dor FJ, et al. Heart transplantation in baboons using α1,3-galactosyltransferase gene-knockout pigs as donors: Initial experience. *Nat Med* 2005;**11**:29.

Mauiyyedi S, Pelle PD, Saidman S, et al. Chronic humoral rejection: identification of antibody-mediated chronic renal allograft rejection by C4d deposits in peritubular capillaries. *J Am Soc Nephrol* 2001;**12**:574–82.

Najafian N, Albin MJ, Newell KA. How can we measure immunologic tolerance in humans? *J Am Soc Nephrol* 2006;**17**:2652–63.

Racusen LC, Colvin RB, Solez K, et al. Antibody-mediated rejection criteria: An addition to the Banff 97 classification of renal allograft rejection. *Am J Transplant* 2003;**3**: 708–14.

Romagnani S. Regulation of the T cell response. *Clin Exp Allergy* 2006;**36**:1357–66.

Sayegh MH, Carpenter CB. Transplantation 50 years later – progress, challenges, and promises. *N Engl J Med* 2004;**351**:2761–6.

Schwarz BA, Bhandoola A. Trafficking from the bone marrow to the thymus: a prerequisite for thymopoiesis. *Immunol Rev* 2006;**209**:47–57.

Takemoto SK, Zeevi A, Feng S, et al. National conference to assess antibody-mediated rejection in solid organ transplantation. *Am J Transplant* 2004;**4**:1033–41.

Terasaki P, Humoral theory of transplantation. *Am J Transpl* 2003;**3**:665–73.

Tinckam KJ, Chandraker A. Mechanisms and role of HLA and non-HLA alloantibodies. *Clin J Am Soc Nephrol* 2006;**1**:404–14.

Yang YG, Sykes M. Xenotransplantation: current status and a perspective on the future. *Nature Rev Immunol* 2007; **7**:519–31.