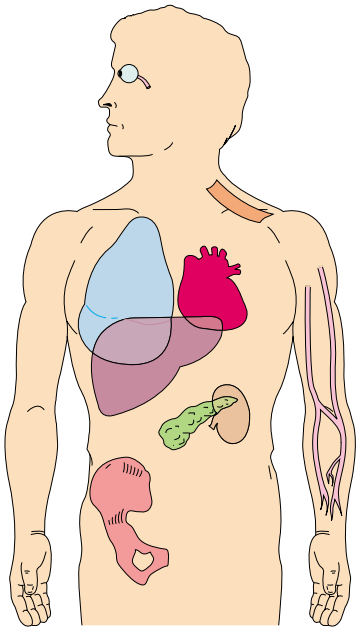
chapter 21

Transplantation Immunology



Transplantations Routinely Used in Clinical Practice

Transplantation, as the term is used in immunology, refers to the act of transferring cells, tissues, or organs from one site to another. The desire to accomplish transplants stems from the realization that many diseases can be cured by implantation of a healthy organ, tissue, or cells (a graft) from one individual (the donor) to another in need of the transplant (the recipient or host). The development of surgical techniques that allow the facile reimplantation of organs has removed one barrier to successful transplantation, but others remain. One is the lack of organs for transplantation. Although a supply of organs is provided by accident victims and, in some cases, living donors, there are more patients in need of transplants than there are organs available. The seriousness of the donor-organ shortage is reflected in the fact that, as of November 2000, an estimated 73,000 patients in the United States were on the waiting list for an organ transplantation. The majority of those on the list (~70%) require a kidney; at present, the waiting period for this organ averages over 800 days. While the lack of organs for transplantation is a serious issue, the most formidable barrier to making transplantation a routine medical treatment is the immune system. The immune system has evolved elaborate and effective mechanisms to protect the organism from attack by foreign agents, and these same mechanisms cause rejection of grafts from anyone who is not genetically identical to the recipient.

Alexis Carrel reported the first systematic study of transplantation in 1908; he interchanged both kidneys in a series of nine cats. Some of those receiving kidneys from other cats maintained urinary output for up to 25 days. Although all the cats eventually died, the experiment established that a transplanted organ could carry out its normal function in the recipient. The first human kidney transplant, attempted in 1935 by a Russian surgeon, failed because there was a mismatch of blood types between donor and recipient. This incompatibility caused almost immediate rejection of the kidney, and the patient died without establishing renal function. The rapid immune response experienced here, termed hyperacute rejection, is mediated by antibodies and will be described in this chapter. The first successful human kidney transplant, which was between identical twins, was accomplished in Boston in 1954. Today, kidney, pancreas, heart, lung, liver, bone-marrow, and cornea transplantations are performed among nonidentical individuals with ever-increasing frequency and success.

A variety of immunosuppressive agents can aid in the survival of the transplants, including drugs and specific antibodies developed to diminish the immunologic attack on grafts, but the majority of these agents have an overall immunosuppressive effect, and their long-term use is deleterious. New methods of inducing specific tolerance to the graft without suppressing other immune responses are being developed and promise longer survival of transplants without compromise of host immunity. This chapter describes the mechanisms underlying graft rejection, various procedures that are used to prolong graft survival, and a summary of the current status of transplantation as a clinical tool. A Clinical Focus section examines the use of organs from nonhuman species (xenotransplants) to circumvent the shortage of organs available for patients in need of them.

Immunologic Basis of Graft Rejection

The degree of immune response to a graft varies with the type of graft. The following terms are used to denote different types of transplants:

- **Autograft** is self-tissue transferred from one body site to another in the same individual. Transferring healthy skin to a burned area in burn patients and use of healthy blood vessels to replace blocked coronary arteries are examples of frequently used autografts.
- **Isograft** is tissue transferred between genetically identical individuals. In inbred strains of mice, an isograft can be performed from one mouse to another syngeneic mouse. In humans, an isograft can be performed between genetically identical (monozygotic) twins.
- **Allograft** is tissue transferred between genetically different members of the same species. In mice, an allograft is performed by transferring tissue or an organ from one strain to another. In humans, organ grafts from one individual to another are allografts unless the donor and recipient are identical twins.
- **Xenograft** is tissue transferred between different species (e.g., the graft of a baboon heart into a human). Because of significant shortages in donated organs, raising animals for the specific purpose of serving as organ donors for humans is under serious consideration.

Autografts and isografts are usually accepted, owing to the genetic identity between graft and host (Figure 21-1a). Because an allograft is genetically dissimilar to the host, it is often recognized as foreign by the immune system and is rejected. Obviously, xenografts exhibit the greatest genetic disparity and therefore engender a vigorous graft rejection.

Allograft Rejection Displays Specificity and Memory

The rate of allograft rejection varies according to the tissue involved. In general, skin grafts are rejected faster than other tissues such as kidney or heart. Despite these time differences, the immune response culminating in graft rejection always displays the attributes of specificity and memory. If an inbred mouse of strain A is grafted with skin from strain B, primary graft rejection, known as first-set rejection, occurs (Figure 21-1b). The skin first becomes revascularized between days 3 and 7; as the reaction develops, the vascularized transplant becomes infiltrated with lymphocytes, monocytes, neutrophils, and other inflammatory cells. There is decreased vascularization of the transplanted tissue by 7–10 days, visible necrosis by 10 days, and complete rejection by 12–14 days.

Immunologic memory is demonstrated when a second strain-B graft is transferred to a previously grafted strain-A mouse. In this case, a graft-rejection reaction develops more quickly, with complete rejection occurring within 5–6 days; this secondary response is designated second-set rejection (Figure 21-1c). The specificity of second-set rejection can be demonstrated by grafting an unrelated strain-C graft at the same time as the second strain-B graft. Rejection of the strain-C graft proceeds according to first-set rejection kinetics, whereas the strain-B graft is rejected in an accelerated second-set fashion.

T Cells Play a Key Role in Allograft Rejection

In the early 1950s, Avrion Mitchison showed in adoptive-transfer experiments that lymphocytes, but not serum antibody, could transfer allograft immunity. Later studies implicated T cells in allograft rejection. For example, nude mice, which lack a thymus and consequently lack functional T cells, were found to be incapable of allograft rejection; indeed, these mice even accept xenografts. In other studies, T cells derived from an allograft-primed mouse were shown to transfer second-set allograft rejection to an unprimed syngeneic recipient, as long as that recipient was grafted with the same allogeneic tissue (Figure 21-2).

Analysis of the T-cell subpopulations involved in allograft rejection has implicated both $CD4^+$ and $CD8^+$ populations. In one study, mice were injected with monoclonal antibodies to deplete one or both types of T cells and then the rate of graft rejection was measured. As shown in Figure 21-3, removal of the $CD8^+$ population alone had no effect on graft survival, and the graft was rejected at the same rate as in control mice (15 days). Removal of the $CD4^+$ T-cell population alone prolonged graft survival from 15 days to 30 days. However, removal of both the $CD4^+$ and the $CD8^+$ T cells resulted in long-term survival (up to 60 days) of the allografts. This study indicated that both $CD4^+$ and $CD8^+$ T-cells participated in rejection and that the collaboration of both subpopulations resulted in more pronounced graft rejection.

Similar Antigenic Profiles Foster Allograft Acceptance

Tissues that are antigenically similar are said to be **histocompatible;** such tissues do not induce an immunologic response that leads to tissue rejection. Tissues that display significant antigenic differences are *histoincompatible* and induce an immune response that leads to tissue rejection. The various antigens that determine histocompatibility are encoded by more than 40 different loci, but the loci responsible for the most vigorous allograft-rejection reactions are located within the **major histocompatibility complex (MHC).** The organization of the MHC—called the H-2 complex in mice and the HLA complex in humans—was described in Chapter 7 (see Figure 7-1). Because the MHC loci are closely linked, they are usually inherited as a complete set, called a **haplotype,** from each parent.

VISUALIZING CONCEPTS

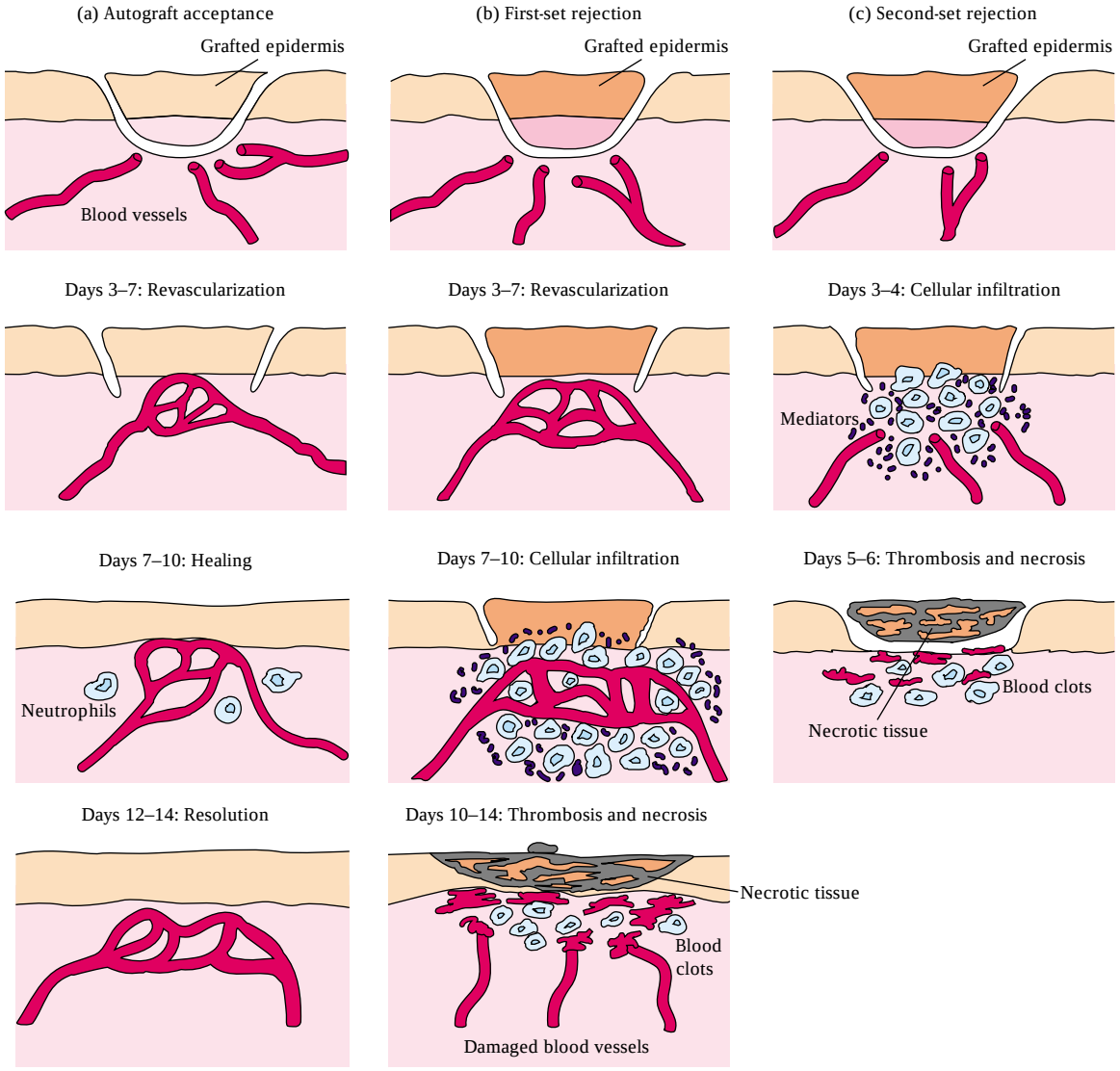


FIGURE 21-1 Schematic diagrams of the process of graft acceptance and rejection. (a) Acceptance of an autograft is completed within 12–14 days. (b) First-set rejection of an allograft begins 7–10 days after grafting, with full rejection occurring by 10–14 days. (c) Second-set rejection of an allograft begins within 3–4 days, with full rejection by 5–6 days. The cellular infiltrate that invades an allograft (b, c) contains lymphocytes, phagocytes, and other inflammatory cells.

Within an inbred strain of mice, all animals are homozygous at each MHC locus. When mice from two different inbred strains, with haplotypes *b* and *k*, for example, are mated, all the F_1 progeny inherit one haplotype from each parent (see Figure 7-2a). These F_1 offspring have the MHC type *b/k* and can accept grafts from either parent. Neither of the parental strains, however, can accept grafts from the F_1 offspring because each parent lacks one of the F_1 haplotypes. MHC inheritance in outbred populations is more complex, because the high degree of polymorphism exhibited at each MHC locus

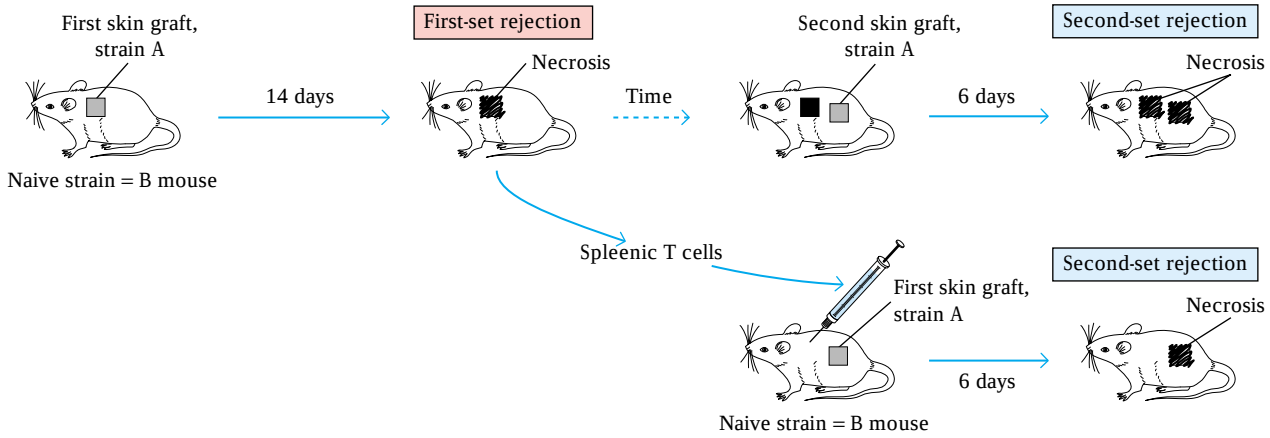


FIGURE 21-2 Experimental demonstration that T cells can transfer allograft rejection. When T cells derived from an allograft-primed mouse are transferred to an unprimed syngeneic mouse, the recipient mounts a second-set rejection to an initial allograft from the original allogeneic strain.

gives a high probability of heterozygosity at most loci. In matings between members of an outbred species, there is only a 25% chance that any two offspring will inherit identical MHC haplotypes (see Figure 7-2c), unless the parents share one or more haplotypes. Therefore, for purposes of organ or bone-marrow grafts, it can be assumed that there is a 25% chance of identity within the MHC between siblings. With parent-to-child grafts, the donor and recipient will always have one haplotype in common but are nearly always mismatched for the haplotype inherited from the other parent.

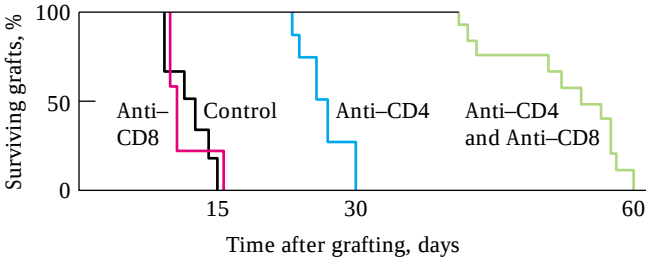


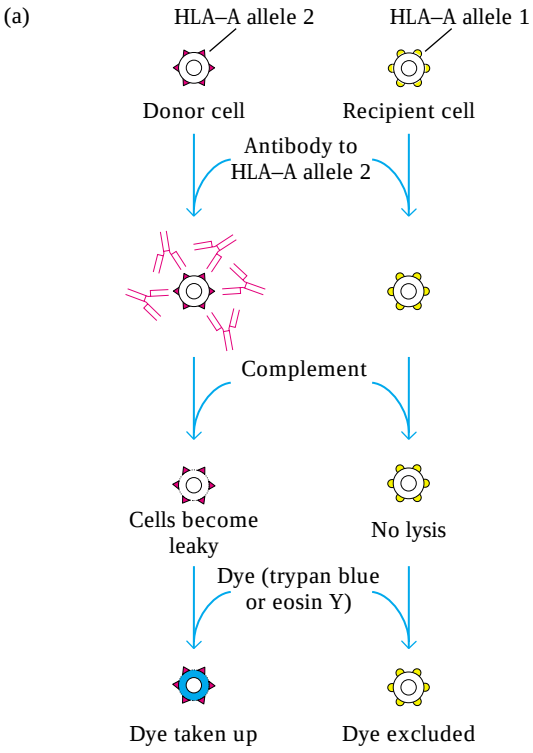
FIGURE 21-3 The role of $CD4^+$ and $CD8^+$ T cells in allograft rejection is demonstrated by the curves showing survival times of skin grafts between mice mismatched at the MHC. Animals in which the $CD8^+$ T cells were removed by treatment with an anti-CD8 monoclonal antibody (red) showed little difference from untreated control mice (black). Treatment with monoclonal anti-CD4 (blue) improved graft survival significantly, and treatment with both anti-CD4 and anti-CD8 antibody prolonged graft survival most dramatically (green). *[Adapted from S. P. Cobbold et al., 1986,* Nature ***323****:165.]*

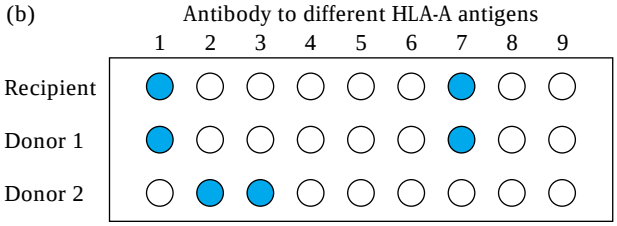
Graft Donors and Recipients Are Typed for RBC and MHC Antigens

Since differences in blood group and major histocompatibility antigens are responsible for the most intense graft-rejection reactions, various tissue-typing procedures to identify these antigens have been developed to screen potential donor and recipient cells. Initially, donor and recipient are screened for ABO blood-group compatibility. The blood-group antigens are expressed on RBCs, epithelial cells, and endothelial cells. Antibodies produced in the recipient to any of these antigens that are present on transplanted tissue will induce antibody-mediated complement lysis of the incompatible donor cells.

HLA typing of potential donors and a recipient can be accomplished with a microcytotoxicity test (Figure 21-4a, b). In this test, white blood cells from the potential donors and recipient are distributed into a series of wells on a microtiter plate, and then antibodies specific for various class I and class II MHC alleles are added to different wells. After incubation, complement is added to the wells, and cytotoxicity is assessed by the uptake or exclusion of various dyes (e.g., trypan blue or eosin Y) by the cells. If the white blood cells express the MHC allele for which a particular monoclonal antibody is specific, then the cells will be lysed upon addition of complement, and these dead cells will take up a dye such as trypan blue. HLA typing based on antibody-mediated microcytotoxicity can thus indicate the presence or absence of various MHC alleles.

Even when a fully HLA-compatible donor is not available, transplantation may be successful. In this situation, a one-way mixed-lymphocyte reaction (MLR) can be used to quantify the degree of class II MHC compatibility between potential





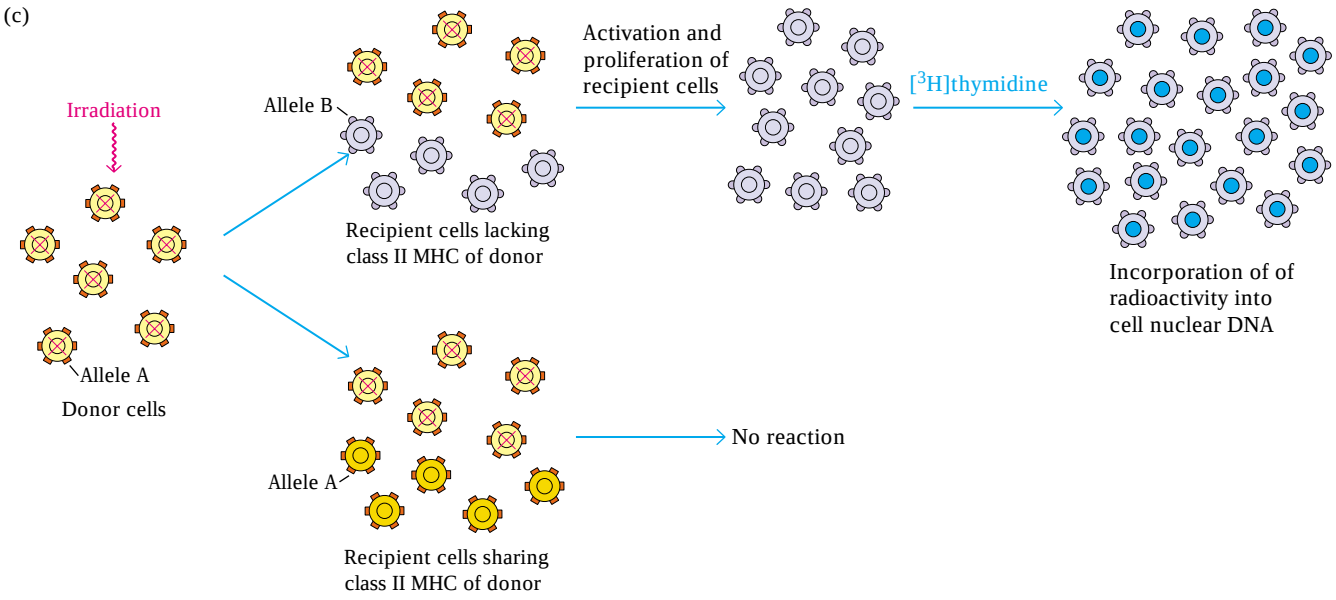


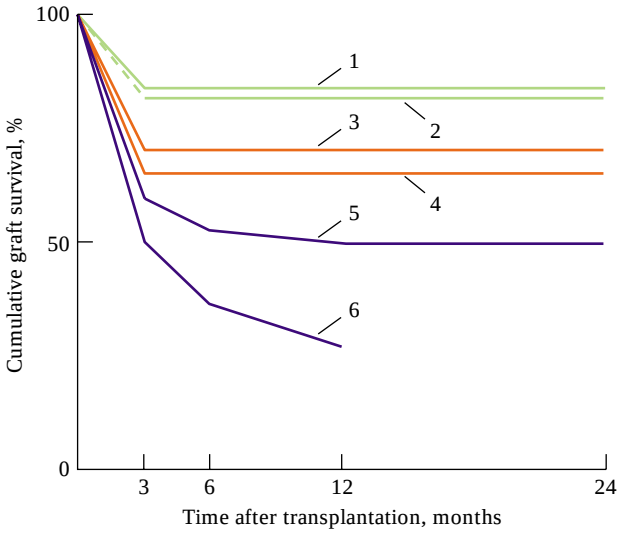
FIGURE 21-4 Typing procedures for HLA antigens. (a, b) HLA typing by microcytotoxicity. (a) White blood cells from potential donors and the recipient are added to separate wells of a microtiter plate. The example depicts the reaction of donor and recipient cells with a single antibody directed against an HLA-A antigen. The reaction sequence shows that if the antigen is present on the lymphocytes, addition of complement will cause them to become porous and unable to exclude the added dye. (b) Because cells express numerous HLA antigens, they are tested separately with a battery of antibodies specific for various HLA-A antigens. Here, donor 1 shares HLA-A antigens recognized by antisera in wells 1 and 7 with the recipient, whereas donor 2 has none of HLA-A antigens in common with the recipient. (c) Mixed lymphocyte reaction to determine identity of class II HLA antigens between a potential donor and recipient. Lymphocytes from the donor are irradiated or treated with mitomycin C to prevent cell division and then added to cells from the recipient. If the class II antigens on the two cell populations are different, the recipient cells will divide rapidly and take up large quantities of radioactive nucleotides into the newly synthesized nuclear DNA. The amount of radioactive nucleotide uptake is roughly proportionate to the MHC class II differences between the donor and recipient lymphocytes.

donors and a recipient (Figure 21-4c). Lymphocytes from a potential donor that have been x-irradiated or treated with mitomycin C serve as the stimulator cells, and lymphocytes from the recipient serve as responder cells. Proliferation of the recipient T cells, which indicates T-cell activation, is measured by the uptake of [^{3}H]thymidine into cell DNA. The greater the class II MHC differences between the donor and recipient cells, the more [^{3}H]thymidine uptake will be observed in an MLR assay. Intense proliferation of the recipient lymphocytes indicates a poor prognosis for graft survival. The advantage of the MLR over microcytotoxicity typing is that it gives a better indication of the degree of T_H-cell activation generated in response to the class II MHC antigens of the potential graft. The disadvantage of the MLR is that it takes several days to run the assay. If the potential donor is a cadaver, for example, it is not possible to wait for the results of the MLR, because the organ must be used soon after removal from the cadaver. In that case, the microcytotoxicity test, which can be performed within a few hours, must be relied on.

The importance of MHC matching for acceptance of allografts is confirmed by data gathered from recipients of kidney transplants. The data in Figure 21-5 reveal that survival of kidney grafts depends primarily on donor-recipient matching of the HLA class II antigens. Matching or mismatching of the class I antigens has a lesser effect on graft survival unless there also is mismatching of the class II antigens. A two-year survival rate of 90% is seen for kidney transplants in which one or two class I HLA loci are mismatched, while transplanted kidneys with differences in the class II MHC have only a 70% chance of lasting for this period. Those with greater numbers of mismatches have a very low survival rate at one year after transplant. As described below, HLA matching is most important for kidney and bone-marrow transplants; liver and heart transplants may survive with greater mismatching.

Current understanding of the killer-inhibitory receptors (KIR) on the NK cell (see Chapter 14) suggests that absence of a class I antigen recognized by the KIR molecules could lead to killing of the foreign cell. Rejection was observed in experimental bone-marrow transplants where the class I molecule recognized by the recipient NK-inhibitory receptor is absent on donor cells. The effects of such class I mismatching on solid organ grafts may be less marked.

MHC identity of donor and host is not the sole factor determining tissue acceptance. When tissue is transplanted between genetically different individuals, even if their MHC antigens are identical, the transplanted tissue can be rejected because of differences at various **minor histocompatibility loci.** As described in Chapter 10, the major histocompatibility antigens are recognized directly by T_H and T_C cells, a phenomenon termed *alloreactivity*. In contrast, minor histocompatibility antigens are recognized only when they are presented in the context of self-MHC molecules. The tissue rejection induced by minor histocompatibility differences



Curve no.	HLA mismatches (no.) Class I	Class II
1	0	0
2	1 or 2	0
3	3 or 4	0
4	0	1 or 2
5	1 or 2	1 or 2
6	3 or 4	1 or 2

FIGURE 21-5 The effect of HLA class I and class II antigen matching on survival of kidney grafts. Mismatching of one or two class I (HLA-A or HLA-B) antigens has little effect on graft survival. A single class II difference (line 4) has the same effect as 3 or 4 differences in class I antigens (line 3). When both class I and class II antigens are mismatched, rejection is accelerated. *[Adapted from T. Moen et al., 1980,* N. Engl. J. Med. ***303:****850.]*

is usually less vigorous than that induced by major histocompatibility differences. Still, reaction to these minor tissue differences often results in graft rejection. For this reason, successful transplantation even between HLA-identical individuals requires some degree of immune suppression.

Cell-Mediated Graft Rejection Occurs in Two Stages

Graft rejection is caused principally by a cell-mediated immune response to alloantigens (primarily, MHC molecules) expressed on cells of the graft. Both delayed-type hypersensitive and cell-mediated cytotoxicity reactions have been implicated. The process of graft rejection can be divided into two stages: (1) a sensitization phase, in which antigen-reactive lymphocytes of the recipient proliferate in response to allo-

antigens on the graft, and (2) an effector stage, in which immune destruction of the graft takes place.

SENSITIZATION STAGE

During the sensitization phase, $CD4^+$ and $CD8^+$ T cells recognize alloantigens expressed on cells of the foreign graft and proliferate in response. Both major and minor histocompatibility alloantigens can be recognized. In general, the response to minor histocompatibility antigens is weak, although the combined response to several minor differences can sometimes be quite vigorous. The response to major histocompatibility antigens involves recognition of both the donor MHC molecule and an associated peptide ligand in the cleft of the MHC molecule. The peptides present in the groove of allogeneic class I MHC molecules are derived from proteins synthesized within the allogeneic cell. The peptides present in the groove of allogeneic class II MHC molecules are generally proteins taken up and processed through the endocytic pathway of the allogeneic antigen-presenting cell.

A host T_H cell becomes activated when it interacts with an antigen-presenting cell (APC) that both expresses an appropriate antigenic ligand–MHC molecule complex and provides the requisite co-stimulatory signal. Depending on the tissue, different populations of cells within a graft may function as APCs. Because dendritic cells are found in most tissues and because they constitutively express high levels of class II MHC molecules, dendritic cells generally serve as the major APC in grafts. APCs of host origin can also migrate into a graft and endocytose the foreign alloantigens (both major and minor histocompatibility molecules) and present them as processed peptides together with self-MHC molecules.

In some organ and tissue grafts (e.g., grafts of kidney, thymus, and pancreatic islets), a population of donor APCs called *passenger leukocytes* has been shown to migrate from the graft to the regional lymph nodes. These passenger leukocytes are dendritic cells, which express high levels of class II MHC molecules (together with normal levels of class I MHC molecules) and are widespread in mammalian tissues, with the chief exception of the brain. Because passenger leukocytes express the allogeneic MHC antigens of the donor graft, they are recognized as foreign and therefore can stimulate immune activation of T lymphocytes in the lymph node. In some experimental situations, the passenger cells have been shown to induce tolerance to their surface antigens by deletion of thymic T-cell populations with receptors specific for them. Consistent with the notion that exposure to donor cells can induce tolerance are data showing that blood tranfusions from the donor prior to transplantation can aid acceptance of the graft.

Passenger leukocytes are not the only cells involved in immune stimulation. For example, they do not seem to play any role in skin grafts. Other cell types that have been implicated in alloantigen presentation to the immune system include Langerhans cells and endothelial cells lining the blood vessels. Both of these cell types express class I and class II MHC antigens.

Recognition of the alloantigens expressed on the cells of a graft induces vigorous T-cell proliferation in the host. This proliferation can be demonstrated in vitro in a mixed-lymphocyte reaction (see Figure 21-4c). Both dendritic cells and vascular endothelial cells from an allogeneic graft induce host T-cell proliferation. The major proliferating cell is the $CD4^+$ T cell, which recognizes class II alloantigens directly or alloantigen peptides presented by host antigen-presenting cells. This amplified population of activated T_H cells is thought to play a central role in inducing the various effector mechanisms of allograft rejection.

EFFECTOR STAGE

A variety of effector mechanisms participate in allograft rejection (Figure 21-6). The most common are cell-mediated reactions involving delayed-type hypersensitivity and CTL-mediated cytotoxicity; less common mechanisms are antibody-plus-complement lysis and destruction by antibody-dependent cell-mediated cytotoxicity (ADCC). The hallmark of graft rejection involving cell-mediated reactions is an influx of T cells and macrophages into the graft. Histologically, the infiltration in many cases resembles that seen during a delayed-type hypersensitive response, in which cytokines produced by T_{DTH} cells promote macrophage infiltration (see Figure 14-15). Recognition of foreign class I alloantigens on the graft by host $CD8^+$ cells can lead to CTL-mediated killing (see Figure 14-4). In some cases, $CD4^+$ T cells that function as class II MHC–restricted cytotoxic cells mediate graft rejection.

In each of these effector mechanisms, cytokines secreted by T_H cells play a central role (see Figure 21-6). For example, IL-2, IFN-γ, and TNF-β have each been shown to be important mediators of graft rejection. IL-2 promotes T-cell proliferation and generally is necessary for the generation of effector CTLs (see Figure 14-1). IFN-γ is central to the development of a DTH response, promoting the influx of macrophages into the graft and their subsequent activation into more destructive cells. TNF-β has been shown to have a direct cytotoxic effect on the cells of a graft. A number of cytokines promote graft rejection by inducing expression of class I or class II MHC molecules on graft cells. The interferons (α, β, and γ), TNF-α, and TNF-β all increase class I MHC expression, and IFN-γ increases class II MHC expression as well. During a rejection episode, the levels of these cytokines increase, inducing a variety of cell types within the graft to express class I or class II MHC molecules. In rat cardiac allografts, for example, dendritic cells are initially the only cells that express class II MHC molecules. However, as an allograft reaction begins, localized production of IFN-γ in the graft induces vascular endothelial cells and myocytes to express class II MHC molecules as well, making these cells targets for CTL attack.

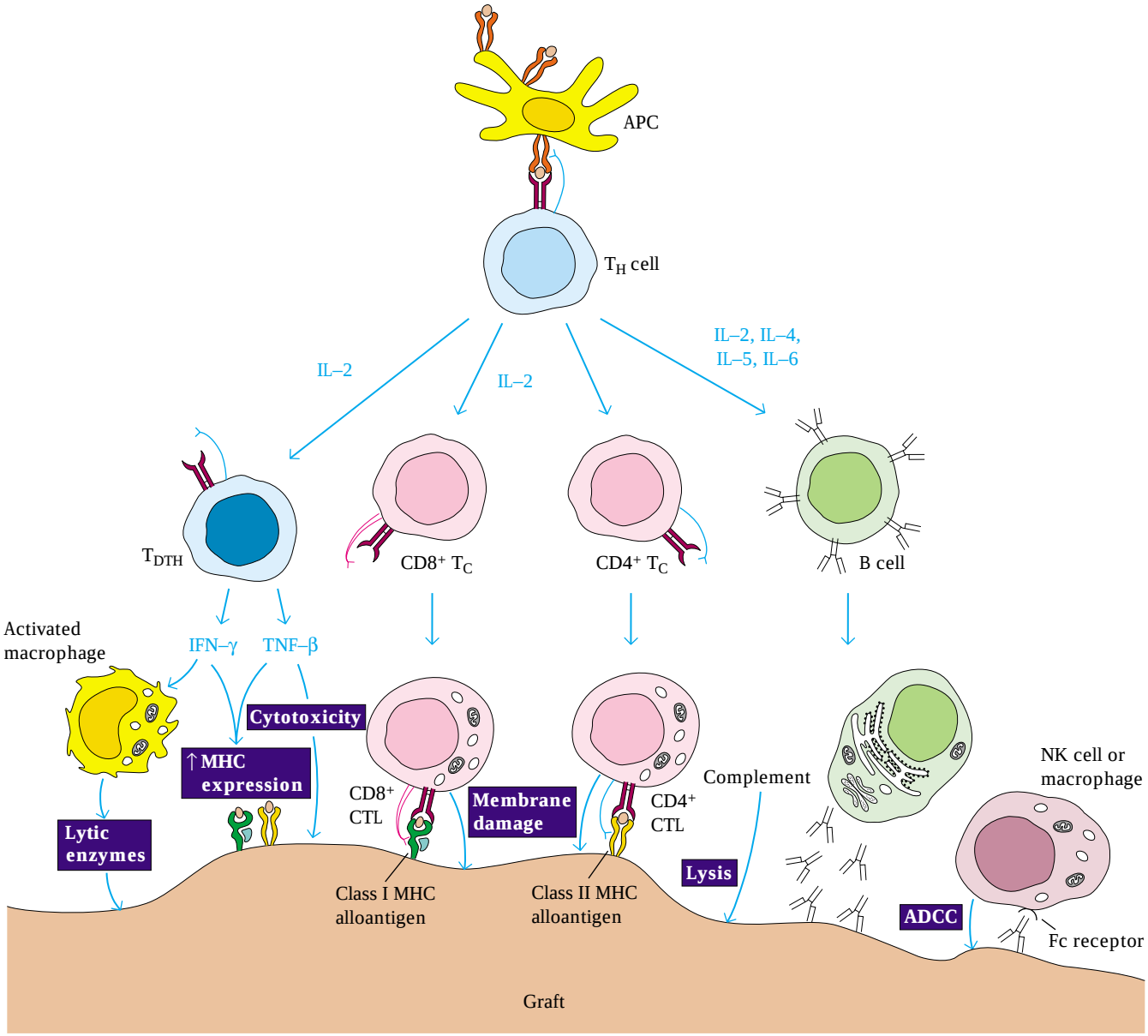


FIGURE 21-6 Effector mechanisms (purple blocks) involved in allograft rejection. The generation or activity of various effector cells depends directly or indirectly on cytokines (blue) secreted by activated T_H cells. ADCC = antibody-dependent cell-mediated cytotoxicity.

Clinical Manifestations of Graft Rejection

Graft-rejection reactions have various time courses depending upon the type of tissue or organ grafted and the immune response involved. Hyperacute rejection reactions occur within the first 24 hours after transplantation; acute rejection reactions usually begin in the first few weeks after transplantation; and chronic rejection reactions can occur from months to years after transplantation.

Pre-Existing Recipient Antibodies Mediate Hyperacute Rejection

In rare instances, a transplant is rejected so quickly that the grafted tissue never becomes vascularized. These hyperacute reactions are caused by preexisting host serum antibodies specific for antigens of the graft. The antigen-antibody complexes that form activate the complement system, resulting in an intense infiltration of neutrophils into the grafted tissue. The ensuing inflammatory reaction causes massive blood clots within the capillaries, preventing vascularization of the graft (Figure 21-7).

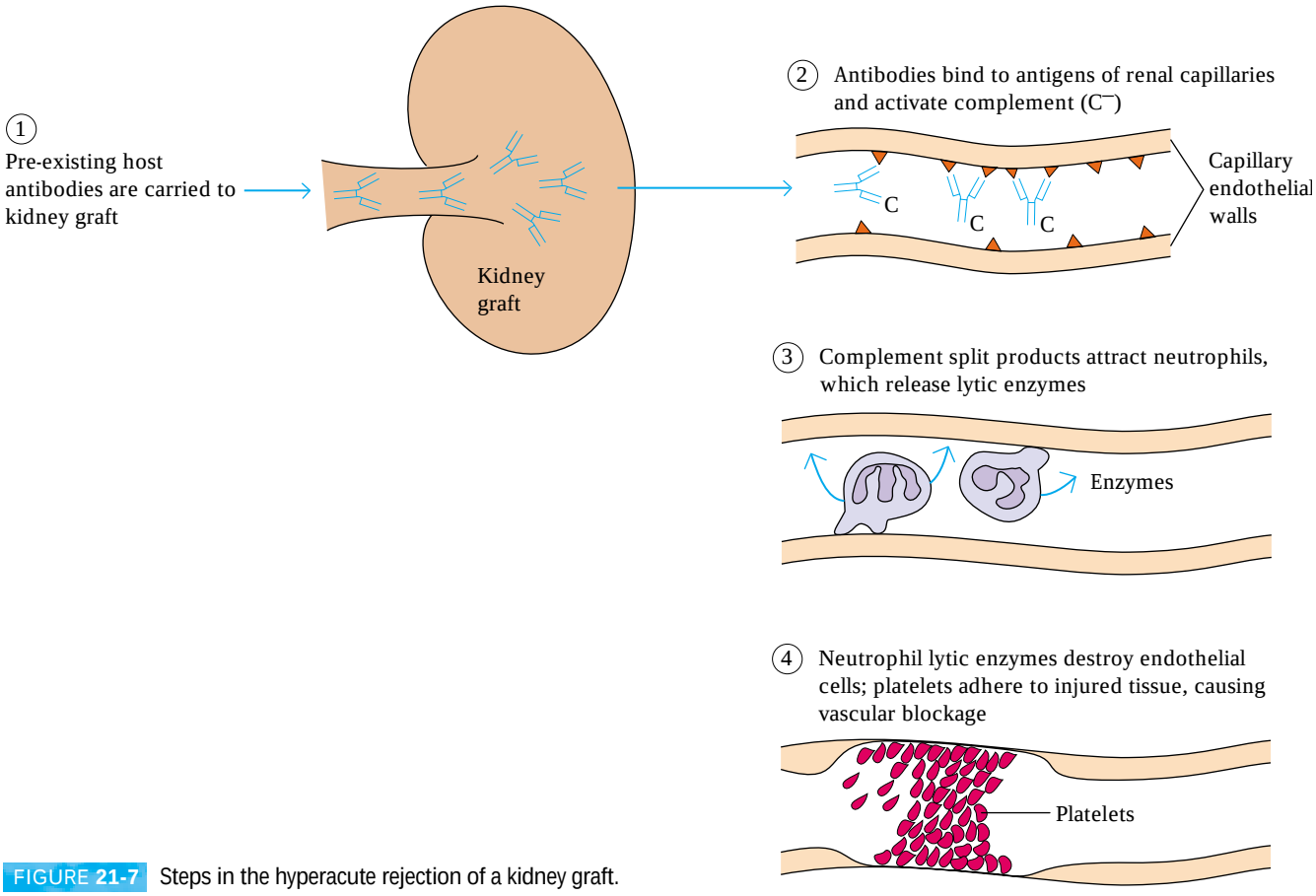


FIGURE 21-7 Steps in the hyperacute rejection of a kidney graft.

Several mechanisms can account for the presence of preexisting antibodies specific for allogeneic MHC antigens. Recipients of repeated blood transfusions sometimes develop significant levels of antibodies to MHC antigens expressed on white blood cells present in the transfused blood. If some of these MHC antigens are the same as those on a subsequent graft, then the antibodies can react with the graft, inducing a hyperacute rejection reaction. With repeated pregnancies, women are exposed to the paternal alloantigens of the fetus and may develop antibodies to these antigens. Finally, individuals who have had a previous graft sometimes have high levels of antibodies to the allogeneic MHC antigens of that graft.

In some cases, the preexisting antibodies participating in hyperacute graft rejection may be specific for blood-group antigens in the graft. If tissue typing and ABO blood-group typing are performed prior to transplantation, these preexisting antibodies can be detected and grafts that would result in hyperacute rejection can be avoided. Xenotransplants are often rejected in a hyperacute manner because of antibodies to cellular antigens of the donor species that are not present in the recipient species. Such an antigen is discussed in the Clinical Focus section of this chapter.

In addition to the hyperacute rejection mediated by preexisting antibodies, there is a less frequent form of rejection termed *accelerated rejection* caused by antibodies that are produced immediately after transplantation.

Acute Rejection Is Mediated by T-Cell Responses

Cell-mediated allograft rejection manifests as an acute rejection of the graft beginning about 10 days after transplantation (see Figure 21-1b). Histopathologic examination reveals a massive infiltration of macrophages and lymphocytes at the site of tissue destruction, suggestive of T_H-cell activation and proliferation. Acute graft rejection is effected by the mechanisms described previously (see Figure 21-6).

Chronic Rejection Occurs Months or Years Post-Transplant

Chronic rejection reactions develop months or years after acute rejection reactions have subsided. The mechanisms of chronic rejection include both humoral and cell-mediated responses by the recipient. While the use of immunosuppressive drugs and the application of tissue-typing methods to obtain optimum match of donor and recipient have dramatically increased survival of allografts during the first years after engraftment, little

CLINICAL FOCUS

Is There a Clinical Future for Xenotransplantation?

Unless organ donations increase drastically, most of the 72,000 U.S. patients on the waiting list for a transplant will not receive one. The majority (47,000) need a kidney, but last year only 12,500 kidneys were transplanted. A solution to this shortfall is to utilize animal organs. Some argue that xenografts bring the risk of introducing pathogenic retroviruses into the human population; others object based on ethical grounds relating to animal rights. Nevertheless, the use of pigs to supply organs for humans is under serious consideration. Pigs breed rapidly, have large litters, can be housed in pathogen-free environments, and share considerable anatomic and physiologic similarity with humans. In fact, pigs have served as donors of cardiac valves for humans for years. Primates are more closely related to humans than pigs are, but the availability of large primates as transplant donors is, and will continue to be, extremely limited.

Balancing the advantages of pig donors are serious difficulites. For example, if a pig kidney were implanted into a human by techniques standard for human transplants, it would likely fail in a rapid and dramatic fashion due to hyperacute rejection. This antibody-mediated rejection is due to the presence on the pig cells (and those of most mammals other than humans and the highest nonhuman primates) of a disaccharide antigen (galactosyl-1,3-α-galactose) that is not present on human cells. The presence of this antigen on many microorganisms means that nearly everyone has been exposed to it and has formed antibodies against it. The preexisting antibodies react with pig cells, which are then lysed rapidly by complement. The absence of human regulators of complement activity on the pig cells, including human decay-accelerating factor (DAF) and human membrane-cofactor protein (MCP), intensifies the complement lysis cycle. (See Chapter 13 for descriptions of DAF and MCP.)

How can this major obstacle be circumvented? Being tested are strategies for absorbing the antibodies from the circulation on solid supports, and using soluble gal-gal disaccharides to block antibody reactions. A more elegant solution involves genetically engineering pigs to knock out the gene for the enzyme that synthesizes galactosyl-1,3-α-galactose. Solving the immediate rejection reaction by interfering with the specific reaction against this antigen may not prevent all antibody-mediated rejection. Certainly other antigenic differences to which human recipients have antibodies will be present in some if not all donor/recipient pairs. However, any antibody attack on the pig cells may be blunted if human DAF is present on the targeted cell to dampen the complement reaction. The lack of human DAF is remedied by producing transgenic pigs that express this protein. Addition of human complement regulators to the pig represents a universal solution, in that any cell that might become a target in the transplant will resist complement lysis.

An additional concern is that pig endogenous retroviruses will be introduced into humans as a result of xenotransplantation and cause disease. Opponents of xenotransplantation raise the specter of another HIV-type epidemic resulting from human infection by a new animal retrovirus. Recently, a Boston-based company announced development of pigs free of endogenous pig retroviruses, reducing the possibility of this bleak outcome.

Will we see the use of pig kidneys in humans in the near future? The increasing demand for organs is driving the commercial development of colonies of pigs suitable to become organ donors. While kidneys are the most sought-after organ at present, other organs and cells from the specially bred and engineered animals will find use if they are proven to be safe and effective. A statement from the American Society of Transplantation and the American Society of Transplant Surgeons endorses the use of xenotransplants if certain conditions are met (*Xenotransplantation* **7:**235). These include the demonstration of feasibility in a nonhuman primate model, proven benefit to the patient, and lack of infectious-disease risk. Barriers remain to the clinical use of xenotransplants, but serious efforts are in motion to overcome them.

progress has been made in long-term survival. The use of immunosuppressive drugs, which are described below, greatly increases the short-term survival of the transplant, but chronic rejection is not prevented in most cases. Data for rejection of kidney transplants since 1975 indicates an increase from 40% to over 80% in one-year survival of grafts. However, in the same period long-term survival has risen only slightly; as in 1975, about 50% of transplanted kidneys are still functioning at 10 years after transplant. Chronic rejection reactions are difficult to manage with immunosuppressive drugs and may necessitate another transplantation.

General Immunosuppressive Therapy

Allogeneic transplantation requires some degree of immunosuppression if the transplant is to survive. Most of the immunosuppressive treatments that have been developed have the disadvantage of being nonspecific; that is, they result in generalized immunosuppression of responses to all antigens, not just those of the allograft, which places the recipient at increased risk of infection. In addition, many

immunosuppressive measures are aimed at slowing the proliferation of activated lymphocytes. However, because any rapidly dividing nonimmune cells (e.g., epithelial cells of the gut or bone-marrow hematopoietic stem cells) are also affected, serious or even life-threatening complications can occur. Patients on long-term immunosuppressive therapy are at increased risk of cancer, hypertension, and metabolic bone disease.

Mitotic Inhibitors Thwart T-Cell Proliferation

Azathioprine (Imuran), a potent mitotic inhibitor, is often given just before and after transplantation to diminish T-cell proliferation in response to the alloantigens of the graft. Azathioprine acts on cells in the S phase of the cell cycle to block synthesis of inosinic acid, which is a precursor of the purines adenylic and guanylic acid. Both B-cell and T-cell proliferation is diminished in the presence of azathioprine. Functional immune assays such as the MLR, CML, and skin test show a significant decline after azathioprine treatment, indicating an overall decrease in T-cell numbers.

Two other mitotic inhibitors that are sometimes used in conjunction with other immunosuppressive agents are cyclophosphamide and methotrexate. Cyclophosphamide is an alkylating agent that inserts into the DNA helix and becomes cross-linked, leading to disruption of the DNA chain. It is especially effective against rapidly dividing cells and therefore is sometimes given at the time of grafting to block T-cell proliferation. Methotrexate acts as a folic-acid antagonist to block purine biosynthesis. The fact that the mitotic inhibitors act on all rapidly dividing cells and not specifically on those involved in immune response against the allograft can lead to deleterious side reactions by thwarting division of other functional cells in the body.

Corticosteroids Suppress Inflammation

As described at the end of Chapter 15, corticosteroids, such as prednisone and dexamethasone, are potent anti-inflammatory agents that exert their effects at many levels of the immune response. These drugs are often given to transplant recipients together with a mitotic inhibitor such as azathioprine to prevent acute episodes of graft rejection.

Certain Fungal Metabolites Are Immunosuppressants

Cyclosporin A (CsA), FK506 (tacrolimus), and rapamycin (sirolimus) are fungal metabolites with immunosuppressive properties. Although chemically unrelated, CsA and FK506 have similar actions. Both drugs block activation of resting T cells by inhibiting the transcription of genes encoding IL-2 and the high-affinity IL-2 receptor (IL-2R), which are essential for activation. CsA and FK506 exert this effect by binding to cytoplasmic proteins called immunophilins, forming a complex that blocks the phosphatase activity of calcineurin. This prevents the formation and nuclear translocation of the cytoplasmic subunit NFATc and its subsequent assembly into NFAT, a DNA-binding protein necessary for transcription of the genes encoding a number of molecules important to T-cell activation (see Figure 10-11). Rapamycin is structurally similar to FK506 and also binds to an immunophilin. However, the rapamycin-immunophilin complex does not inhibit calcineurin activity; instead, it blocks the proliferation and differentiation of activated T_H cells in the G_1 phase of the cell cycle. All three drugs, by inhibiting T_H-cell proliferation and thus T_H-cell cytokine expression, reduce the subsequent activation of various effector populations involved in graft rejection, including T_H cells, T_C cells, NK cells, macrophages, and B cells.

The profound immunosuppressive properties of these three agents have made them a mainstay of heart, liver, kidney, and bone-marrow transplantation. Cyclosporin A has been shown to prolong graft survival in kidney, liver, heart, and heart-lung transplants. In one study of 209 kidney transplants from cadaver donors, the 1-year survival rate was 64% among recipients receiving other immunosuppressive treatments and 80% among those receiving cyclosporin A. Similar results have been obtained with liver transplants (Figure 21-8). Despite these impressive results, CsA does have some negative side effects, the most notable of which is toxicity to the kidneys. Acute nephrotoxicity is quite common, in some cases progressing to chronic nephrotoxicity and drug-induced kidney failure. FK506 and rapamycin are 10–100 times more potent as immune suppressants than CsA, and therefore can be administered at lower doses and with fewer side effects than CsA.

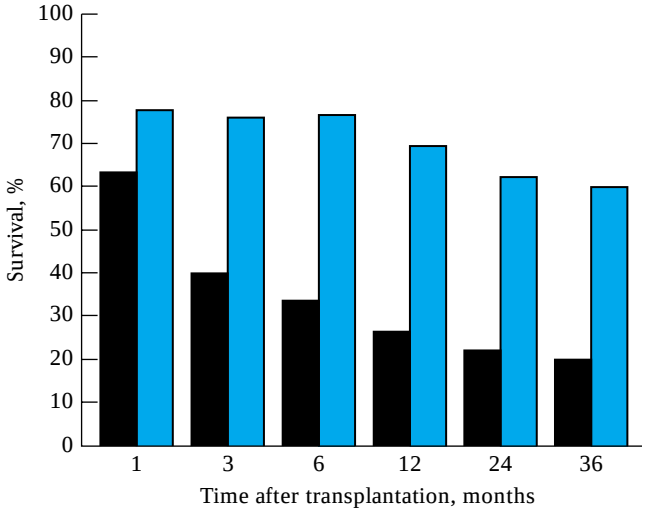


FIGURE 21-8 Comparison of the survival rate of liver transplants in 84 patients who were immunosuppressed with azathioprine and corticosteroids (black) with the survival rate in 55 patients who were immunosuppressed with cyclosporin A and corticosteroids (blue). *[Adapted from S. M. Sabesin and J. W. Williams, 1987,* Hosp. Pract. ***15****(July):75.]*

Total Lymphoid Irradiation Eliminates Lymphocytes

Because lymphocytes are extremely sensitive to x-rays, x-irradiation can be used to eliminate them in the transplant recipient just before grafting. In total lymphoid x-irradiation, the recipient receives multiple x-ray exposures to the thymus, spleen, and lymph nodes before the transplant surgery. The typical protocol is daily x-irradiation treatments of about 200 rads per day for several weeks until a total of 3400 rads has been administered. The recipient is grafted in this immunosuppressed state. Because the bone marrow is not x-irradiated, lymphoid stem cells proliferate and renew the population of recirculating lymphocytes. These newly formed lymphocytes appear to be more tolerant to the antigens of the graft.

Specific Immunosuppressive Therapy

In addition to harmful side effects peculiar to the various immunosuppressive treatments described above, a major limitation common to all is that they lack specificity, thus producing a more-or-less generalized immunosuppression and increasing the recipient's risk for infection. What is needed ideally is an antigen-specific immunosuppressant that reduces the immune response to the alloantigens of the graft while preserving the recipient's ability to respond to other foreign antigens. Although this goal has not yet been achieved in human transplants, recent successes in animal experiments indicate that it may be possible. Specific immunosuppression to allografts has been achieved in animal experiments using antibodies or soluble ligands reactive with cell-surface molecules.

Monoclonal Antibodies Can Suppress Graft-Rejection Responses

Monoclonal antibodies directed against various surface molecules on cells of the immune system have been used successfully to suppress T-cell activity in general or to suppress the activity of subpopulations of T cells. Results from studies with animal models suggest further that certain monoclonals may be used to suppress only T cells that are activated. Successes with animal models and trials with humans give reason to believe that two types of strategies involving antibodies to suppress rejection will find broad clinical use. Monoclonal antibodies may be used to deplete the recipient of a certain broad or specific cell population; alternatively, they may be used to block co-stimulatory signals. In the latter case, a state of anergy is induced in those T cells that react to antigens present on the allograft.

A strategy to deplete immune cells involves use of a monoclonal antibody to the CD3 molecule of the TCR complex. Injection of such monoclonal antibodies results in a rapid depletion of mature T cells from the circulation. This depletion appears to be caused by binding of antibody-coated T cells to Fc receptors on phagocytic cells, which then phagocytose and clear the T cells from the circulation. In a further refinement of this strategy, a cytotoxic agent such as diphtheria toxin is coupled with the antibody. The cell with which the antibody reacts internalizes the toxin, causing its death. Another depletion strategy used to increase graft survival uses monoclonal antibodies specific for the high-affinity IL-2 receptor (anti-TAC). Since the high-affinity IL-2 receptor is expressed only on activated T cells, exposure to anti-TAC after the graft specifically blocks proliferation of T cells activated in response to the alloantigens of the graft.

Monoclonal-antibody therapy, which was initially employed to deplete T cells in graft recipients, also has been used to treat donors' bone marrow before it is transplanted. Such treatment is designed to deplete the immunocompetent T cells in the bone-marrow transplant; these are the cells that react with the recipient tissues, causing graft-versus-host disease (described below). Monoclonal antibodies with isotypes that activate the complement system are most effective in all cell-depletion strategies.

The CD3 receptor and the high-affinity IL-2 receptor are targets present on all activated T cells; molecules present on particular T-cell subpopulations may also be targeted for immunosuppressive therapy. For example, a monoclonal antibody to CD4 has been shown to prolong graft survival. In one study, monkeys were given a single large dose of anti-CD4 just before they received a kidney transplant. Graft survival in the treated animals was markedly increased over that in untreated control animals. Interestingly, the anti-CD4 did not reduce the $CD4^+$ T-cell count, but instead appeared to induce the T cells to enter an immunosuppressed state. This is an example of a nondepleting antibody.

Other targets for monoclonal-antibody therapy are the cell-surface adhesion molecules. Simultaneous treatment with monoclonal antibodies to the adhesion molecules ICAM-1 and LFA-1 for 6 days after transplantation has permitted indefinite survival of cardiac grafts between allogeneic mice. However, when either monoclonal antibody was administered alone, the cardiac transplant was rejected. The requirement that both monoclonal antibodies be given at the same time probably reflects redundancy of the adhesion molecules: LFA-1 is known to bind to ICAM-2 in addition to ICAM-1; and ICAM-1 is known to bind to Mac-1 and CD43 in addition to LFA-1. Only when all possible pairings among these adhesins are blocked at the same time is adhesion and signal transduction through this ligand pair blocked.

A practical difficulty with using monoclonal antibodies to prolong graft survival in humans is that they are generally of mouse origin. Many recipients develop an antibody response to the mouse monoclonal antibody, rapidly clearing it from the body. This limitation has been overcome by the construction of human monoclonal antibodies and mouse-human chimeric antibodies (see Figure 5-25 and Clinical Focus in Chapter 5).

Because cytokines appear to play an important role in allograft rejection, another strategy for prolonging graft survival is to inject animals with monoclonal antibodies specific for the implicated cytokines, particularly TNF-α, IFN-γ, and IL-2. Monoclonal antibodies to TNF-α have been shown to prolong bone-marrow transplants in mice and to reduce the incidence of graft-versus-host disease. Monoclonal antibodies to IFN-γ and to IL-2 have each been reported in some cases to prolong cardiac transplants in rats.

Blocking Co-Stimulatory Signals Can Induce Anergy

As described in Chapter 10, T_H-cell activation requires a co-stimulatory signal in addition to the signal mediated by the T-cell receptor. The interaction between the B7 molecule on the membrane of antigen-presenting cells and the CD28 or CTLA-4 molecule on T cells provides one such signal (see Figure 10-13). Lacking a co-stimulatory signal, antigen-activated T cells become anergic (see Figure 10-15). CD28 is expressed on both resting and activated T cells and binds B7 with a moderate affinity; CTLA-4 is expressed at much lower levels and only on activated T cells but binds B7 with a 20-fold higher affinity. A second pair of co-stimulatory molecules required for T-cell activation are CD40, which is present on the APC, and CD40 ligand (CD40L or CD154), which is present on the T cell.

D. J. Lenschow, J. A. Bluestone, and colleagues demonstrated that blocking the B7-mediated co-stimulatory signal with CTLA-4 after transplantation would cause the host's T cells directed against the grafted tissue to become anergic, thus enabling it to survive. In their experiment, human pancreatic islets were transplanted into mice injected with CTLA-4Ig, a soluble fusion protein consisting of the extracellular domains of CTLA4 and the constant region of the IgG1 heavy chain (see Figure 10-14). Including the IgG1 heavy-chain constant region increases the half-life of the soluble fusion protein. The xenogeneic graft exhibited long-term survival in treated mice but was quickly rejected in untreated controls. The fact that the soluble form of the CTLA-4 receptor was able to block the rejection of the human tissue transplant in the recipient mice is evidence that blocking co-stimulatory signals in vivo is a viable strategy (Figure 21-9).

These exciting results were extended to transplantation of kidneys mismatched for class I and class II antigens in monkeys by Allan Kirk, David Harlan, and their colleagues. The recipients were treated for about 4 weeks after transplantation with either CTLA4-Ig or a monoclonal antibody directed against CD40L, or both in combination. Untreated control animals rejected the mismatched kidneys within 5–8 days; those treated with a single agent retained their grafts for 20–98 days. However, the animals given both reagents showed no evidence of rejection at 150 days after transplantation. This suppression of allograft rejection did not lead to a state of general immunosuppression; peripheral T-cell counts remained normal and other immune functions were present, including mixed lymphocyte reactivity between donor and recipients. Human clinical trials of the procedures developed for monkeys are planned; if successful, they could revolutionize clinical transplantation procedures. The ability to block

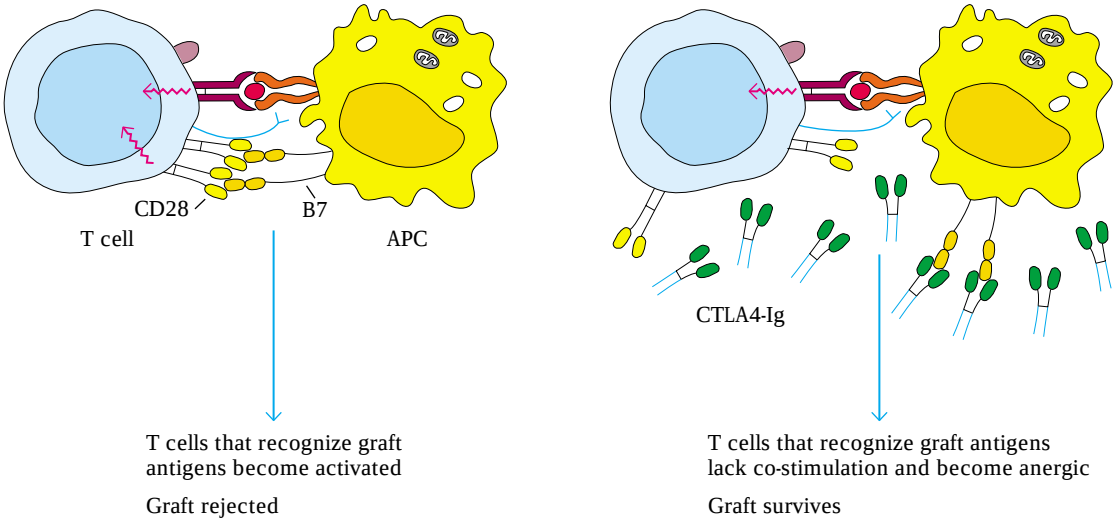


FIGURE 21-9 Blocking co-stimulatory signals at the time of transplantation can cause anergy instead of activation of the T cells reactive against the graft. T-cell activation requires both the interaction of the TCR with its ligand and the reaction of co-stimulatory receptors with their ligands (a). In (b), contact between one of the co-stimulatory receptors, CD28 on the T cell, and its ligand, B7 on the APC, is blocked by reaction of B7 with the soluble ligand CTLA-4Ig. The CTLA4 is coupled to an Ig H chain, which slows its clearance from the circulation. This process specifically suppresses graft rejection without inhibiting the immune response to other antigens.

allograft rejection without general immunosuppression and without the deleterious side effects of suppressive drugs would enable recipients to lead normal lives.

Immune Tolerance to Allografts

There are instances in which an allograft may be accepted without the use of immunosuppressive measures. Obviously, in the case of tissues that lack alloantigens, such as cartilage or heart valves, there is no immunologic barrier to transplantation. However, there are also instances in which the strong predicted response to an allograft does not occur. There are two general cases in which an allograft may be accepted. One is when cells or tissue are grafted to a so-called privileged site that is sequestered from immune-system surveillance. The second is when a state of tolerance has been induced biologically, usually by previous exposure to the antigens of the donor in a manner that causes immune tolerance rather than sensitization in the recipient. Each of these exceptions is considered below.

Privileged Sites Accept Antigenic Mismatches

In immunologically privileged sites, an allograft can be placed without engendering a rejection reaction. These sites include the anterior chamber of the eye, the cornea, the uterus, the testes, and the brain. The cheek pouch of the Syrian hamster is a privileged site used in experimental situations. Each of these sites is characterized by an absence of lymphatic vessels and in some cases by an absence of blood vessels as well. Consequently, the alloantigens of the graft are not able to sensitize the recipient's lymphocytes, and the graft has an increased likelihood of acceptance even when HLA antigens are not matched.

The privileged location of the cornea has allowed corneal transplants to be highly successful. The brain is an immunologically privileged site because the blood-brain barrier prevents the entry or exit of many molecules, including antibodies. The successful transplantation of allogeneic pancreatic islet cells into the thymus in a rat model of diabetes suggests that the thymus may also be an immunologically privileged site.

Immunologically privileged sites fail to induce an immune response because they are effectively sequestered from the cells of the immune system. This suggests the possibility of physically sequestering grafted cells. In one study, pancreatic islet cells were encapsulated in semipermeable membranes (fabricated from an acrylic copolymer) and then transplanted into diabetic mice. The islet cells survived and produced insulin. The transplanted cells were not rejected, because the recipient's immune cells could not penetrate the membrane. This novel transplant method enabled the diabetic mice to produce normal levels of insulin and may have application for treatment of human diabetics.

Early Exposure to Alloantigens Can Induce Specific Tolerance

In 1945, Ray Owen reported that nonidentical twins in cattle retained the ability to accept cells or tissue from the genetically distinct sibling throughout their lives, unlike nonidentical twins of other mammalian species. A shared placenta in cattle allows free circulation of cells from one twin to the other throughout the embryonic period. Although the twins may have inherited distinct paternal and maternal antigens, they do not recognize those of their placental partner as foreign and can accept grafts from them.

Experimental support for the notion that tolerance comes from exposure of the developing organism to alloantigens came from mouse experiments. If neonates of mouse strain A are injected with cells from strain C they will accept grafts from C strain as adults. Immunocompetence of the injected A-strain mice and specificity of the tolerance is shown by the fact that they reject grafts from other strains as rapidly as their untreated littermates. While no human experimental data demonstrate such specific tolerance, anecdotal data suggests that it may operate in humans as well. There are examples in which allografts, mismatched at a single HLA locus are accepted with little or no immune suppression. In cases where the mismatched antigen is expressed by the mother, but not inherited by the offspring, there is the possibility that perinatal exposure induced subsequent tolerance to this antigen. Because human maternal cells do not normally cross the placental barrier, such specific tolerance to noninherited maternal antigens would be an exception rather than a commonplace event.

Clinical Transplantation

For a number of illnesses, a transplant is the only means of therapy. Figure 21-10 summarizes the major organ and cell transplants being performed at the present time. In addition, certain combinations of organs, such as heart and lung or kidney and pancreas, are being transplanted simultaneously with increasing frequency. Since the first kidney transplant was performed in the 1950s, approximately 400,000 kidneys have been transplanted worldwide. The next most frequently transplanted solid organ is the liver (52,000), followed by the heart (42,000) and, more distantly, by the lung (6,000) and pancreas (2,000). Bone-marrow transplants number around 80,000. Although the clinical results of transplantation of various cells, tissues, and organs in humans have improved considerably in the past few years, major obstacles to the use of this treatment exist. As explained above, the use of immunosuppressive drugs greatly increases the short-term survival of the transplant, but medical problems arise from use of these drugs, and chronic rejection is not prevented in most cases. The need for additional transplants after rejection exacerbates the shortage of organs which is a major obstacle to the

VISUALIZING CONCEPTS

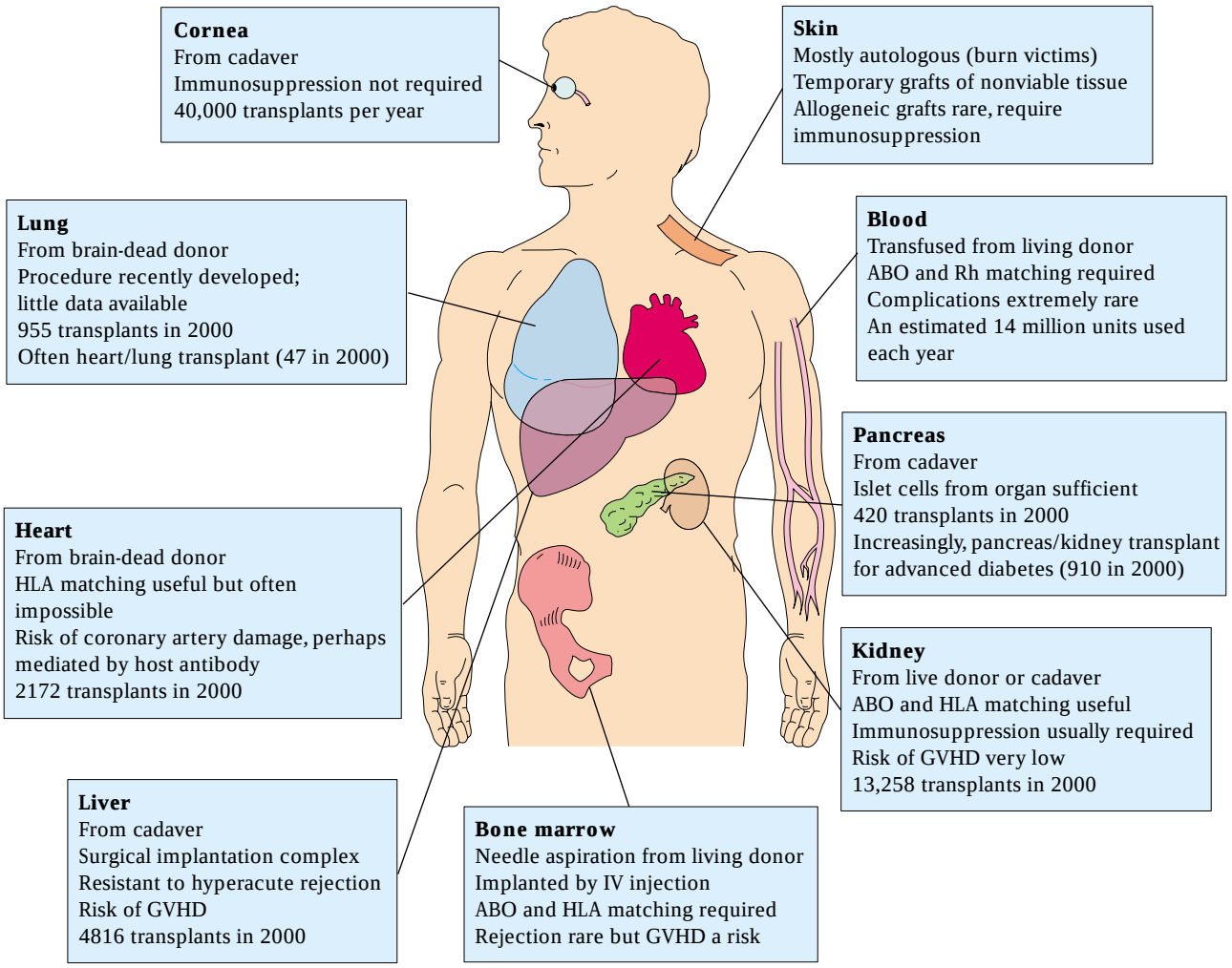


FIGURE 21-10 Transplantations routinely used in clinical practice. For the solid organs, the number of transplants performed in the United States in 2000 is indicated. Estimates are included for other transplants if available.

widespread use of transplantation. Several of the organ systems for which transplantation is a common treatment are considered below. The frequency with which a given organ or tissue is transplanted depends on a number of factors:

- Clinical situations in which transplantation is indicated
- Availability of tissue or organs
- Difficulty in performing transplantation and caring for post-transplantation patients
- Specific factors that aid or hinder acceptance of the particular transplant

The urgency of the transplantation may depend on the affected organ. In the case of the heart, lung, and liver, few alternative procedures can keep the patient alive when these organs cease to function. Although dialysis may be used to maintain a patient awaiting a kidney transplant, there are no comparable measures for the heart or lungs if the allograft fails. Research on artificial organs is ongoing but there are no reports of long-term successes.

The Most Commonly Transplanted Organ Is the Kidney

As mentioned above, the most commonly transplanted organ is the kidney; in 2000, there were 13,258 kidney transplants performed in the United States. Major factors contributing to this number are the numerous clinical indications for kidney transplantation. Many common diseases, such as diabetes and various types of nephritis, result in kidney failure that can be alleviated by transplantation. With respect to availability, kidneys can be obtained not only from cadavers but also from living relatives or volunteers, because it is possible to donate a kidney and live a normal life with the remaining kidney. In 1999, 4457 of the 12,483 kidneys transplanted in the U.S. came from living donors. Surgical procedures for transplantation are straightforward; technically, the kidney is simpler to reimplant than the liver or heart. Because many kidney transplants have been done, patient-care procedures have been worked out in detail. Matching of blood and histocompatibility groups is advantageous in kidney transplantation because the organ is heavily vascularized, but the kidney presents no special problems that promote rejection or graft-versus-host disease (GVHD), as the bone marrow or liver do.

Two major problems are faced by patients waiting for a kidney. One is the short supply of available organs, and the second is the increasing number of sensitized recipients. The latter problem stems from rejection of a first transplant, which then sensitizes the individual and leads to the formation of antibodies and activation of cellular mechanisms directed against kidney antigens. Any subsequent graft containing antigens in common with the first would be quickly rejected. Therefore, detailed tissue typing procedures must be used to ascertain that the patient has no antibodies or active cellular mechanisms directed against the potential donor's kidney. In many cases, patients can never again find a match after one or two rejection episodes. It is almost always necessary to maintain kidney-transplant patients on some form of immunosuppression, usually for their entire lives. Unfortunately, this gives rise to complications, including risks of cancer and infection as well as other side effects such as hypertension and metabolic bone disease.

Bone-Marrow Transplants Are Used for Leukemia, Anemia, and Immunodeficiency

After the kidney, bone marrow is the most frequent transplant. Since the early 1980s, bone-marrow transplantation has been increasingly adopted as a therapy for a number of malignant and nonmalignant hematologic diseases, including leukemia, lymphoma, aplastic anemia, thalassemia major, and immunodeficiency diseases, especially severe combined immunodeficiency, or SCID (see Chapter 19). The bone marrow, which is obtained from a living donor by multiple needle aspirations, consists of erythroid, myeloid, monocytoid, megakaryocytic, and lymphocytic lineages. The graft, usually about 10^9 cells per kilogram of host body weight, is injected intravenously into the recipient. The first successful bone-marrow transplantations were performed between identical twins. However, development of the tissue-typing procedures described earlier now makes it possible to identify allogeneic donors who have HLA antigens identical or near-identical to those of the recipients. While the supply of bone marrow for transplantation is not a problem, finding a matched donor may be one.

In the usual procedure, the recipient of a bone-marrow transplant is immunologically suppressed before grafting. Leukemia patients, for example, are often treated with cyclophosphamide and total-body irradiation to kill all cancerous cells. The immune-suppressed state of the recipient makes graft rejection rare; however, because the donor bone marrow contains immunocompetent cells, the graft may reject the host, causing **graft-versus-host disease (GVHD).** GVHD affects 50%–70% of bone-marrow-transplant patients; it develops as donor T cells recognize alloantigens on the host cells. The activation and proliferation of these T cells and the subsequent production of cytokines generate inflammatory reactions in the skin, gastrointestinal tract, and liver. In severe cases, GVHD can result in generalized erythroderma of the skin, gastrointestinal hemorrhage, and liver failure.

Various treatments are used to prevent GVHD in bone-marrow transplantation. The transplant recipient is usually placed on a regimen of immunosuppressive drugs, often including cyclosporin A and methotrexate, in order to inhibit the immune responses of the donor cells. In another approach, the donor bone marrow is treated with anti-T-cell antisera or monoclonal antibodies specific for T cells before transplantation, thereby depleting the offending T cells. Complete T-cell depletion from donor bone marrow, however, increases the likelihood that the marrow will be rejected, and so the usual procedure now is a partial T-cell depletion. Apparently, a low level of donor T-cell activity, which results in a low-level GVHD, is actually beneficial because the donor cells kill any host T cells that survive the immunosuppression treatment. This prevents residual recipient cells from becoming sensitized and causing rejection of the graft. In leukemia patients, low-level GVHD also seems to result in destruction of host leukemic cells, thus making it less likely for the leukemia to recur.

Heart Transplantation Is a Challenging Operation

Perhaps the most dramatic form of transplantation is that of the heart; once the damaged heart has been removed, the patient must be kept alive by wholly artificial means until the transplanted heart is in place and beating. Heart-lung machines are available to circulate and aerate the patient's blood after the heart is removed. The donor's heart must be maintained in such a manner that it will begin beating when it is placed in the recipient. It has been found that a human heart can be kept viable for a limited period in ice-cold buffer solutions that effectively short circuit the electric impulses

that control the rhythmic beating, which could damage the isolated organ. The surgical methods of implanting a heart have been available for a number of years. The first heart transplant was carried out in South Africa by Dr. Christian Barnard, in 1964. Since then, the one-year survival rate for transplantation of the heart has become greater than 80%. In 2000, 2172 heart transplants were performed in the United States and about 3500 worldwide. An issue peculiar to heart transplantation has been a new type of atherosclerotic disease in the coronary arteries of the implanted organ. There is some possibility that host antibodies mediate injury to the vessels in the donated heart.

Although a heart transplant may greatly benefit patients with various types of heart disease or damage, there is obviously a strict limit on the number of available hearts. Accident victims who are declared brain dead but have an intact circulatory system and a functioning heart are the normal source of these organs. HLA matching is desirable but not often possible, because of the limited supply of hearts and the urgency of the procedure.

Lung Transplants Are on the Increase

In recent years, lung transplantation, either by itself or in conjunction with heart transplantation, has been used to treat diseases such as cystic fibrosis and emphysema or acute damage to the lungs such as that caused by smoke inhalation. In 2000, 945 lung and 47 heart/lung transplants were performed. First-year survival rate for lung transplants is reported at about 60%.

Liver Transplants Treat Congenital Defects and Damage from Viral or Chemical Agents

The liver is a large organ that performs a number of functions related to clearance and detoxification of chemical and biological substances. Liver malfunction can be caused by damage to the organ from viral diseases such as hepatitis or by exposure to harmful chemicals, as in chronic alcoholism. Damage to the liver may correct itself and the damaged tissue can regenerate after the causative injurious agent is cleared. If the liver tissue does not regenerate, damage may be fatal. The majority of liver transplants are used as a therapy for congenital abnormalities of the liver. Because the liver is large and has a complicated circulation, re-implantation of the liver initially posed a technical problem. Techniques have been developed to overcome this major surgical challenge, and the recent one-year survival rate has risen to approximately 65%. In 2000, 4816 livers were transplanted in the United States. Increasingly, a liver from a single donor may be split and given to two recipients; normally, a child will receive the smaller portion and an adult the larger.

The immunology of liver transplantation is interesting because the organ appears to resist rejection by hyperacute antibody-mediated mechanisms. It has been shown that even transplantation across blood-group barriers, which would be expected to trigger hyperacute rejection, can be successful in the short term. However, leukocytes within the donor organ together with anti–blood-group antibodies can mediate antibody-dependent hemolysis of recipient red blood cells if there is a mismatch of the blood groups. In addition, manifestations of GVHD have occurred in liver transplants even when donor and recipient are blood-group compatible. These reactions are obviously caused by donor lymphocytes carried by the transplanted liver.

Pancreas Transplantation Offers a Cure for Diabetes Mellitus

One of the more common diseases in the United States is diabetes mellitus. This disease is caused by malfunction of insulin-producing islet cells in the pancreas. Transplantation of a pancreas could provide the appropriately regulated levels of insulin necessary to make the diabetic individual normal. Recently, one-year success rates for pancreas transplantation of about 55% have been reported. Transplantation of the complete pancreas is not necessary to restore the function needed to produce insulin in a controlled fashion; transplantation of the islet cells alone could restore function. Kidney failure is a frequent complication of advanced diabetes occurring in about 30% of diabetics, therefore kidney and pancreas transplants are indicated. In 2000, there were 420 pancreas transplants and 904 simultaneous kidney/pancreas transplants. A group at the University of Wisconsin reports that they have overcome surgical and medical barriers to the dual transplant and have achieved survival rates of 87% at one year and 78% at five years for the 381 cases in their study. Whether it is better to carry out simultaneous kidney-pancreas transplants or to transplant separately remains an issue to be resolved on a case-to-case basis.

Skin Grafts Are Used to Treat Burn Victims

Most skin transplantation in humans is done with autologous tissue. However, in cases of severe burn, grafts of foreign skin thawed from frozen deposits in tissue banks may be used. These grafts generally act as biologic dressings, because the cellular elements are no longer viable and the graft does not grow in the new host; the grafts are left in place for several days but are regularly replaced. True allogeneic skin grafting using fresh viable donor skin has been undertaken in some cases, but rejection must be prevented by the use of immunosuppressive therapy. This is not desirable because a major problem with burn victims is the high risk of infection, and immunosuppressive therapy accentuates this risk.

The above list of common transplants is by no means all-inclusive and is expected to grow in future years. For example, intracerebral neural-cell grafts have restored functionality in victims of Parkinson's disease. In studies conducted thus far, the source of neural donor cells was human embryos; the possibility of using those from other animal species is being tested.

Xenotransplantation May Be the Answer to the Shortage of Donor Organs

While the immune system represents a formidable barrier to the use of transplantation, there has been significant progress in overcoming this obstacle. However, there has not been comparable progress in solving the complex problem of finding organs for those who need them. The insufficient supply of available organs means that a large percentage of patients die while waiting for a transplant. The need for an alternative source of donor organs has focused attention on xenotransplantation. The larger nonhuman primates (chimpanzees and baboons) have served as the main transplant donors, and, as discussed in the Clinical Focus section, the use of the pig as a source of organs is under serious consideration.

The earliest transplants of chimpanzee kidneys into humans date back to 1964. Since that time, sporadic attempts at kidney, heart, liver, and bone-marrow transplantation from primates into humans have been made. No attempt has met with great success but several have received some attention. In 1993, T. E. Starzl performed two liver transplants from baboons into patients suffering from liver failure. Both patients died, one after 26 days and the other after 70 days. In 1994, a pig liver was transplanted into a 26-year-old suffering from acute hepatic failure. The liver functioned only 30 hours before it was rejected by a hyperacute rejection reaction. In 1995, baboon bone marrow was infused into an HIV-infected man with the aim of boosting his weakened immune system with the baboon immune cells, which do not become infected with the virus. Although there were no complications from the transplant, the baboon bone marrow did not appear to establish itself in the recipient.

A major problem with xenotransplants is that immune rejection is often quite vigorous, even when recipients are treated with potent immunosuppressive drugs such as FK506 or rapamycin. The major response involves the action of humoral antibody and complement, leading to the development of a hyperacute rejection reaction. In addition to the problem of rejection, there is general concern that xenotransplantation has the potential of spreading pathogens from the donor to the recipient. These pathogens could potentially cause diseases, called xenozoonoses, that are fatal for humans. For example, certain viruses, including close relatives of HIV-1 found in chimpanzees and HIV-2 and herpesvirus B, which occur in several primate species, cause limited pathogenesis in their primate hosts but can lead to deadly infections in humans. In addition, there is the fear that primate retroviruses (see Chapter 19), such as SIV, may recombine with human variants to produce new agents of disease. The possibility of introducing new viruses into humans may be greater for transplants from closely related species, such as primates, and less in the case of more distantly related species, such as pigs, because viruses are less likely to replicate in cells from unrelated species.

SUMMARY

- Graft rejection is an immunologic response displaying the attributes of specificity, memory, and self/nonself recognition. There are three major types of rejection reactions:
  - Hyperacute rejection mediated by preexisting host antibodies to graft antigens.
  - Acute graft rejection in which T_H cells and/or CTLs mediate tissue damage.
  - Chronic rejection, which involves both cellular and humoral immune components.
- The immune response to tissue antigens encoded within the major histocompatibility complex is the strongest force in rejection.
- The match between a recipient and potential graft donors is assessed by typing MHC class I and class II tissue antigens.
- The process of graft rejection can be divided into a sensitization stage, in which T cells are stimulated, and an effector stage, in which they attack the graft.
- In most clinical situations, graft rejection is suppressed by nonspecific immunosuppressive agents or by total lymphoid x-irradiation.
- Experimental approaches using monoclonal antibodies offer the possibility of specific immunosuppression. These antibodies may act by:
  - Deleting populations of reactive cells.
  - Inhibiting co-stimulatory signals leading to anergy in specifically reactive cells.
- Certain sites in the body, including the cornea of the eye, brain, testes, and uterus, do not reject transplants despite genetic mismatch between donor and recipient.
- Specific tolerance to alloantigens is induced by exposure to them in utero or by injection of neonates.
- A major complication in bone-marrow transplantation is graft-versus-host reaction mediated by the lymphocytes contained within the donor marrow.
- The critical shortage of organs available for transplantation may be solved in the future by using organs from nonhuman species (xenotransplants).

References

Adams, D. H. 2000. Cardiac xenotransplantation: clinical experience and future direction. *Ann. Thoracic Surg.* **70:**320.

Auchincloss, H., M. Sykes, and D. H. Sachs. 1999. Transplantation immunology, in *Fundamental Immunology,* 4th ed. W. E. Paul, ed. Lippincott-Raven, Philadelphia. p. 1175.

Fox, A., and L. C. Harrison. 2000. Innate immunity and graft rejection. *Immunol. Rev.* **173:**141.

Grover, F. L., et al. 1997. The past, present, and future of lung transplantation. *Am. J. Surg.* **173:**523.

Harlan, D. M., and A. D. Kirk. 1999. The future of organ and tissue transplantation: can T-cell co-stimulatory pathway modifiers revolutionize the prevention of graft rejection? *JAMA* **282:**1076.

Hirose, R., and F. Vincenti. Review of transplantation—1999. *Clin. Transplants* **1999:**295.

Hong J. C., and B. D. Kahan. 2000. Immunosuppressive agents in transplantation: past, present, and future. *Sem. Nephrol.* **20:** 108.

Kirk, A. D., et al. 1997. CTLA4-Ig and anti-CD40 ligand prevent renal allograft rejection in primates. *Proc. Natl. Acad. Sci. USA* **94:**8789.

Lenschow, D. J., et al. 1992. Long-term survival of xenogeneic pancreatic islets induced by CTLA4-Ig. *Science* **257:**789.

Markees, T. G., et al. 1997. Prolonged survival of mouse skin allografts in recipients treated with donor splenocytes and antibody to CD40 ligand. *Transplantation* **64:**329.

Mollnes, T. E., and A. E. Fiane. 1999. Xenotransplantation: how to overcome the complement obstacle? *Mol. Immunol.* **36:**269.

Rayhill, S. C., et al. 1996. Simultaneous pancreas-kidney transplantation: recent experience at the University of Wisconsin. *Exp. Clin. Endocrinol. Diabetes* **104:**353.

Woo, S. B., S. J. Lee, and M. M. Schubert. 1997. Graft-vs-host disease. *Crit. Rev. Oral Biol. Med.* **8:**201.

USEFUL WEB SITES

http://www.transweb.org

Links to hundreds of sites giving information on all aspects of organ transplantation.

http//www.unos.org

United Network for Organ Sharing site has information concerning solid-organ transplantation for patients, families, doctors, and teachers.

http://www.marrow.org

The National Marrow Donor Program Web site contains information about all aspects of bone-marrow transplantation.

Study Questions

CLINICAL FOCUS QUESTION What features would you include in an ideal animal donor for xenotransplantation? How would you test your model prior to doing clinical trials in humans?

1. Indicate whether each of the following statements is true or false. If you think a statement is false, explain why.

a. Acute rejection is mediated by preexisting host antibodies specific for antigens on the grafted tissue.
b. Second-set rejection is a manifestation of immunologic memory.
c. Passenger leukocytes are host dendritic cells that migrate into grafted tissue and act as antigen-presenting cells.
d. All allografts between individuals with identical HLA haplotypes will be accepted.
e. Cytokines produced by host T_H cells activated in response to alloantigens play a major role in graft rejection.

2. You are a pediatrician treating a child who needs a kidney transplant. The child does not have an identical twin, but both parents and several siblings are willing to donate a kidney if the MHC match with the patient is good.

a. What is the best possible MHC match that could be achieved in this situation?
b. In which relative(s) might you find it? Why?
c. What test(s) would you perform in order to find the best-matched kidney?

3. Indicate in the Response column in the table on page 500 whether a skin graft from each donor to each recipient listed would result in a rejection (R) or an acceptance (A) response. If you believe a rejection reaction would occur, then indicate in the right-hand column whether it would be a first-set rejection (FSR), occurring in 12–14 days, or a second-set rejection (SSR), occurring in 5–6 days. All the mouse strains listed in the table have different H-2 haplotypes.

4. Graft-versus-host disease (GVHD) frequently develops after certain types of transplantations.

a. Briefly outline the mechanisms involved in GVHD.
b. Under what conditions is GVHD likely to occur?
c. Some researchers have found that GVHD can be diminished by prior treatment of the graft with monoclonal antibody plus complement or with monoclonal antibody conjugated with toxins. List at least two cell-surface antigens to which monoclonal antibodies could be prepared and used for this purpose, and give the rationale for your choices.

5. A child who requires a kidney transplant has been offered a kidney from both parents and from five siblings.

a. Cells from the potential donors are screened with monoclonal antibodies to the HLA-A, -B, and -C antigens in a microcytotoxicity assay. In addition, ABO blood-group typing is performed. Based on the results in the table on page 500, a kidney graft from which donor(s) is most likely to survive?
b. Now a one-way MLR is performed using various combinations of mitomycin-treated lymphocytes. The results, expressed as counts per minute of [^{3}H]thymidine incorporated, are shown in the table on page 500; the stimulation index (ratio of the experimental value to the control in which identical leukocytes are mixed) is listed below in parentheses. Based on these data, a graft from which donor(s) is most likely to be accepted?

6. What is the biologic basis for attempting to use soluble CTL4A or anti-CD40L to block allograft rejection? Why might this be better than treating a graft recipient with CsA or FK506?

Go to www.whfreeman.com/immunology Self-Test
Review and quiz of key terms

For use with Question 3:

Donor	Recipient	Response	Type of rejection
BALB/c	C3H		
BALB/c	Rat		
BALB/c	Nude mouse		
BALB/c	C3H, had previous BALB/c graft		
BALB/c	C3H, had previous C57BL/6 graft		
BALB/c	BALB/c		
BALB/c	(BALB/c × C3H)F_1		
BALB/c	(C3H × C57BL/6)F_1		
(BALB/c × C3H)F_1	BALB/c		
(BALB/c × C3H)F_1	BALB/c, had previous F_1 graft		

For use with Question 5a:

	ABO type	HLA-A type	HLA-B type	HLA-C type
Recipient	O	*A1/A2*	*B8/B12*	*Cw3*
Potential donors				
Mother	A	*A1/A2*	*B8/B12*	*Cw1/Cw3*
Father	O	*A2*	*B12/B15*	*Cw3*
Sibling A	O	*A1/A2*	*B8/B15*	*Cw3*
Sibling B	O	*A2*	*B12*	*Cw1/Cw3*
Sibling C	O	*A1/A2*	*B8/B12*	*Cw3*
Sibling D	A	*A1/A2*	*B8/B12*	*Cw3*
Sibling E	O	*A1/A2*	*B8/B15*	*Cw3*

For use with Question 5b:

Respondent cells	Mytomycin C-treated stimulator cells					
	Patient	Sibling A	Sibling B	Sibling C	Sibling D	Sibling E
Patient	1,672 (1.0)	1,800 (1.1)	13,479 (8.1)	5,210 (3.1)	13,927 (8.3)	13,808 (8.3)
Sibling A	1,495 (1.6)	933 (1.0)	11,606 (12.4)	8,443 (9.1)	11,708 (12.6)	13,430 (14.4)
Sibling B	25,418 (9.9)	26,209 (10.2)	2,570 (1.0)	13,170 (5.1)	19,722 (7.7)	4,510 (1.8)
Sibling C	10,722 (6.2)	10,714 (5.9)	13,032 (7.5)	1,731 (1.0)	1,740 (1.0)	14,365 (8.3)
Sibling D	15,988 (5.1)	13,492 (4.2)	18,519 (5.9)	3,300 (1.1)	3,151 (1.0)	18,334 (5.9)
Sibling E	5,777 (6.5)	8,053 (9.1)	2,024 (2.3)	6,895 (7.8)	10,720 (12.1)	888 (1.0)