AJKD
Core Curriculum in Nephrology

Transplant Immunology and Immunosuppression: Core Curriculum 2015

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Kidney transplantation is the kidney replacement therapy of choice for patients with kidney failure as long as there are no comorbid conditions that preclude the surgery or interfere with the ability to adhere to long-term immunosuppression. In acceptable candidates, kidney transplantation is more cost-effective than dialysis and is associated with lower mortality rates than those observed in waitlisted individuals. During the past 3 decades, kidney transplant recipients have benefitted from remarkable improvements in short-term outcomes compared with recipients in earlier eras. Specifically, 1-year kidney transplant survival rates are now ~95%, and the incidence of acute rejection during the first year after transplantation is now ~15%. Long-term outcomes have improved much less impressively. Alloimmune reactions resulting in acute and/or chronic rejection remain the primary barrier to long-term survival of the kidney transplant. Immunologic tolerance can be achieved with relative ease in small animals. However, the human immune system is complex, containing redundant pathways that make tolerance difficult to achieve. Thus, in the current era, transplant rejection still constitutes the major threat to long-term survival of transplanted kidneys, and nearly all transplant recipients require life-long treatment with immunosuppression to mollify alloimmune responses and allow for long-term transplant survival. This review focuses on immune mechanisms of kidney transplant injury and treatments currently used to prevent or treat transplant rejection (see Box 1 for outline of topics).

MECHANISMS OF TRANSPLANT REJECTION

The main responsibility of the immune response is to defend against infectious pathogens, a role that requires both recognition of pathogens and subsequent activation of immune cells and soluble mediators of immunity. Similarly, the immune responses that lead to recognition and destruction of a transplant require mononuclear cells with migration capacity, antigen-presenting cells (APCs), soluble mediators such as cytokines, and effector cells that target and injure the transplant. It is useful to consider the cellular and noncellular components of alloimmunity (Box 2) when attempting to understand the effects and limitations of current therapeutics in transplantation.

Allorecognition

The major histocompatibility complex (MHC) comprises cell-surface proteins encoded by a gene family located on chromosome 6. The primary immunologic role of MHC gene products is to present antigens—in the form of fragments of foreign proteins—so that they can be recognized by T lymphocytes through their antigen-specific receptors. MHC molecules are required for presentation of foreign antigens because T cells are not capable of responding to soluble proteins.

Antigen presentation begins with binding of a peptide antigen by MHC (Fig 1). MHC molecules are composed of one highly polymorphic polypeptide α chain and a monomorphic β chain, consisting of β_2-microglobulin in the case of class I MHC. Allospecificity of class I MHC molecules, constitutively expressed on all nucleated cells, resides in the α chain, a polypeptide with a prominent groove or pocket in which foreign peptides bind for presentation to T cells. Class II MHC molecules are constitutively expressed only on APCs, including dendritic cells,

Box 1. Overview of Transplant Immunology and Immunosuppression

- Mechanisms of transplant rejection
  - Allorecognition
  - T-cell activation and differentiation
  - Effector mechanisms
  - Role of B cells
- Types of rejection
  - Hyperacute rejection
  - Acute cellular rejection
  - Acute humoral rejection
  - Chronic rejection
- Prevention of rejection
  - Desensitization protocols
  - Induction therapy
  - Maintenance immunosuppression
- Treatment of rejection
  - Acute cellular rejection
  - Acute humoral rejection
  - Chronic rejection
- Strategies for achieving tolerance

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Box 2. Components of the Alloimmune Response

- Antigen (peptide)
- Major histocompatibility complex (MHC)
- Antigen-presenting cells (APCs)
- T and B cells
- Costimulatory factors and cytokines
- Effector cells, inflammation, and injury

macrophages, and B cells. For these molecules, adjacent portions of the highly variable α chain and a nonvariable β chain create the peptide groove. For either class, the size of the grooves is too small to bind large intact proteins. Thus, native proteins must be processed into smaller fragments that can bind to MHC molecules. The highly variable amino acid residues located in the groove determine the specificity of peptide binding and T-cell antigen recognition. Functionally, the same T-cell receptor (TCR) can recognize either class I or class II MHC molecules, but restrictions are imposed by the engagement of the T-cell surface molecules CD4 and CD8 to class II and class I molecules, respectively (Fig 2A). Thus, CD4-positive T cells primarily engage peptides presented by class II MHC, whereas CD8-positive T cells engage peptides presented by class I MHC.

Immediately following vascularization of a transplanted organ, donor antigens enter the systemic circulation and travel to the lymph nodes and spleen, where naive T cells become activated. At the same time, recipient cells enter the transplant. Direct allorecognition (Fig 2A) occurs in either the secondary lymphoid system or the transplant. In the lymphoid system, this happens when the recipient's naive lymphocytes are engaged with donor APCs that have traveled to the lymph nodes or spleen. In the transplant, direct allorecognition occurs when donor APCs engage with recipient lymphocytes. Indirect allorecognition (Fig 2B) occurs in the secondary lymphatic system when donor proteins or peptides are processed by recipient APCs and presented to the TCR. In the transplant, indirect allorecognition occurs when recipient APCs process donor peptides and engage recipient lymphocytes by presenting those processed peptides. The direct pathway of allorecognition plays a dominant role in early T-cell–mediated acute rejection episodes, whereas the indirect pathway is believed to be more important in mediating chronic rejection.

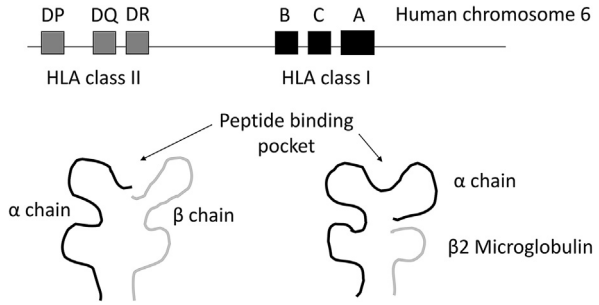

Figure 1. Schematic of the peptide-binding pockets for class II molecules derived from the DP, DQ, or DR loci of the major histocompatibility complex of chromosome 6 and for class I molecules derived from the B, C, or A loci. Figure courtesy of Dr P. Heeger; adapted from Schröppel and Heeger (*Transplantation Immunology*. In: Hricik DE, ed. *Kidney Transplantation*. 2nd ed. London: Remedica, 2007:9-38) with permission of Remedica Medical Education and Publishing.

T-Cell Activation and Differentiation

The TCR is a heterodimer that consists of 2 linked polypeptide chains, α and β. The TCR is then linked to another group of cell-surface molecules known as CD3, a complex that consists of at least 5 covalently bound peptide chains: γ, δ, ε, ζ, and η. When the TCR binds to an MHC-presented antigen, there is a conformational change in CD3 that activates intracellular signal pathways, including tyrosine kinases on the intracytoplasmic tails of the CD3 complex, as well as the CD4 and CD8 accessory molecules. This antigen-driven signal that is transduced by the TCR-CD3 complex to the T-cell cytoplasm has been called "signal 1." This signal is essential, but not sufficient, for full activation of T cells.

A second antigen-independent signal (signal 2) must be provided through additional accessory molecules that costimulate the T cell. Although the family of known costimulatory ligands continues to grow, the most important are ligands between 2 T-cell surface molecules, B28 and CD154 (CD40 ligand), and the APC surface molecules B7 and CD40, respectively (Fig 3). The provision of signals through the TCR alone (without costimulation) leads to clonal and antigen-specific anergy. The T cell does not produce cytokines or undergo cell division and it becomes unresponsive to appropriate stimulation or apoptosis (undergoes programmed cell death).

With adequate costimulation, T-cell activation continues and signals are transduced to the nucleus. A key step in activating T cells is phosphorylation of proteins that form the signaling chain for gene transcription. The immediate effect is phosphotyrosine kinase–mediated phosphorylation of tyrosine residues of several proteins. The phosphorylation modification activates an enzyme that catalyzes the breakdown of plasma membrane phospholipids and the generation of the second messengers inositol 1,4,5 triphosphate (IP3) and diacylglycerol (DAG). IP3 triggers the release of ionized calcium from intracellular stores while DAG activates protein kinase C, leading to the synthesis of nuclear regulatory elements such as the proto-oncogenes c-fos and c-jun. Released cytoplasmic calcium forms a complex with calmodulin, a calcium-dependent regulatory protein. These calcium-calmodulin complexes activate other kinases and

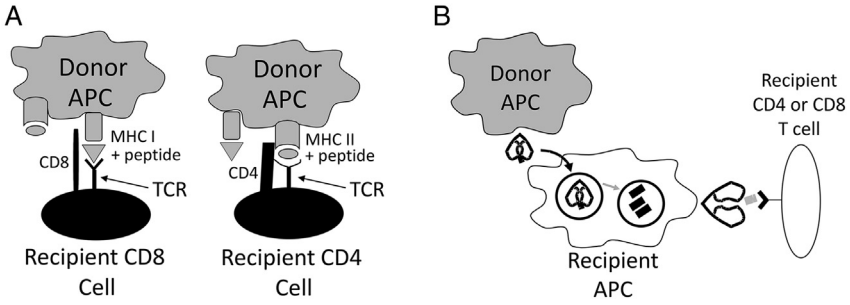

Figure 2. (A) Depiction of direct allorecognition in which a donor antigen-presenting cell (APC) presents peptide to the T-cell receptor (TCR) within the context of donor major histocompatibility complex (MHC) molecule. (Left side) Presentation of a peptide within a class I MHC molecule to a CD8-positive T cell. (Right side) Presentation of a peptide within a class II MHC molecule to a CD4-positive T cell. (B) Depiction of indirect allorecognition in which an antigen is first processed by a recipient APC and then presented within the context of a recipient MHC molecule to either a CD4- or CD8-positive T cell. Figure courtesy of P. Heeger.

phosphatases, including calcineurin. Calcineurin, a calcium-calmodulin complex–dependent phosphatase, plays a key role in the activation of factors required for interleukin 2 (IL-2) gene transcription.

The transcription of IL-2 and other cytokines ultimately drive cell-cycle progression (signal 3) and proliferation (Fig 4) with help from a series of kinases, including members of the target of rapamycin (TOR) pathway. The final result of activation is the proliferation of CD4-positive helper T (T_H) cells and the maturation of CD8-positive cytotoxic T cells. Activated T cells can ultimately differentiate into a number of other phenotypes, including memory cells, which can respond quickly and robustly to the original antigen many years after it was first presented, and regulatory cells (Tregs), which can suppress immune responses and promote tolerance.

Both CD4- and CD8-positive T cells can be divided into subsets defined by the cytokines they produce following activation. The cytokines act as potent biological response modifiers; thus, CD4-positive T_H cells may be of the T_H1 subset (producing IL-2 and interferon γ [IFN-γ]) or the T_H2 subset (producing IL-4 and IL-5). The latter group of cytokines is involved in B-cell help. Other subsets of T cells express the cytokine transforming growth factor β (TGFβ).

Effector Mechanisms

Humans possess an innate immune system designed to quickly stymie the spread of infectious pathogens. Innate immunity (designed to destroy a pathogen) is often initiated by Toll-like receptors, modulated by complement activation, and mediated by macrophages and natural killer cells. Recently there has been interest in the concept that this pathway may interact with alloimmune mechanisms, thus forming a potential link between nonspecific injury (eg, ischemia-reperfusion injury or infections) and acute rejection.

In contrast, T and B cells provide finely tuned specificity; however, developing this adaptive immunity requires days to weeks. Although transplant rejection requires T-cell activation, it is important to recognize that other cells of the immune system can contribute to transplant injury. For example, endothelial cells participate in the maturation of dendritic cells. Mesangial cells in the kidney produce matrix that regulates immune responses. Tubular epithelial cells express TGFβ and other cytokines that can contribute to fibrosis. The complement system serves as an important interface between innate and adaptive immunity. The terminal components of complement are important effectors of transplant destruction, leading to membrane injury, neutrophil infiltration, and damage to epithelial and endothelial cells. However, the complement system is also involved in T- and B-cell stimulation.

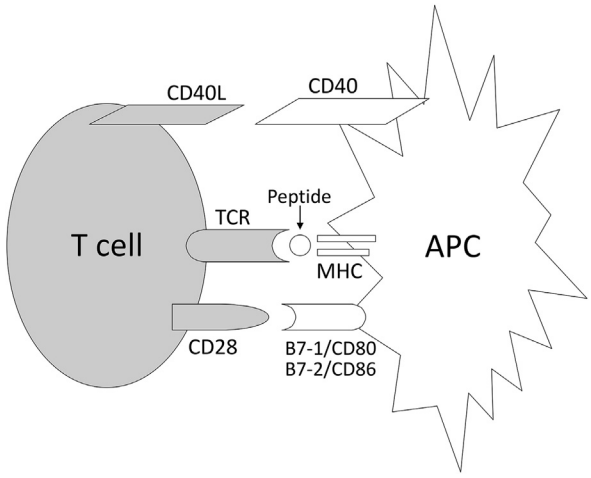

Figure 3. Depiction of 2 costimulatory signals, CD40:CD40 ligand (CD40L) and B7:CD28, which are required for full T-cell activation, beginning with presentation of a peptide by an antigen-presenting cell (APC) to the T-cell receptor (TCR). The B7 accessory molecule consists of either CD80 or CD86, which binds to CD28 with different affinities. Abbreviation: MHC, major histocompatibility complex. Figure courtesy of P. Heeger.

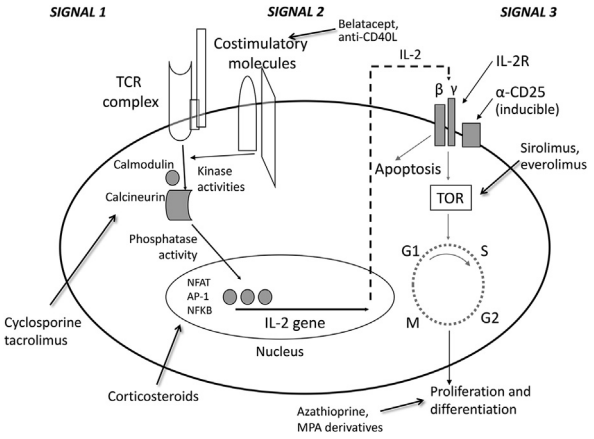

Figure 4. Schematic of the 3 signals required for full activation and proliferation of T cells and the sites of action for commonly used maintenance immunosuppressive drugs. See text for details. Abbreviations: IL-2R, interleukin 2 receptor; MPA, mycophenolic acid; TCR, T cell receptor; TOR, target of rapamycin. Figure courtesy of P. Heeger; adapted from Schröppel and Heeger (*Transplantation Immunology*. In: Hricik DE, ed. *Kidney Transplantation*. 2nd ed. London: Remedica, 2007:9-38) with permission of Remedica Medical Education and Publishing.

The Fas/Fas ligand (FasL) pathway is another important effector mechanism. Fas is ubiquitously expressed on parenchymal cells, whereas FasL is induced upon activation of CD4-positive T cells. Cross-linking of Fas with trimerized FasL ultimately activates caspase 8 and propagates a death signal that ends in apoptosis.

Activation of caspase enzymes that eventually lead to irreversible cell injury and death can also occur independently of cell-surface receptors. CD8-positive T cells express cytotoxic molecules that are lethal to cells. One of these, granzyme B, requires a pore structure to gain access to the cell. That pore is formed by perforin, another product of cytotoxic T cells, which is inserted into the target cell membrane in a calcium-dependent process. When granzyme B enters the target cell cytosol, it triggers pathways that result in cell death through apoptosis. Natural killer cells are effector cells that also produce perforin and granzyme B. In addition, they produce IFN-γ, thus promoting inflammation.

Role of B Cells

With the help of T_H2 T cells, bone marrow–derived B cells can differentiate into plasma cells that ultimately produce antibodies specific for the original peptide antigen presented to the T cell. Recently, several growth factors necessary for this differentiation have been identified and may ultimately serve as therapeutic targets. Mature B cells are found mainly in lymphoid follicles and bone marrow and in low numbers in the circulation. Differentiated plasma cells generate antitransplant antibodies that can act by fixing complement or opsonizing cells that are then killed by cell-mediated lympholysis. B cells also serve as excellent APCs.

Recently, alloantibodies have been re-identified as major effectors of both acute and chronic transplant injury. Alloantibodies are primarily directed against HLA antigens. However, a number of less common alloantibodies to non-HLA antigens (eg, endothelial or epithelial antigens) have been identified and occasionally cause transplant injury. Preformed antibodies to HLA antigens most commonly occur in patients who have had previous transplants, pregnancy, or blood transfusions. Less commonly, they develop cross-reactively after exposure to vaccines, viruses, or other pathogens. Preformed anti-HLA antibodies are measured by a variety of cross-matching techniques beyond the scope of this review. Mixing recipient serum with the cells or with HLA antigens of a specific donor performs a donor-specific cross-match. When the serum of a potential transplant recipient is "cross-matched" with cells from a large panel of potential donors, the test is referred to as a panel-reactive antibody (PRA) test. Patients with a high PRA score (ie, preformed anti-HLA antibodies against a large number of potential donors) are said to be sensitized and generally exhibit transplant outcomes that are inferior to nonsensitized patients. In theory, only donor-specific antibodies (DSAs) are responsible for transplant injury. Transplantation is usually avoided in patients with pre-existing DSAs. However, very low titers may escape detection by even the most sensitive of cross-matching techniques. Moreover, de novo DSAs develop in as many as 15% of kidney transplant recipients during the first year after transplantation and increase in frequency with the passage of time; they are now recognized as a major cause of late transplant injury and transplant loss.

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TYPES OF REJECTION

Transplant rejection can be classified based on clinicopathologic criteria into hyperacute, acute, and chronic forms (Box 3). Although acute forms of rejection are usually divided into cellular and humoral types, there are sometimes components of both cellular- and antibody-mediated damage in a single tissue specimen. The term chronic rejection was considered obsolete a decade ago, but has made a comeback with increasing recognition of the role of antibodies in mediating immune damage (which was once encompassed under the broader term chronic allograft nephropathy). The Banff criteria are histologic criteria used to assess the type and severity of rejection. Although the criteria have been revised often, traditionally they have been most useful in assessing the severity of acute forms of kidney transplant rejection (Box 4). Banff indexes for chronic injury were historically more helpful in assessing the severity of interstitial fibrosis and tubular atrophy. However, the criteria continue to be revised to allow grading of acute and chronic humoral rejection.

Biopsy of the transplanted kidney remains the gold standard for the diagnosis of rejection. Subclinical rejection is found in 5% to 25% of protocol biopsies performed during the first 6 posttransplantation months in patients with stable kidney function, an observation suggesting that immune injury can occur before kidney function begins decreasing. This is one line of evidence that has fueled interest in developing immune biomarkers that can be used as surrogates for invasive biopsies or as predictors of imminent rejection, to potentially allow therapeutic intervention before the immune injury affects transplant function. A number of promising urine biomarkers have been reported in recent years, but have not yet achieved widespread use in clinical practice.

Box 3. Types of Kidney Transplant Rejection

- Hyperacute
- Acute cellular
  - Interstitial
  - Vascular
- Acute humoral
- Mixed (features of both cellular and humoral)
- Chronic

Box 4. Banff 2007 Classification for Acute Kidney Transplant Rejection

Acute Cellular Rejection
- "Borderline changes": mild form of T-cell–mediated rejection with no intimal arteritis but minor interstitial inflammation with foci of tubulitis
- Grades
  - Grade IA: Interstitial inflammation in at least 25% of parenchyma and moderate tubulitis
  - Grade IB: Like IA but more extensive tubulitis
  - Grade IIA: Mild to moderate intimal arteritis
  - Grade IIB: Severe intimal arteritis comprising at least 25% of luminal area
  - Grade III: Transmural arteritis

Acute Antibody-Mediated Rejection
- Requires positive C4d staining and the presence of circulating donor-specific antibodies with 1 of 3 histologic variants:
  - An ATN-like picture
  - Capillary involvement
  - Arterial involvement

Based on Solez et al (*Am J Transplant.* 2008;8:753-760).
Abbreviation: ATN, acute tubular necrosis.

Hyperacute Rejection

This form of rejection occurs in recipients with high titers of preformed DSAs and is a rare occurrence in the era of modern highly sensitive cross-matching techniques. Clinically, hyperacute rejection most often presents immediately after revascularization of the transplant, with overt necrosis of the transplant before completion of the operation, usually mandating immediate transplant nephrectomy. Delayed hyperacute rejection, presumably reflecting lower DSA titers and less immediate destruction of the transplant, has been described, necessitating nephrectomy hours to days after the initial operation. The histology of hyperacute rejection is characterized by fibrinoid necrosis of arterioles, reflecting complement-mediated antibody damage to endothelial cells.

Acute Cellular Rejection

Acute cellular rejection accounts for 90% of early rejection episodes (ie, those occurring in the first 3 months posttransplantation) and is mediated by the activation and proliferation of T cells. Use of induction antibody therapy may delay the onset of cellular rejection. Moreover, lack of adherence to or intentional withdrawal of maintenance immunosuppression can result in acute cellular rejection even years after transplantation. In the modern era, acute cellular rejection is rarely symptomatic and most often is recognized by declining kidney function (ie, increasing serum creatinine concentration or decreasing glomerular filtration rate). Histologically, it is characterized by interstitial inflammation (a mixture of lymphocytes, macrophages, and occasional eosinophils; Fig 5A and B), vascular inflammation

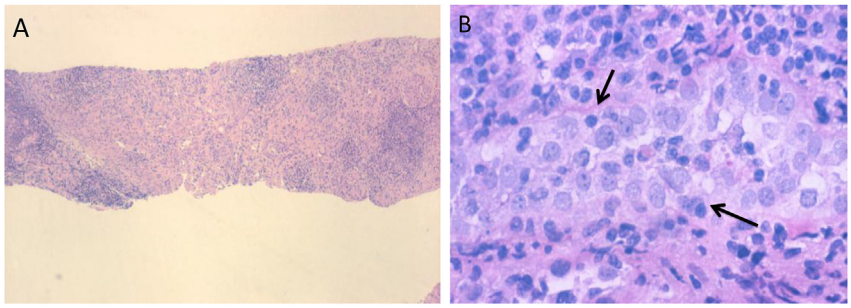

Figure 5. (A) Low-power view of a transplant kidney biopsy specimen shows interstitial inflammation affecting >25% of the core in a acute cellular rejection (hematoxylin and eosin stain). (B) High-power view of the same specimen shows infiltration of the interstitium with round cells and macrophages, and "tubulitis" characterized by lymphocytic invasion of tubular basement membranes and entrance into the tubular lumen (arrows) (hematoxylin and eosin stain).

(lymphocytic infiltration of vascular intima), or both. Vascular involvement (Banff grade II) reflects a more severe variant than isolated interstitial disease (Banff grade I), as evidenced by a poorer response to therapy and worse long-term transplant survival. Clinical factors associated with high rates of acute cellular rejection include retransplantation, humoral sensitization (PRA score > 50%), history of delayed graft function, African American ethnicity, and treatment nonadherence.

Acute Humoral Rejection

Acute humoral rejection can occur early after transplantation in highly sensitized patients (including those who have been treated with desensitization protocols) or any time after transplantation in patients who develop de novo DSAs. Symptoms are rare and patients usually present with either declining kidney function or an increase in urine protein excretion. The histologic hallmark of acute humoral rejection is peritubular capillaritis manifested by infiltration of these capillaries with lymphocytes and especially with neutrophils (Fig 6). In severe cases, fibrinoid necrosis may be seen in peritubular capillaries and glomeruli. C4d is a complement component that binds to renal tissue after complement activation and thus represents a sign of recent attack by complement-dependent antibodies. The presence of C4d in peritubular capillaries, detected either by standard immunofluorescence techniques or peroxidase staining, is highly suggestive of acute humoral rejection (especially when the staining is dense and linear) and is closely associated with the presence of circulating DSAs. However, the specificity of C4d positivity is suboptimal. Histologic signs of humoral rejection in the absence of C4d staining remain concerning, whereas in the absence of histologic changes, the significance of C4d staining (especially if patchy and nonlinear) is not always certain.

Chronic Rejection

Recently, the term chronic rejection has taken on new meanings. Kidney biopsies performed on patients with late declines in kidney function (ie, >1 year after transplantation) often show some combination of interstitial fibrosis, tubular atrophy, and glomerular changes characterized by the term transplant glomerulopathy. The isolated findings of interstitial fibrosis/tubular atrophy (IFTA) have traditionally been attributed to nonimmune mechanisms such as hyperfiltration in a solitary kidney or the toxic effects of calcineurin inhibitors (CNIs). The fibrotic effects of CNIs have been overplayed because recent prospective studies of patients with isolated fibrosis have demonstrated remarkably little adverse effect on long-term transplant survival. However, the presence of inflammation (ie, inflammatory infiltrates not meeting Banff criteria for acute rejection), with or without IFTA, appears to compromise long-term transplant survival and will be the focus of future interventional trials. Transplant glomerulopathy occurs more commonly in patients with positive staining for C4d and/or detectable DSAs and confers an ominous prognosis, with transplant survival rates far lower than those observed in patients with negative study results.

Histologically, transplant glomerulopathy strongly resembles idiopathic membranoproliferative glomerulonephritis, complete with mesangial proliferation

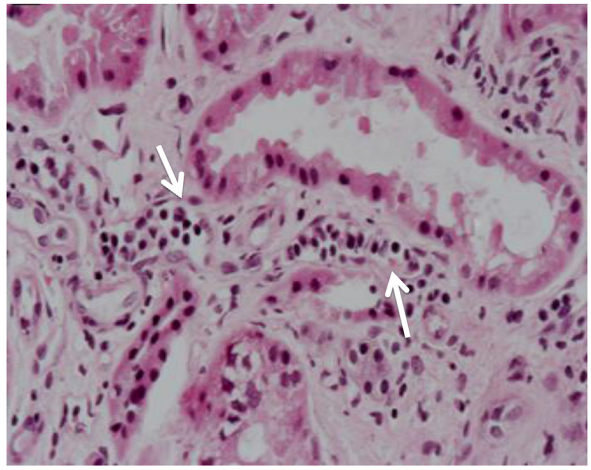

Figure 6. "Capillaritis" in a biopsy specimen from a patient with acute humoral rejection. Lymphocytes and granulocytes are present within peritubular capillaries (white arrows) (hematoxylin and eosin stain).

and duplication of glomerular basement membranes (ie, "tram-tracking"). When performed, electron microscopy typically reveals duplication of glomerular basement membranes. The number of basement membrane layers may be directly correlated with worse transplant outcomes. In some cases, transplant glomerulopathy appears more like idiopathic membranous nephropathy. Not surprisingly, analyses of subepithelial deposits in such cases sometimes reveal HLA antigens complexed with anti-HLA antibodies. These observations suggest that some cases of "de novo" membranous nephropathy after kidney transplantation actually represent a forme fruste of antibody-mediated transplant glomerulopathy.

Gene microarray analysis of tissue obtained from serial transplant biopsies has been useful in depicting the typical sequence of immune injuries after kidney transplantation. Messenger RNA transcripts suggesting T-cell injury occur early after transplantation (first year) and appear to be reversible with treatment. Transcripts suggesting evidence for B-cell activity increase progressively beyond the first 6 months after transplantation, indicating the increasing importance of antibody-mediated injury over time. Nonadherence to immunosuppressive medication regimens plays a role in almost 50% of cases of late humoral rejection.

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PREVENTION OF REJECTION

Desensitization Protocols

Two types of immunologically incompatible donor-recipient combinations have emerged: those that are blood group type ABO incompatible and those in which recipients have significant preformed HLA antibodies to their donors. Active intervention to address the clinical problems of sensitization developed in the mid-1990s. These have included desensitization protocols and paired donor exchange programs (though the latter topic is outside the scope of this review).

ABO-Incompatible Desensitization

ABO-incompatible donor-recipient combinations have been successfully performed for more than 20 years. Patients with the A_2 subtype of blood group A express markedly reduced levels of A antigen on cell surfaces, which when first recognized led to the use of A_2 donor organs for B and O recipients. The A_1 red blood cell surface contains about 1 million A antigens, whereas the A_2 erythrocyte contains only about 250,000. For potential recipients with low levels of anti-A antibodies, kidneys from A_2 donors behave more like blood group O donors and often can be transplanted without implementing specialized recipient preconditioning.

Transplantation across unacceptable ABO disparities in the absence of an A_2 donor kidney or in the recipient with a high level of anti-A antibodies requires recipient desensitization to the donor. Desensitization protocols all implement similar principles for recipient management: minimizing or eliminating the circulating DSAs, usually with plasmapheresis; attenuating the subsequent immune response with some combination of immunosuppression, intravenous immunoglobulin (IVIG), rituximab (ie, monoclonal anti-CD20 antibody), or splenectomy; and active surveillance and early re-treatment if DSAs re-emerge.

Preformed Anti-HLA Antibody Desensitization

Implementing desensitization protocols for potential transplant recipients who exhibit preformed donor-specific anti-HLA antibodies may be less rewarding. Defining the antibody and its titer can often predict the probability of successful desensitization. It may be possible to successfully perform transplantation on a recipient with a low antibody titer against single donor antigen without pretreatment; however, in the recipient with a high PRA score ($>30\%$) and high titers of antibodies against one or more donor antigens, desensitization can be attempted. The goal of desensitization in this circumstance is the marked reduction of antibody reactivity and not necessarily a negative cross-match. Desensitization is rarely successful when recipients exhibit extremely high DSA titers. Because of variable outcomes and the large associated cost, only a minority of US transplantation centers are currently using desensitization

protocols for highly sensitized patients on a regular basis.

Desensitization for patients with pre-existing anti-HLA antibodies is performed either with high doses of IVIG given regularly (eg, monthly) until a negative cross-match is achieved or with plasmapheresis-based protocols similar to those used for ABO-incompatible pairs, often using lower IVIG doses as adjunctive therapy. The optimal strategy remains a subject of considerable debate and controversy. These protocols work best when a living donor is available for expeditious transplantation, but have also been applied to patients awaiting deceased donor transplantation. Posttransplantation plasmapheresis and IVIG administration are generally continued for up to 2 weeks. Persistently elevated antibody titers after transplantation often portend transplant loss. Desensitization protocols have clearly allowed kidney transplantation in patients who may have not been suitable for transplantation in an earlier era. However, these patients exhibit high rates of acute humoral rejection requiring expensive treatment and may exhibit poor long-term outcomes, raising questions about the cost-effectiveness of this strategy. Proteasome inhibitors and complement inhibitors have been added to desensitization protocols at some centers, but are used more commonly to treat humoral rejection.

Induction Therapy

Induction therapy refers to using specific agents that usually are administered to the transplant recipient on a short-term basis during the perioperative period. Some of these agents have long-lasting effects on the immune system, so their biological actions overlap with those of drugs used during the maintenance phase. The aim of induction therapy is to provide the transplanted organ with the opportunity to survive the initial effector response by the host, a process essential to maintaining transplant function. One advantage of using antilymphocyte antibodies as induction agents is a reduction in the incidence of acute rejection during the early posttransplantation period. In theory, this avoids superimposition of immune injury on a transplanted kidney that already may be injured by perioperative factors, including ischemia-reperfusion injury.

The benefits of using induction therapy must be balanced against the toxicities of the various agents, the costs associated with the treatment, and the side effects of the agent used. Currently, ~80% of patients receiving kidney transplants in US medical centers receive some form of induction antibody therapy. Induction antibodies have been classified as either polyclonal or monoclonal agents. However, antibodies available for use in the United States currently are limited to 2 polyclonal agents (rabbit antithymocyte globulin [ATG] [Thymoglobulin; Sanofi] and horse antithymocyte globulin [ATGAM; Pfizer]) and 2 monoclonal antibodies (basiliximab and alemtuzumab).

Presently, rabbit ATG is the most popular polyclonal antibody used in the United States, but it is technically prescribed off-label for induction therapy: it is approved by the US Food and Drug Administration (FDA) only for the *treatment* of acute rejection. The reasons why rabbit ATG is effective are not fully understood. The preparation includes antibodies against many T-cell markers, including CD2, CD3, CD4, CD8, CD11a, CD18, CD25, CD44, CD45, HLA-DR, and HLA antigen class I heavy chains. Generally, treatment with rabbit ATG is associated with profound lymphopenia, so much so that it has been classified as a T-cell–depleting antibody. The agent effectively suppresses cellular immune responses to a variety of antigenic stimuli, but may be less reliable in preventing antibody-mediated acute rejection.

Currently available monoclonal induction antibodies include basiliximab, which binds to the IL-2 receptor (CD25), and alemtuzumab, which binds to CD52. Of these agents, only basiliximab is FDA approved for induction therapy. Today, alemtuzumab is approved only for the treatment of lymphoma; in transplant recipients, the drug causes lymphopenia that often is more profound and sustained than that induced by polyclonal agents.

Induction immunotherapy sometimes involves the use of large doses of drugs that normally are reserved for maintenance immunosuppression. A majority of centers continue to administer high doses of corticosteroids during and for several days after transplantation. When administered in high doses, these agents may inhibit the generation of inflammatory cytokines and limit the migration of cells into the transplant. In addition, steroids are often administered to mollify the side effects of some of the induction antibodies.

Maintenance Immunosuppression

Maintenance immunosuppression has evolved over the last 50 years, largely because of several serendipitous discoveries of new immunosuppressant drugs. Major improvements in long-term transplant survival occurred during 3 major eras. The first, from 1954 to 1982, was based on a drug regimen that initially consisted of monotherapy with azathioprine, and shortly thereafter, the combination of azathioprine with corticosteroids. With this combination, early acute rejection remained a major obstacle to even short-term transplant survival. In addition, these medications were nonspecific in their ability to suppress immune responses.

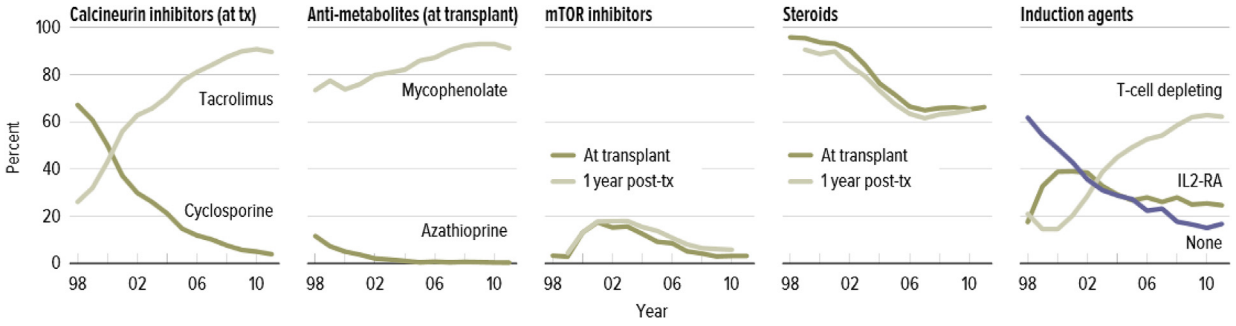

Figure 7. Trends in the use of immunosuppressant drug classes between 1998 and 2011 at the time of hospital discharge (dark lines) and at 1 year post-tx (light lines). Abbreviations: IL2-RA, interleukin 2 receptor antibody; mTOR, mammalian target of rapamycin; tx, transplantation. Reproduced from the 2011 Annual Data Report of the Organ Procurement and Transplantation Network (OPTN) and Scientific Registry of Transplant Recipients (SRTR).

The second era in transplant immunosuppression started in 1982 with the introduction of cyclosporine, a potent CNI that blocks IL-2 production and T-cell activation (signal 1 in the scheme described previously). Using cyclosporine resulted in markedly improved transplant survival rates and a decreased incidence of acute rejection. Between 1994 and 2001, the addition of a second CNI (tacrolimus) and a more selective antimetabolite (mycophenolate mofetil [MMF]) resulted in 1-year patient and transplant survival rates $>$ 95% and $>$90%, respectively. Using combinations of these medications, acute transplant rejection in low-risk nonsensitized patients is often $<$10%, whereas even in high-risk patients, early acute rejection occurs in $<$25%. During this time, the half-lives of kidney transplants slowly increased and chronic rejection replaced acute rejection as the main reason for organ failure and poor long-term function in surviving patients.

The most recent era in the history of transplant immunosuppression, beginning in the early 2000s, saw the introduction of sirolimus, representing a new class of drugs called mammalian TOR (mTOR) inhibitors. Sirolimus is a powerful antiproliferative agent that blocks both lymphocyte and mesenchymal cell proliferation (signal 3). In the same period, enteric-coated mycophenolic acid was introduced as an alternative to MMF, and a second mTOR inhibitor, everolimus, was approved. Another major new drug class introduced during this most recent era is belatacept, a fusion protein that blocks T-cell costimulation (signal 2) mediated by the B7-CD28 ligand. In clinical trials, belatacept was used as an alternative to CNI (ie, cyclosporine) therapy and was associated with better long-term kidney function despite higher rates of acute rejection. This third era in the history of transplant immunosuppression also has been marked by the proliferation of generic equivalents for cyclosporine, tacrolimus, and MMF.

The availability of multiple immunosuppressive agents has allowed transplantation physicians to combine medications to take advantage of disparate mechanisms of action, with the goal of reducing morbidity and optimizing outcomes. This has resulted in significant controversy about which regimen of immunosuppressive medications may be the most efficacious. Figure 7 depicts data from the 2011 Annual Report of the Scientific Registry of Transplant Recipients. Several trends are obvious. First, tacrolimus has emerged as the CNI of choice. Second, MMF has largely replaced azathioprine. Third, the mTOR inhibitors have declined in popularity as de novo agents. Finally, almost 40% of patients are now managed without the use of corticosteroids. As of 2011, the combination of tacrolimus and a mycophenolate acid derivative, with or without steroids, was used for maintenance therapy in just under 80% of kidney transplant recipients in the United States.

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TREATMENT OF REJECTION

Acute Cellular Rejection

Acute cellular rejection that is believed to be clinically or histologically mild is frequently treated at first with large "pulse" doses of corticosteroids. Antilymphocyte preparations are used in patients for whom pulse steroid therapy is not effective or for those initially presenting with clinically or histologically severe rejection. In the current era, rabbit ATG is the most commonly used agent.

Acute Humoral Rejection

Algorithms for treating acute antibody-mediated rejection are less well established and vary widely from center to center. Traditional antilymphocyte antibodies are often used adjunctively to treat antibody-mediated rejection, especially when there is suspicion that cellular rejection might also be occurring. However, plasmapheresis, anti-CD20 antibodies (ie, rituximab), and/or IVIG are now the modalities most commonly used for primary treatment of humoral rejection. The duration of pheresis varies widely, but most often is dictated by changes in the titers of the offending DSAs. IVIG may be administered intermittently (eg, after each plasmapheresis session) or occasionally in larger doses only after completing a course of pheresis. The mechanisms underlying the immunomodulatory effects of IVIG are incompletely understood, but may include Fc-receptor blockade, regulation of complement components, modulation of cytokine secretion, suppression of natural killer cell activity, downregulation of NFκB (nuclear factor-κB) activation, and attenuation of T-cell stimulation.

Two classes of agents, proteasome inhibitors and complement inhibitors, are now being used experimentally as additional adjuncts to treat acute humoral rejection. Proteasomes are cellular complexes that serve to contain fragments of cellular proteins that otherwise would lead to apoptosis of the cell. Proteasome inhibitors effectively induce apoptosis in cells, such as plasma cells, that produce abundant amounts of proteins. Bortezemib, a proteasome inhibitor currently approved for treating multiple myeloma, has been used in combination with traditional therapies for treatment of acute humoral rejection and appears to have its largest benefit in early cases (occurring in the first 6 months after transplantation). Complement inhibitors offer the theoretical benefit of inhibiting complement-dependent antibody injury even if the antibodies are present. Eculizumab, currently approved only for treating paroxysmal nocturnal hemoglobinuria, is a monoclonal anti-C5 antibody that has shown some benefit as an adjunctive treatment for acute humoral rejection.

Chronic Rejection

Treatment of chronic antibody-mediated rejection has been disappointing, possibly because the pathophysiology remains poorly understood and lesions such as transplant glomerulopathy often coexist with other chronic histologic changes (eg, IFTA). Virtually all the measures described in the preceding for treating acute rejection (described here) have been used for treating chronic rejection with variable and often unsatisfactory results. Use of angiotensin-converting enzyme inhibitors should be routine in patients with heavy proteinuria. In patients with circulating DSAs, minimizing or eliminating CNIs is not recommended because reduced exposure to these agents may promote further antibody development.

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STRATEGIES FOR ACHIEVING TOLERANCE

One ultimate goal of transplantation is to induce immunologic tolerance to the transplant such that the host's immune system is intact (can respond normally to immune stimuli) without immunosuppression and with the specific absence of a detrimental immune response directed at the transplanted organ. Studies in animal models have suggested that tolerance to a transplant can be achieved under certain conditions, including removal of the donor-reactive immune cells (deletion), induction of immunologic ignorance (so the immune system never "sees" the transplant antigens), induction of anergy (nonresponsiveness), or active inhibition by Tregs. Immunologic tolerance has been achieved in human kidney transplant recipients when bone marrow transplantation has been performed between HLA antigen–identical donors, followed by kidney transplantation using the same donor. Based on these experiments of nature, several groups have attempted to use bone marrow ablation with either marrow or stem cell transplantation and adjunctive cocktails of early immunosuppression in an effort to achieve long-term tolerance. Results have been variably successful, but the cost-effectiveness and safety of these strategies have been open to question.

Tregs suppress immune responses, potentially by local cytokine production and prevention of dendritic cell activation. The recent recognition of multiple phenotypes of Treg (eg, those that are CD25-, CD4-, and Foxp3-positive) as well as newly developed methods for inducing Treg expansion in vitro and in vivo, has excited the transplant community. While only limited success has been achieved in developing human allograft tolerance in humans, multiple groups are studying whether and how Tregs can be exploited to prolong transplant survival and potentially induce robust transplant tolerance.

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