The Allograft Immune Response

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Graft 2003; 6; 71
DOI: 10.1177/1522162803256700

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This article describes the interaction between the host immune system and the allograft. Histocompatibility antigens, in particular, the class I and II major histocompatibility antigens (MHC), distinguish self and non-self. Recipient T cells recognize MHC antigens displayed on the surface of antigen-presenting cells to trigger T cell activation and proliferation. CD4+ Tcells, also known as helper Tcells, are the dominant phenotype in acute cellular allograft rejection. CD8+ T cells, known as cytotoxic T cells, are responsible for cell-directed cytotoxicity. Under the influence of selectins, integrins, and the immunoglobulin superfamily, recipient leukocytes migrate to the graft. Macrophages activated by CD4+ Tcells release cytotoxic cytokines that cause tissue destruction. The types of allograft rejection are hyperacute, acute cellular, and chronic ductopenic.

Keywords: liver transplantation; histocompatibility antigens; Th; CD4 T cells; CD8 T cells

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DOI: 10.1177/1522162803256700

The Alloimmune Response

In this article, there is a simplified account of the interaction between the host immune system and the allograft.

Recognition of Self and Non-Self

The histocompatibility antigens are a set of protein products that constitute the self-identity that is unique in each individual. T cells are educated to identify and tolerate self antigen, whereas the encounter with any non-self antigen will lead to the initiation of immune response. The most recognized histocompatibility antigens are the class I and class II glycoproteins of the major histocompatibility complex (MHC) (Fig. 1, Table 1). Class I is expressed on all nucleated cells and in general is responsible for activating T cells bearing the CD8 surface molecule (CD8+). Of the several class I genes, A and B are the most important for clinical transplantation. MHC class II glycoproteins are expressed primarily on dendritic cells, B cells, and macrophages. As a group, these cells are also referred to as antigen presenting cells, due to their avidity by which they display peptide in conjunction with MHC.

In general, the greater the divergence between donor and recipient MHC antigens, the stronger the immune response. Before being transported to the plasma membrane of the cell, MHC class I and class II undergo intracellular processing and are loaded with peptides in the antigen presenting cell (Fig. 2). Peptides that are derived from the allograft are recognized as non-self by T-cells, leading to initiation of immune response against the transplanted organ. The peptides that stimulate the immune response involved in acute cellular rejection of the liver allograft remain to be determined. The diagnosis of acute cellular rejection in liver allografts is dependent on the demonstration of a mixed inflammatory cell infiltrate in the portal triads. Biliary epithelium and venous endothelium are the early targets of cellular rejection on account of their rich expression of class I and II MHC antigens. In contrast, hepatocytes that express few class I or II MHC antigens are rarely the target of early acute cellular rejection.

Recipient T- and B-Cell Activation

Recipient T cells are required for allograft rejection. The T-cell receptor is able to recognize donor

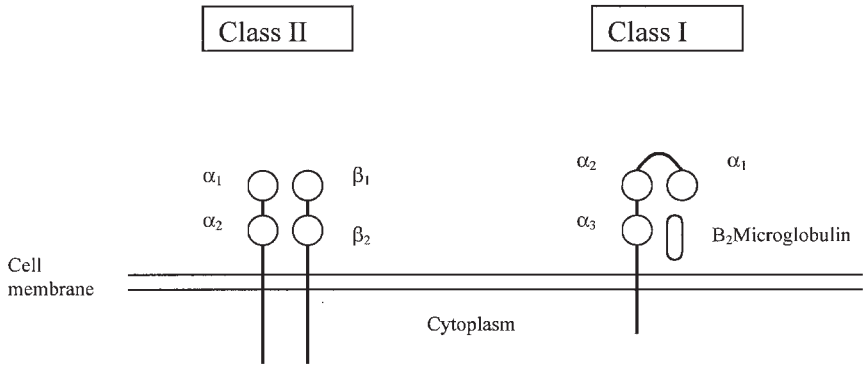

Figure 1. Schematic structure of MHC class I and class II.

Table 1 | **Characteristics of MHC Molecules**

Common Features of MHC Class I and II glycoproteins
• allelic diversity
• antigen presentation
Differing characteristics
Class I MHC molecules:
• expressed on all nucleated cells
• activate T cells bearing the CD8 surface molecule (CD8+)
Class II MHC molecules:
• expressed on dendritic cells, B-cells, and macrophages
• activate T cells bearing the CD4 antigen (CD4+)

HISTOCOMPATIBILITY ANTIGENS

A set of protein products unique to each individual that constitute self-identity

ThHelper T cells divided into two classes depending on their cytokine expression pattern

MHC antigens displayed on the surface of the antigen-presenting cell. T-cell recognition is associated with the initiation of complex intracellular signaling pathways that result in activation and proliferation of the T cell (Fig. 3).

There are two classes of T cells based on the surface expression of CD4 or CD8 molecules. CD4 and CD8 molecules bind to the same MHC on the antigen-presenting cell as the T cell receptor. CD4+ cells are known as helper cells and play an important role in initiating and directing the immune response (Fig. 4). CD8+ cells also known as cytotoxic T cells are responsible for cell-directed cytotoxicity.

Whether a T cell, whose receptor has bound the MHC-peptide displayed on the antigen presenting cell, becomes activated depends on receiving a second set of signals (co-stimulation) from the antigen-presenting cell. These costimulatory interactions act directly through cell surface receptor-ligand interactions and soluble cytokines that are linked to intracellular signaling pathways (Fig. 4).

CD4+ T cells are the dominant phenotype initiating acute cellular allograft rejection. Once activated, the CD4+ T cells undergo clonal expansion and differentiation. In doing so, they secrete cytokines that attract other leukocytes to activate other T cells and facilitate the differentiation of B cells to plasma and memory cells.

The pattern of cytokines elaborated by subsets of CD4+ and CD8+ stimulated T cells is important in directing the alloimmune response. CD4 bearing Th lymphocytes (T helper) cells have been stratified into two classes of Th cells depending on the type of cytokines elaborated by the cells in question. The subdivision of Th cells is called the Th1 and Th2 paradigm. Precursor CD4 cells producing interleukin-12 promote Th1 cells, whereas

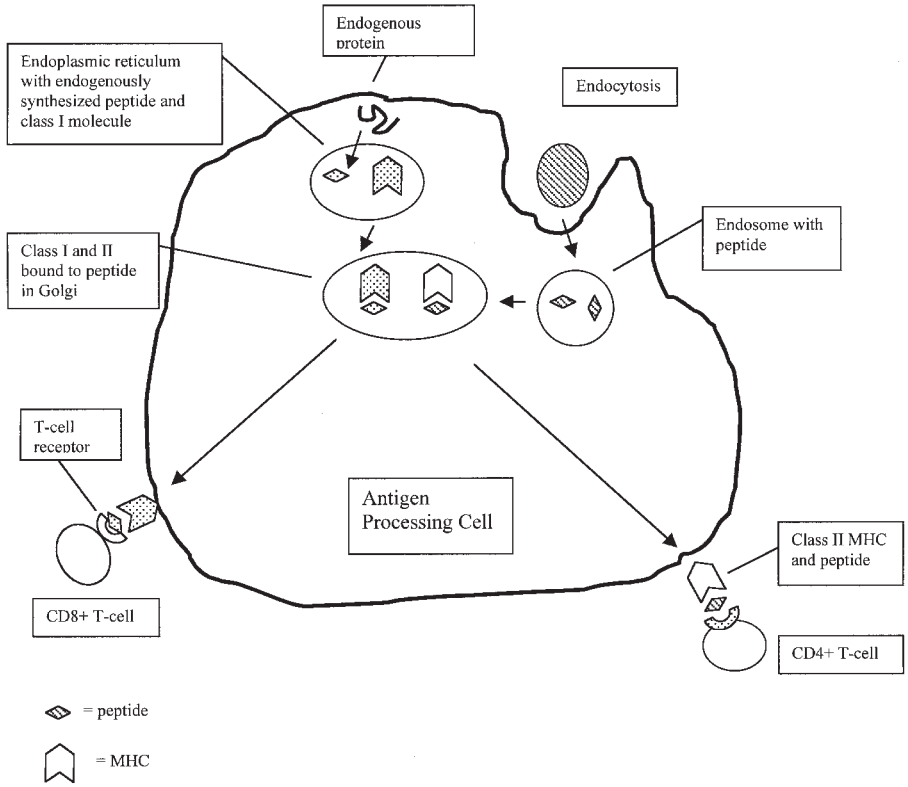

Figure 2. Antigen processing and presentation. The antigen-presenting cell presents exogenous protein bound to class II molecules to CD4+ T cells and also processes intracellular protein and presents it with MHC class I to CD8+ T cells. The T-cell receptors recognize peptide bound to MHC.

CD4 T CELLS

The dominant phenotype and initiates acute cellular allograft rejection

precursor CD4 cells producing interleukin-4 (IL-4) promote Th2 cells. Th1 cells secrete IFN-γ, (IL-2), which promote cell-mediated cytotoxicity by activating macrophages and cytotoxic Tcells. Th2 cells secrete IL-4 and IL-6, cytokines that promote allergic inflammation and stimulate B cells to produce antibodies. Furthermore, cytokines from Th1 cells inhibit Th2 cells whereas cytokines from Th2 cells inhibit Th1 cells. It appears in certain experimental situations that a Th2 predominance is associated with the prolongation of graft survival or even tolerance (Table 2).

While much attention has been given to T-cell activation, B cells are also involved in the response. Donor antigen shed from the graft binds to surface Ig and is then internalized by the Bcell. The antigen is processed and presented on the B cell surface in conjunction with class II to recruit antigen-specific T-cell help. The B-cell undergoes clonal expansion and differentiation becoming a plasma cell capable of producing soluble Ig (Fig. 5). Other B cells will become memory cells.

Stages of Allograft Response

The immunologic events surrounding transplantation of an allograft can be conceptualized as a series of steps starting with changes in the graft prior to transplantation and extending to the time of rejection.

Antigen Presentation and Allorecognition

When first transplanted, the liver allograft has the immune phenotype of the donor. Initially, therefore, the liver allograft expresses the MHC molecules of the donor, resulting in two pathways of antigen recognition. Whereas in the nontransplant setting, T cells recognize foreign (or non-self) peptides bound to native (or self) MHC molecules, the MHC molecules in the allogeneic liver are non-self,

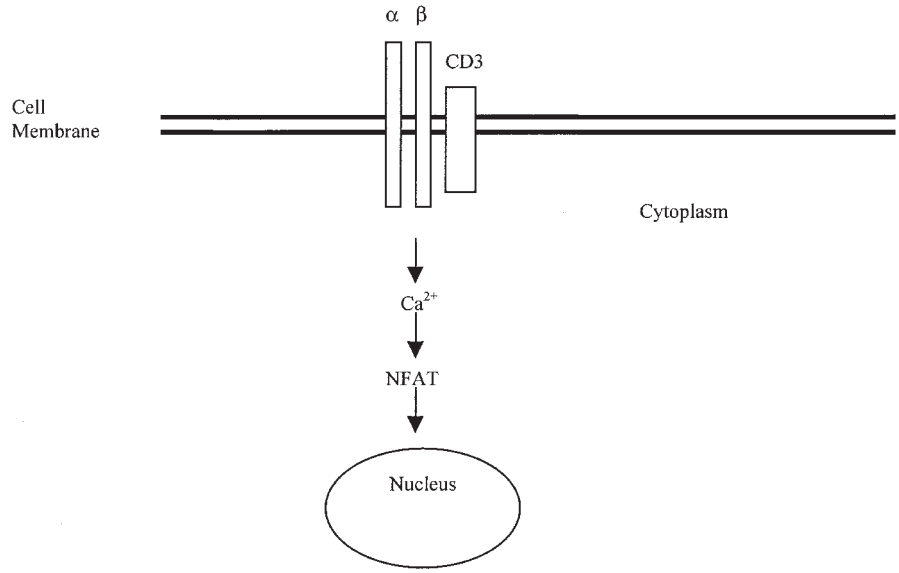

Figure 3. T-cell receptor. The T-cell receptor (TCR) is composed of two subunits and is associated with CD3 proteins. Transcriptional activity is initiated in the nucleus via signaling pathways. NFAT = nuclear factor of activated T cells.

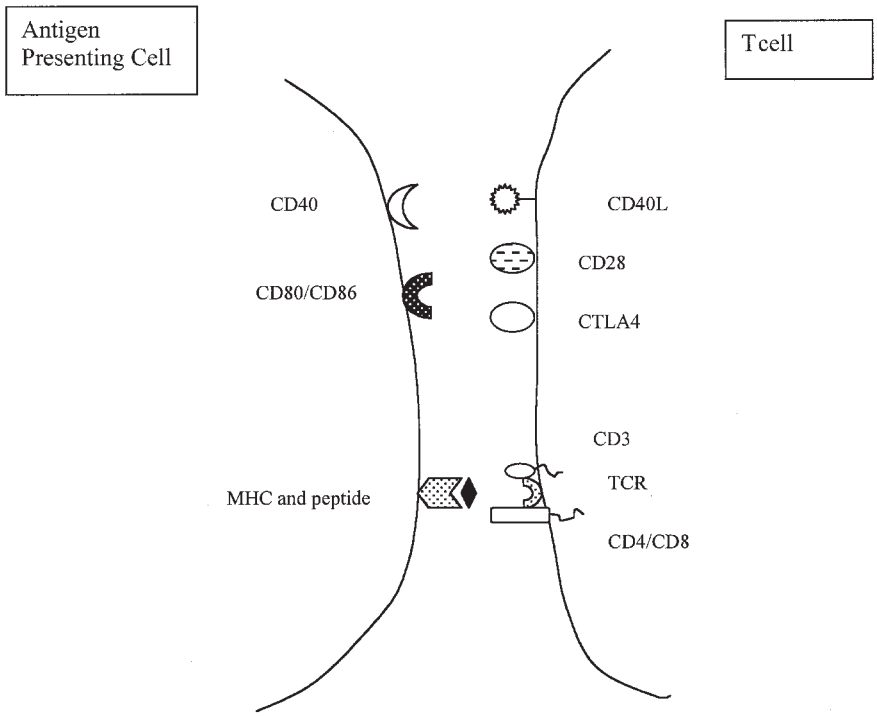

Figure 4. Cell-surface proteins involved in T-cell activation. The T-cell receptor complex, including CD3 and CD4 or CD8 bind to an APC displaying MHC and peptide. Several costimulatory molecules such as CD28 are required for T-cell activation.

Table 2 | **TH1 AND TH2 PARADIGM**

	Differentiated by	Cytokines Produced	Function
Th1	IL-12	IL-2, IFN-γ	Cell-mediated cytotoxicity suppresses Th2-cell response
Th2	IL-4	IL-4, Il-5, IL-6, IL-10	Suppresses Th1-cell response Promotes B-cell expansion

and it is presumed that recipient T cells recognize intact donor MHC molecules as non-self because their three-dimensional stoichiometry resembles a self MHC bound to a foreign peptide, a concept referred to as molecular mimicry. This process is called *direct antigen recognition* and is thought to be the main mechanism for the immune response in acute cellular rejection. Later, there is migration of donor dendritic cells into the host, and migration of recipient APCs into the donor liver. This leads to a second pathway for alloimmune recognition, in which peptides derived from catabolism of the donor MHC molecules are presented by self MHC on recipient APCs.

It is unclear whether the nonimmunologic injury incurred by the donor liver in the process of organ retrieval, preservation, and reperfusion contributes to the initiation or maintenance of the alloimmune response. The period of cold preservation, ischemia, and reperfusion leads to the differential expression of endothelial cell surface molecules and cytokines. These include adhesion molecules, interleukins, and chemokines, which attract inflammatory cells. Oxygen radicals produced during ischemia and reperfusion directly harm the graft.

Helper T cells are thought to be the most important cells for initiating allograft cellular rejection. They are responsible for the production of cytokines, such as interleukin 2, which are necessary for clonal expansion of activated lymphocytes. The cytokines act in an autocrine fashion on CD4-expressing surface molecules (Th cells) and as a paracrine stimulus on other effector cells such as cytotoxic T cells (CD8 cells), macrophages, and B cells.

Leukocyte Migration into the Allograft

As part of the early evolution of the allograft immune response, recipient leukocytes are recruited to the donor allograft. This involves the elaboration of a series of soluble molecules as well as cell-to-cell interactions. Three main classes of receptors are credited with leukocyte migration.

- Selectins: primarily responsible for allowing the leukocyte to gently adhere to the endothelial surface
- Integrins
- Members of the immunoglobulin superfamily: responsible for extravasation of leukocytes into the allograft

Graft Destruction

CD8+ T cells are the main effectors of graft destruction and cause cell death through direct cell contact. When activated by membrane binding to the allograft, they release cytotoxic molecules termed *perforin* and *granzyme*. Perforins create holes in the target cell membrane, and granzymes disrupt intracellular processing. Cytotoxic T cells also have a cell surface protein termed *Fas ligand*, which when bound to a receptor protein called Fas, which is present on target cells, results in death of the target cell by the process of apoptosis.

Macrophages that have been activated by CD4+ T cells are capable of causing tissue destruction through the release of cytotoxic cytokines or through direct cell lysis. The role of NK cells in organ allograft rejection is unclear. B cells secrete specific antibody that binds to the allograft cell surface. The antibody induces tissue damage through the activation of the complement system (Fig. 6).

Classification of Allograft Rejection

Allograft rejection is classified into three types based on the nature of the immune response after transplantation:

1. Hyperacute rejection
2. Acute cellular rejection
3. Chronic ductopenic rejection

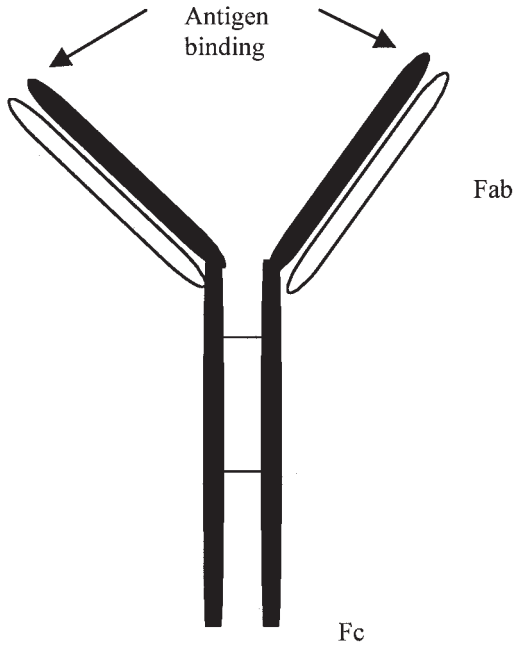

Figure 5. Antibody structure. Heavy chains are in dark; light chains are white. The antigen binding sites are composed of heavy and light chains.

CD8 T CELLS

Responsible for cell-directed cytotoxicity

Hyperacute rejection involves preformed antibodies
Acute cellular rejection is mediated by the recipient T-cell response to donor MHC antigens
Chronic ductopenic rejection involves obliterative vasculopathy and bile duct loss

Hyperacute Rejection

Hyperacute rejection is characterized by a rapid response of the host immune system to the allograft. Within minutes to hours of the transplant, preformed antibodies engage class I MHC or the ABO blood group antigens on the graft. The antibodies facilitate complement-mediated lysis of the endothelium and initiate an inflammatory cell infiltrate. Hyperacute rejection is rarely observed in liver transplants, even among those with a positive crossmatch.

Acute Cellular Rejection

Acute cellular rejection is due to an immune reaction mediated by recipient T lymphocytes' response to donor MHC antigens. Antibodies and cytokines also contribute to the immunologic attack. The biliary epithelium and venous endothelium express MHC class I and II molecules and are the focus of the acute cellular rejection response. Hepatocytes that express few class I or II MHC antigens are rarely the target of acute cellular rejection.

The principal clinical features of acute cellular rejection are

1. Elevated liver transaminases and/or bilirubin
2. Lymphocytic infiltrates in the portal triads seen on biopsy
3. The development of graft dysfunction manifested by hyperbilirubinemia and subsequent impaired liver synthetic function

Acute cellular rejection is usually controlled with additional corticosteroid-based immunosuppression with no significant impact on graft or patient survival.

Chronic Ductopenic Rejection

Chronic ductopenic rejection occurs months to years after transplantation. The mechanisms that underlie chronic rejection in any of the solid organs are less well understood than acute cellular rejection. Both immunologic and nonimmunologic processes are implicated. The impact of chronic rejection on the liver allograft is on the intralobular bile ducts, a phenomenon termed the "vanishing bile duct syndrome," and is associated with chronic graft failure. It is believed that many if not all episodes of chronic ductopenic rejection are preceded by acute cellular rejection.

Mechanisms of Immunosuppressive Drug Action

The rational design and use of drugs is based on an understanding of the immune response to the donor organ. These agents can be divided by classes based on their mechanism of action. The antimetabolites include azathioprine and mycophenolate mofetil. Both interfere with purine synthesis and clonal expansion of T and B cells (Fig. 7).

Cyclosporine and tacrolimus exert their action by inhibiting calcineurin, a protein responsible for promoting cytokine-induced gene activation. By inhibiting IL-2 production, they prevent activation of lymphocytes. Rapamycin (Sirolimus), one of the most recently approved antirejection drugs, is structurally similar to tacrolimus and appears to inhibit the T-cell response to IL-2.

Glucocorticoids bind to cytoplasmic receptors, which are translocated to the nucleus where they regulate gene transcription by binding to specific

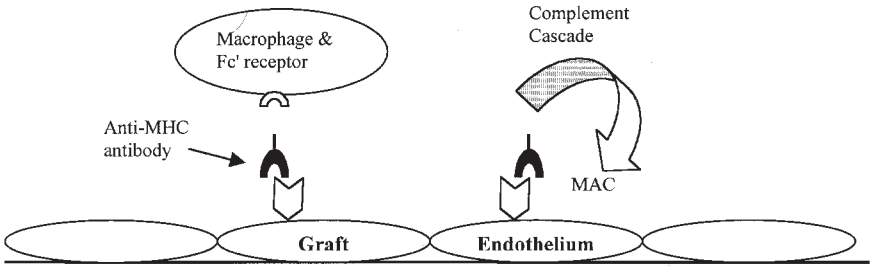

Figure 6. Antibody-mediated damage to graft endothelium. Recipient antibody binds to MHC on graft endothelium. Antibody initiates graft damage through antibody-dependent cellular toxicity (ADCC) and activation of the complement system.

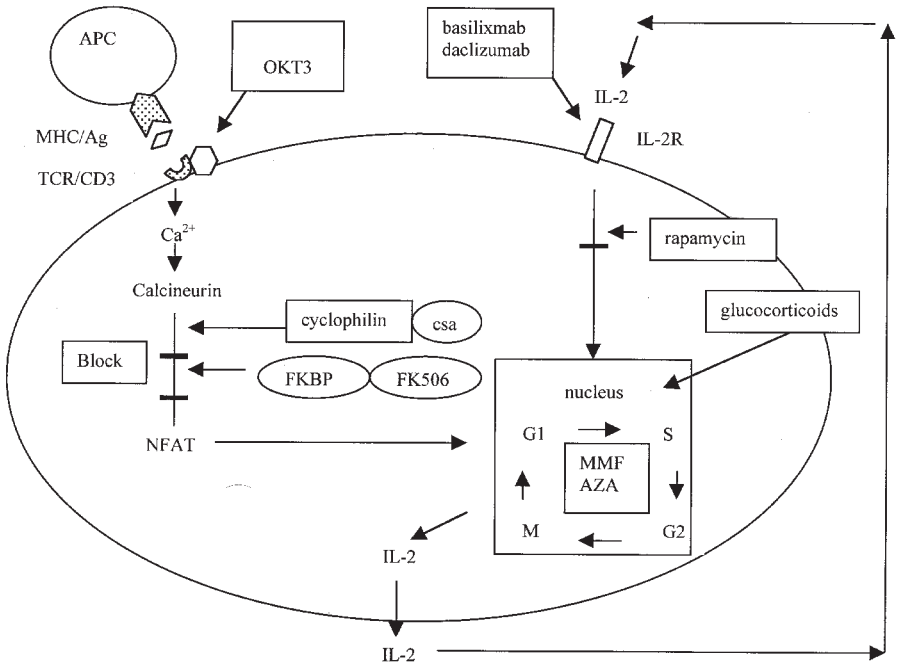

Figure 7. Mechanism of action of commonly used immunosuppressants. Cyclosporine (CSA), tacrolimus (FK506), mycophenolate mofetil (MMF), azathioprine (AZA), FK506 binding protein (FKBP).

gene regulatory regions. They interfere with many aspects of the immune system including the production of IL-1, IL-2, and IFN-γ.

Antibodies recognize many surface antigen epitopes (polyclonal) or single cell surface antigen epitopes (monoclonal). Antithymocyte globulin and thymoglobulin are two polyclonal preparations of immunoglobulin to lymphocytes. OKT-3 is directed against the CD3 receptor on T cells. Basiliximab and daclizumab are two monoclonal antibodies against the chain of the IL-2 receptor. They are used for induction therapy and like the other antibodies result in depletion of the cells that bear the cell surface protein that they bind.

Tolerance

The development of an immunologic state wherein the recipient is unresponsive to donor alloantigen, but yet the immune system is capable of recognizing and responding to other foreign proteins such as bacterial or tumor antigens without the need for immunosuppression, is known as tolerance.

Mechanisms of tolerance can be grouped into suppression, anergy, deletion, and ignorance. Suppression involves the inhibition of donor-reactive T- and B-cell responses by a "suppressor" cell population. While functional examples exist, it has been difficult identifying a suppressor cell. Anergy occurs when T cells encounter peptide-MHC complexes that they recognize, but the T cell does not receive adequate co-stimulatory signals. Deletion, the destruction of alloreactive T cells, is likely to occur in the thymus, and to a lesser extent in the periphery. Ignorance indicates that alloreactive T cells are present but do not respond to stimuli.

GLOSSARY

Acquired immunity: All immune processes utilizing immunological memory (see below). Acquired immunity is the basis of vaccination.

Acute cellular rejection: Inflammation of the allograft elicited by genetic disparity between the donor and recipient, primarily affecting interlobular bile ducts and vascular endothelia, including portal veins and hepatic venules, and occasionally the hepatic artery and its branches.

Allele: Alternative forms of the same gene.

Allogeneic: Genetically dissimilar donor and recipient pair of the same species. The converse is syngeneic.

Allotype: Antigenic determinants that differ among individuals of the same species. Examples include different epitopes of the HLA system.

Anergy: Immunologic tolerance in which lymphocytes become functionally unresponsive.

Antigen presenting cell (APC): Functional descriptor of specialized cells bearing MHC cell surface molecules, by which they "present" peptides, which are the product of intracellular degradation of exogenous proteins recognized as non-self. Activated APCs also express co-stimulatory molecules. Macrophages and dendritic cells are paradigmatic APCs.

Apoptosis: Also called programmed cell death. A specific form of cell death due to enzymatic degradation of DNA, without inflammation.

B cells: Lymphocytes capable of antibody production. Most arise from stem cells in bone marrow. B lymphocytes produce antibodies as circulating proteins or as stationary molecules. The latter, which constitute the B-cell receptor, contain a hydrophobic transgenic sequence that tethers the immune recognition segment of the antibody to the cell surface membrane.

Cell-mediated immunity: Immunologic response based on cellular elements of the immune system.

CD antigen: Cell surface antigens, classified according to "cluster of differentiation" (CD), in which individual molecules are assigned a CD number on the basis of their reactivity with specific monoclonal antibodies.

CD3: A complex of molecules on the cell surface of Tcells that in association with the T-cell receptor (TCR), activate intracellular signal transduction mechanisms when the TCR binds an antigen. Blockade of CD3 by a monoclonal antibody (Orthoclone OKT3) depletes the patient of T cells.

CD4: Cell surface molecule expressed by functionally distinct subset of T lymphocytes. CD4 binds to an invariant part of the MHC class II molecule. CD4-bearing T cells usually act as T helper (Th) cells and recognize antigens processed by APCs and presented in conjunction with MHC class II molecules.

CD8: Cell surface molecule expressed by functionally distinct subset of T lymphocytes. CD8 binds to an invariant part of the MHC class I molecule. CD8-bearing T cells usually act as cytotoxic T lymphocytes (CTLs) and recognize antigens processed by infected or injured nucleated cells and presented in conjunction with MHC class I molecules.

CD28: The best characterized co-stimulatory molecule. Cell surface molecule expressed by T lymphocytes, activated by binding of TCR and antigen ligand. CD 28 has two known ligands (variously named B7-1 or CD80 and B7-2 or CD86), which are expressed on the cell surface of activated APCs.

CTLA-4: Cell surface molecule, structurally similar to CD28, which also binds B7-1 and B7-2. In contrast to the CD28-B7 interaction, linkage of CTLA-4 and B7 leads to an inhibitory signal that terminates the inflammatory response.

Chemokine: Chemotactic cytokines that regulate leukocyte transit. Each type of leukocyte bears chemokine receptors on its cell surface that guides it to chemokines secreted in tissues.

Chronic ductopenic rejection: Defined by two histopathological features: obliterative vasculopathy and bile duct loss

Clone: Genetically identical cells derived from a common ancestor.

Co-stimulatory signal: Non-antigen-specific interaction between lymphocytes and antigen-presenting cells, which uses cell surface molecules expressed on APCs to bind to receptors on lymphocytes (eg, CD 28 on lymphocytes and B7-1 on APC). Co-stimulatory signals enhance the immune response by promoting lymphocyte clonal expansion and cytokine production and are necessary for T-cell activation. Interaction of T lymphocyte and APC in the absence of co-stimulatory signals leads to anergy or apoptosis of the T cell. A parallel receptor ligand interaction that is inhibitory of the immune response is described through CTLA-4.

Cytokine: A large family of low molecular weight soluble proteins involved in regulating cellular activity. Includes the chemokines (see above).

Cytotoxic T lymphocyte (CTL): T lymphocyte that kills its target upon recognizing complexes of peptides and MHC complexes on the target cell membrane. Cytotoxic T cells usually express the CD8 cell surface molecule.

Epitope: The structure within an antigen that is recognized by an antigen receptor (antibody or T-cell receptor).

Graft versus host disease (GVHD): Clinical syndrome caused by immune reaction of allogeneic lymphocytes contained within allograft tissue reacting against alloantigens in the recipient (usually in skin, liver, and gastrointestinal tract).

Haplotype: Closely linked alleles on the same chromosome, usually inherited as a group and linked to inheritance of some phenotypic characteristic.

Helper T cell: T lymphocytes that secrete cytokines required for the immune function of other cells in the immune system. Most helper T cells express the cell surface molecule CD 4.

Human leukocyte antigens (HLA): The major histocompatibility complexes in humans.

Humoral immunity: Immunologic response involving antibodies.

Idiotype: An antigenic determinant within the binding site of an antibody that is recognized by another antibody.

Immunologic memory: The ability of the immune system to recall an encounter with a specific antigen, and to generate a greater response in a subsequent exposure to the same alloantigen. Immunologic memory results from the generation of memory T and B cells during the initial encounter with an alloantigen and is the characteristic feature of "acquired immunity."

Innate immunity: All immunologic defenses that lack immunologic memory. The characteristic feature is that the response remains unchanged however often a specific immunogenic moiety (immunogen) is encountered. This contrasts with "acquired immunity" (see above).

Isograft: Transplant between genetically identical members of the same species such as inborn strain of animals or twins. Also known as a syngeneic transplant.

Major histocompatibility complexes: Histocompatibility antigens expressed on cell surfaces that are the markers by which the immune system distinguishes self from non-self. In humans, the MHC molecules are called HLA (see above).

Memory cells: Cells with lasting response to certain immunologic epitopes.

Natural killer (NK) cell: Lymphocytes that have an innate ability to kill infected or damaged cells, without requiring interaction with MHC surface molecules.

T cell: Lymphocyte that undergoes selection in the thymus. T cells are the only cells essential to the acute cellular rejection response. T cells are distinguished by their cell surface receptor (TCR). T cells are subdivided into categories: T helper cells and T suppressor cells.

Tolerance: An immunologic state in the absence of immunosuppression wherein the recipient is unresponsive to donor alloantigens, while retaining the capacity to recognize and respond to other foreign proteins such as bacteria or tumor antigens.

Vaccination: The exposure of a naive host to a harmless version of a pathogenic immunogen (an altered pathogen, or molecular mimic), which in turn generates memory cells but not the pathologic consequences of the infection itself (the primary immune response). The immune system is thus primed to deliver an enhanced secondary immune response in the event that the host is exposed to the infectious agent in the future.

Xenotransplantation: Transplantation across species. The graft is called a xenograft.

Based on Delves and Roitt, **N Engl J Med** 2000;343:37-49; and Sayegh and Turka, **N Engl J Med** 1998;338:1813-1821.

Suggested Reading

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