IMMUNO TECHNOLOGY

LECTURE 09: IMMUNE MEDIATED HYPERSENSITIVITY

Systemic Autoimmune Diseases

In systemic autoimmune diseases, the response is directed toward a broad range of target antigens and involves a number of organs and tissues. These diseases reflect a general defect in immune regulation that results in hyperactive T cells and B cells. Tissue damage is widespread, both from cell mediated immune responses and from direct cellular damage caused by auto-antibodies or by accumulation of immune complexes.

Systemic Lupus Erythematosus Attacks Many Tissues

One of the best examples of a systemic autoimmune disease is **systemic lupus erythematosus (SLE),** which typically appears in women between 20 and 40 years of age; the ratio of female to male patients is 10:1. SLE is characterized by fever, weakness, arthritis, skin rashes, pleurisy, and kidney dysfunction. Lupus is more frequent in African-American and Hispanic women than in Caucasians, although it is not known why this is so. Affected individuals may produce autoantibodies to a vast array of tissue antigens, such as DNA, histones, RBCs, platelets, leukocytes, and clotting factors; interaction of these auto-antibodies with their specific antigens produces various symptoms. Auto-antibody specific for RBCs and platelets, for example, can lead to complement-mediated lysis, resulting in hemolytic anemia and thrombocytopenia, respectively. When immune complexes of auto-antibodies with various nuclear antigens are deposited along the walls of small blood vessels, a type III hypersensitive reaction develops. The complexes activate the complement system and generate membrane-attack complexes and complement split products that damage the wall of the blood vessel, resulting in vasculitis and glomerulonephritis.

Excessive complement activation in patients with severe SLE produces elevated serum levels of the complement split products C3a and C5a, which may be three to four times higher than normal. C5a induces increased expression of the type 3 complement receptor (CR3) on neutrophils, facilitating neutrophil aggregation and attachment to the vascular endothelium. As neutrophils attach to small blood vessels, the number of circulating neutrophils declines (neutropenia) and various occlusions of the small blood vessels develop (vasculitis). These occlusions can lead to widespread tissue damage. Laboratory diagnosis of SLE focuses on the characteristic antinuclear antibodies, which are directed against double stranded or single-stranded DNA, nucleoprotein, histones, and nucleolar RNA. Indirect immunofluorescent staining with serum from SLE patients produces various characteristic nucleus-staining patterns.

Multiple Sclerosis Attacks the Central Nervous System

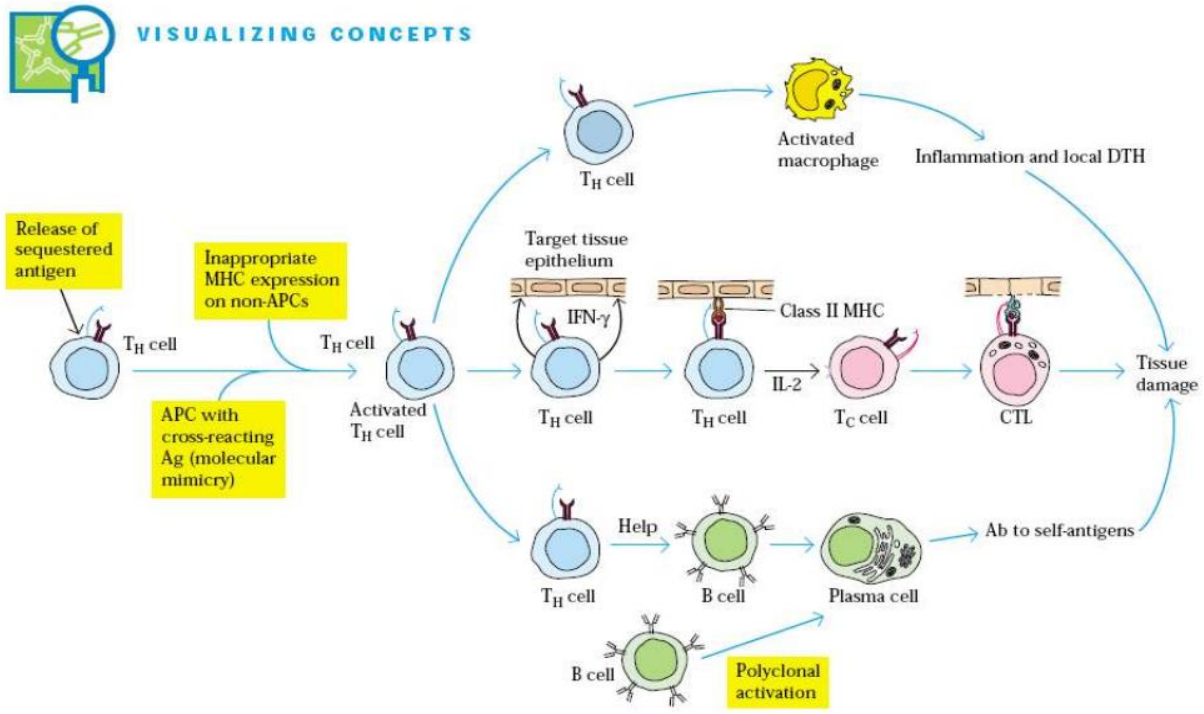
Multiple sclerosis (MS) is the most common cause of neurologic disability associated with disease in Western countries. The symptoms may be mild, such as numbness in the limbs, or severe, such as paralysis or loss of vision. Most people with MS are diagnosed between the ages of 20 and 40. Individuals with this disease produce autoreactive T cells that participate in the formation of inflammatory lesions along the myelin sheath of nerve fibers. The cerebrospinal fluid of patients with active MS contains activated T lymphocytes, which infiltrate the brain tissue and cause characteristic inflammatory lesions, destroying the myelin. Since myelin functions to insulate the nerve fibers, a breakdown in the myelin sheath leads to numerous neurologic dysfunctions. Epidemiological studies indicate that MS is most common in the Northern hemisphere and, interestingly, in the United States. Populations who live north of the 37^{th} parallel have a prevalence of 110–140 cases per 100,000, while those who live south of the 37^{th} parallel show a prevalence of 57–78 per 100,000. And individuals from south of the 37^{th} parallel who move north assume a new risk if the move occurs before 15 years of age. These provocative data suggest that there is an environmental component of the risk of contracting MS. This is not the entire story, however, since genetic influences also are important. While the average person in the United States has about one chance in 1000 of developing MS, close relatives of people with MS, such as children or siblings,

have 1 chance in 50 to 100 of developing MS. The identical twin of a person with MS has a 1 in 3 chance of developing the disease. These data point strongly to the genetic component of the disease. MS affects women two to three times more frequently than men. The cause of MS, like most autoimmune diseases, is not well understood. However, there are some suggestions that infection by certain viruses may predispose a person to MS. Certainly some viruses can cause demyelinating diseases, and it is tempting to speculate that virus infection plays a significant role in MS, but at present there is no definitive data implicating a particular virus.

Rheumatoid Arthritis Attacks Joints

Rheumatoid arthritis is a common autoimmune disorder, most often affecting women from 40 to 60 years old. The major symptom is chronic inflammation of the joints, although the hematologic, cardiovascular, and respiratory systems are also frequently affected. Many individuals with rheumatoid arthritis produce a group of auto-antibodies called **rheumatoid factors** that are reactive with determinants in the Fc region of IgG. The classic rheumatoid factor is an IgM antibody with that reactivity. Such auto-antibodies bind to normal circulating IgG, forming IgM-IgG complexes that are deposited in the joints. These immune complexes can activate the complement cascade, resulting in a type III hypersensitive reaction, which leads to chronic inflammation of the joints.

Proposed Mechanisms for Induction of Autoimmunity



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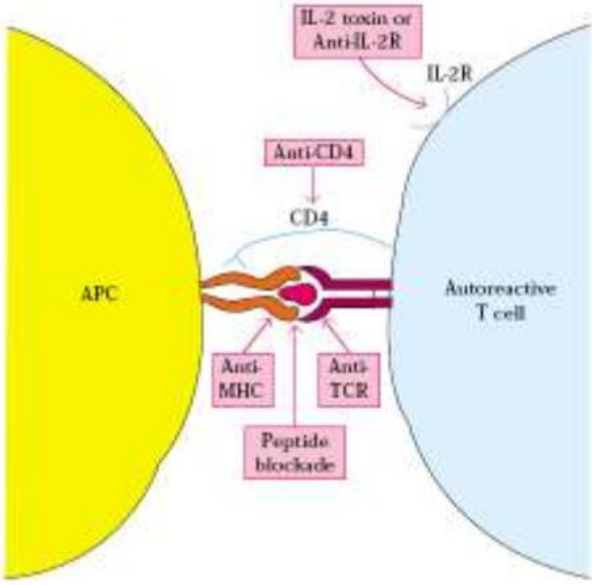
Proposed mechanisms for inducing autoimmune responses. Normal thymic selection appears to generate some self-reactive T_H cells; abnormalities in this process may generate even more self-reactive T_H cells. Activation of these selfreactive T cells in various ways, as well as polyclonal activation of B cells, is thought to induce an autoimmune response, in this case resulting in tissue damage. In all likelihood, several mechanisms are involved in each autoimmune disease.

Treatment of Autoimmune Diseases

Ideally, treatment for autoimmune diseases should be aimed at reducing only the autoimmune response while leaving the rest of the immune system intact. To date, this ideal has not been reached. Current therapies for autoimmune diseases are not cures but merely palliatives, aimed at reducing symptoms to provide the patient with an acceptable quality of life. For the most part, these treatments provide nonspecific suppression of the immune system and thus do not distinguish between a pathologic autoimmune response and a protective immune response.



Immunosuppressive drugs (e.g., corticosteroids, azathioprine, and cyclophosphamide) are often given with the intent of slowing proliferation of lymphocytes. By depressing the immune response in general, such drugs can reduce the severity of autoimmune symptoms. The general reduction in immune responsiveness, however, puts the patient at greater risk for infection or the development of cancer. A somewhat more selective approach employs **cyclosporin A** or FK506 to treat autoimmunity. These agents block signal transduction mediated by the T-cell receptor; thus, they inhibit only antigen-activated T cells while sparing nonactivated ones. Another therapeutic approach that has produced positive results in some cases of myasthenia gravis is removal of the thymus. Because patients with this disease often have thymic abnormalities (e.g., thymic

hyperplasia or thymomas), adult thymectomy often increases the likelihood of remission of symptoms.

Patients with Graves' disease, myasthenia gravis, rheumatoid arthritis, or systemic lupus erythematosus may experience short-term benefit from plasmapheresis. In this process, plasma is removed from a patient's blood by continuous-flow centrifugation. The blood cells are then resuspended in a suitable medium and returned to the patient. Plasmapheresis has been beneficial to patients with autoimmune diseases involving antigen-antibody complexes, which are removed with the plasma. Removal of the complexes, although only temporary, can result in a short-term reduction in symptoms. On the positive side, studies with experimental autoimmune animal models have provided evidence that it is indeed possible to induce specific immunity to the development of autoimmunity.

1. T-Cell Vaccination Is a Possible Therapy
2. Peptide Blockade of MHC Molecules Can Modulate Autoimmune Responses
3. Monoclonal Antibodies May Be Used to Treat Autoimmunity

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Transplantation immunology

Transplantation as the term 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).

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. 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 non identical individuals with ever increasing frequency and success.

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:

1. **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.
2. **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.
3. **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.
4. **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.

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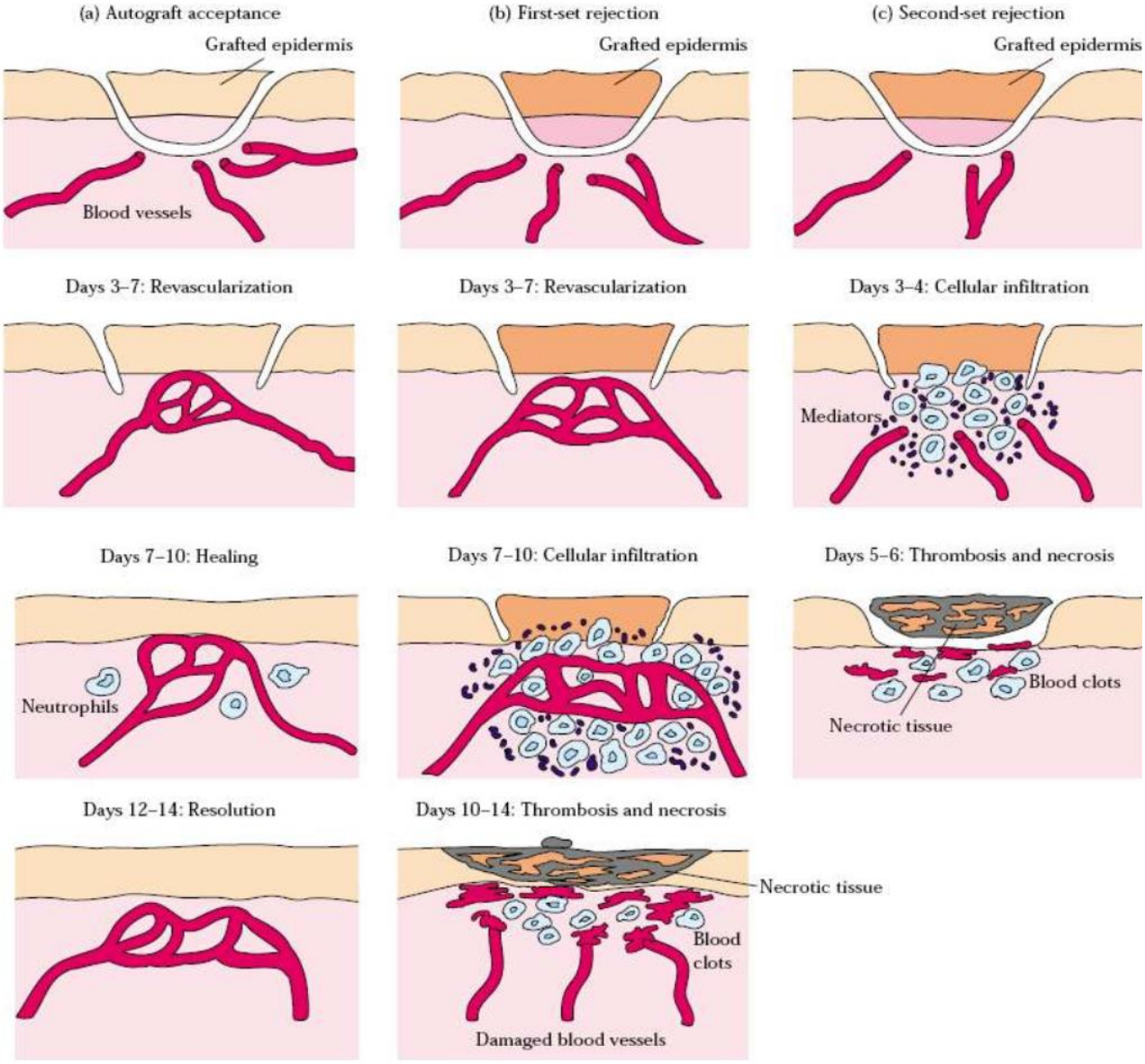
Autografts and isografts are usually accepted, owing to the genetic identity between graft and host (see Figure a). Because an allograft is genetically dissimilar to the host, it is often recognized as foreign by the immune system and is rejected. Obviously, xenografts exhibit the greatest genetic disparity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

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. 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

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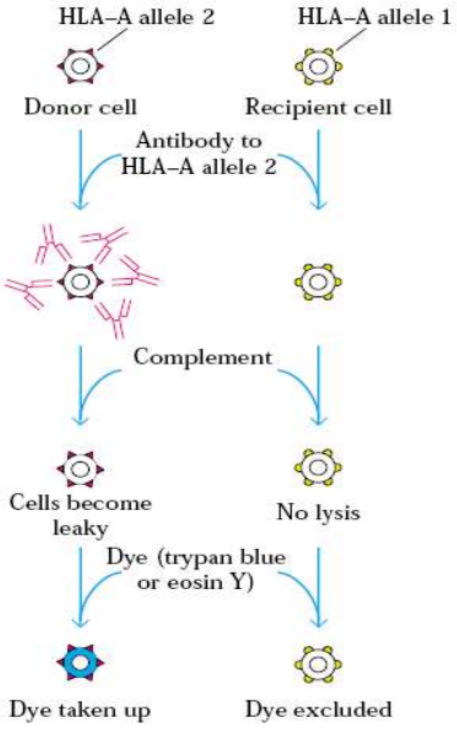
Tissues that are antigenically similar are said to be **histocompatible;** such tissues do not induce an immunologic response that leads to tissue rejection. Tissues that display significant antigenic differences are *histoincompatible* and induce an immune response that leads to tissue rejection. The 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. Because the MHC loci are closely linked, they are usually inherited as a complete set, called a **haplotype,** from each parent.

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 (see Figure).



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.

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 alloantigens 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 transfusions 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.

Effector Stage

A variety of effector mechanisms participate in allograft rejection (see Figure). 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. Recognition of foreign class I alloantigens on the graft by host $CD8^+$ cells can lead to CTL-mediated killing. In some cases, $CD4^+$ T cells that function as class II MHC–restricted cytotoxic cells mediate graft rejection.

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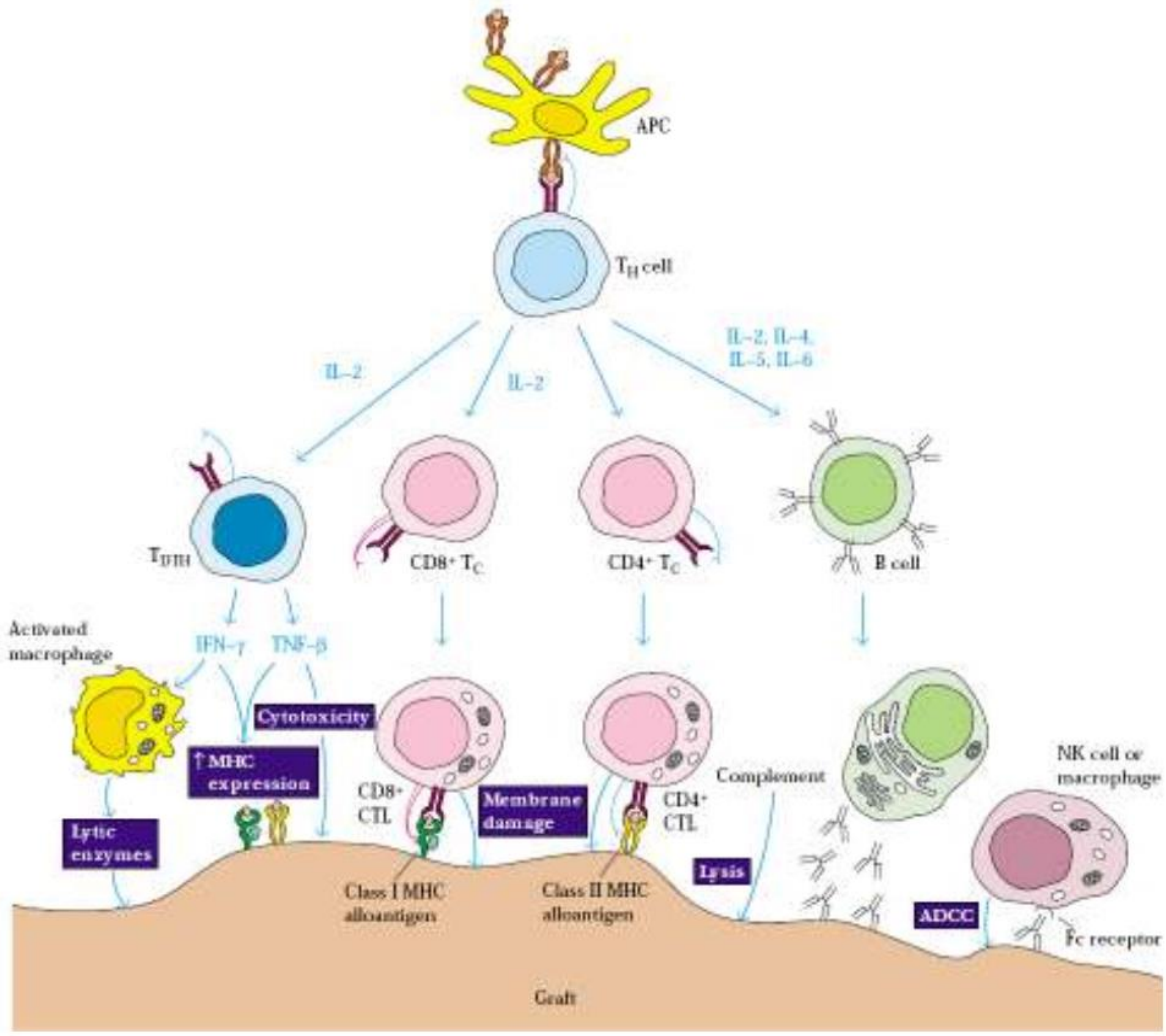

In each of these effector mechanisms, cytokines secreted by TH cells play a central role (see Figure). For example, IL-2, IFN-γ, and TNF-β have each been shown to be important mediators of graft rejection. IL-2 promotes T-cell proliferation and generally is necessary for the generation of effector CTLs (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 those 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.

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.

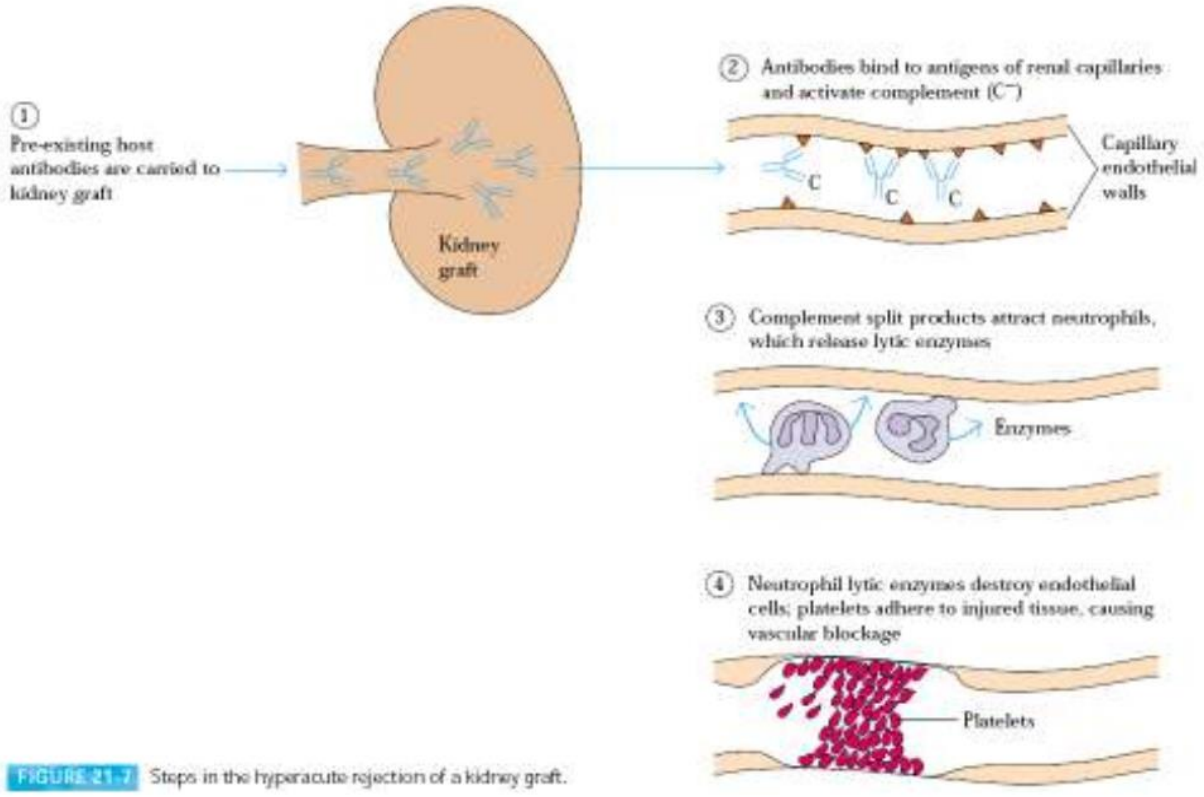

FIGURE 21-7 Steps in the hyperacute rejection of a kidney graft.

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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. 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.

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 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 transplantation.

General Immunosuppressive Therapy

1. Mitotic Inhibitors Thwart T-Cell Proliferation
2. Corticosteroids Suppress Inflammation
3. Certain Fungal Metabolites Are Immunosuppressants
4. Total Lymphoid Irradiation Eliminates Lymphocytes

Specific Immunosuppressive Therapy

- Monoclonal Antibodies Can Suppress
- Graft-Rejection Responses
- Blocking Co-Stimulatory Signals Can Induce Anergy

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.

- Privileged Sites Accept Antigenic Mismatches
- Early Exposure to Alloantigens Can Induce Specific Tolerance