Transplant Immunology for Non-Immunologist

Peter S. Heeger, MD and Rajani Dinavahi, MD

Mount Sinai School of Medicine, New York, NY

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ABSTRACT

Transplantation is the treatment of choice for end-stage kidney, heart, lung, and liver disease. Short-term outcomes in solid-organ transplantation are excellent, but long-term outcomes remain suboptimal. Advances in immune suppression and human leukocyte antigen matching techniques have reduced the acute rejection rate to <10%. Chronic allograft injury remains problematic and is in part immune-mediated. This injury is orchestrated by a complex adaptive and innate immune system that has evolved to protect the organism from infection, but, in the context of transplantation, could result in allograft rejection. Such chronic injury is partially mediated by anti-human leukocyte antigen antibodies. Severe rejections have largely been avoided by the development of tissue-typing techniques and crossmatch testing, which are discussed in detail. Further advances in the understanding of T- and B-cell immunology have led to the development of new immunomodulatory therapies directed at prolonging allograft survival, including those that decrease antibody production as well as those that remove antibodies from circulation. Further application of these immunomodulatory therapies has allowed expansion of the donor pool in some cases by permitting ABO-incompatible transplantation and transplantation in patients with preformed antibodies. Although vast improvements have been made in allograft survival, patients must remain on lifetime immunosuppression. Withdrawal of immunosuppression almost always ultimately leads to allograft rejection. The ultimate dream of transplant biologists is the induction of tolerance, where immune function remains intact but the allograft is not rejected in the face of withdrawn immunosuppression. This, however, has remained a significant challenge in human studies. ***Mt Sinai J Med 79:376–387, 2012.*** *© 2012 Mount Sinai School of Medicine*

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Address Correspondence to:

Rajani Dinavahi
Mount Sinai School of Medicine
New York, NY
Email: rajani.dinavahi@mssm.edu

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Transplantation is the treatment of choice for end-stage kidney, liver, heart, and lung disease, and it is increasingly employed as therapy for intestinal failure and diabetes. The development of new immunosuppressant medications supplemented by improvements in medical care have lowered the morbidity risk of developing acute rejection episodes and improved 1-year graft survival rates (>90% for most organs), but long-term graft survival remains suboptimal.[1] As examples, recent data indicate transplant half-lives are ~8 to 11 years for kidney, 5 to 7 years for heart and <5 years for lung transplants.[2–4] As a result, retransplantation rates have markedly increased. Approximately 20% of current wait-listed kidney-transplant candidates have had failed transplants. Improving long-term graft survival will likely require improving risk-assessment strategies

(who is most likely and least likely to develop chronic injury), developing a better understanding of mechanisms causing transplant injury, and tailoring immunosuppressive therapies to target the specific mechanisms in each patient. From a practical standpoint, clinicians require a basic understanding of transplant immunology to be able to rationally guide changes in therapy to improve patient outcomes and to understand novel risk-assessment and treatment strategies that are being tested in ongoing clinical trials.

INNATE IMMUNITY

The human immune response can be divided into innate and adaptive components. Innate immunity occurs rapidly, with limited specificity, and without memory, and comprises cellular components (eg, neutrophils, macrophages, dendritic cells, natural killer cells)[5] as well as molecular components (toll-like receptors [TLRs], complement proteins, chemokines, and cytokines among others).[6–8] Current concepts are that the innate immune system serves to recognize pathogens, provides signals to activate the more specific adaptive immune response, and provides effector mechanisms for pathogen removal and tissue healing.[9,10] Work performed over the last decade has provided the important insight that activation of the innate immune system can contribute to transplant injury. Complement activation and TLR signaling, among other innate components, are triggered by healing and ischemia reperfusion that occur as a result of the transplant surgery.[11] Downstream effects of these processes contribute to delayed graft function and amplify adaptive immune responses that can negatively impact long-term graft survival. A number of novel treatment strategies that target components of the innate immune system at the time of transplant are being developed and tested in an effort to prevent primary graft dysfunction and to improve long-term outcomes in recipients of deceased-donor organ transplants. Another indication of the importance of innate immunity in transplantation derives from the observation that the outcomes from recipients of living organs that experience limited ischemic injury are better than outcomes in recipients of otherwise similar deceased organs.[12–14]

ADAPTIVE IMMUNITY

Adaptive immune responses, comprising antibody-producing B cells and T cells, develop slower than innate responses but are more specific and result in memory.[9,10] Specificity and memory are essential characteristics for controlling and preventing infections, but as discussed later, are detrimental in the context of transplantation.

Antibody molecules are composed of 2 covalently attached heavy chains and 2 light chains and can be envisioned as being shaped as a Y. The 2 upper portions of the Y, composed of variable regions of heavy and light chains, are highly polymorphic regions capable of binding to extracellular, 3-dimensional, antigenic epitopes. The base of the Y represents the constant, or Fc, region of the antibody (formed by 2 heavy chains) and mediates effector functions, including activating the complement cascade and interacting with macrophages, neutrophils, and natural killer (NK) cells through their Fc receptors (Figure 1A). The consequence of antibody binding to any antigen is to "mark" the molecule or pathogen for removal through the above-noted effector mechanisms. Antibodies can also block receptor ligand interactions and/or transmit stimulatory or inhibitory signals to antigen-expressing cells.

Antibodies are produced by B cells that develop through increasingly well-understood pathways that eliminate strongly self-reactive clones (to prevent autoimmunity). Survival of mature B cells in the periphery is dependent upon cytokines and several survival factors that include BLyS (also known as BAFF) and a proliferation-inducing ligand (APRIL).[15] When mature B cells are appropriately stimulated through their B-cell receptors, they differentiate into memory B cells and into metabolically active, antibody-secreting plasma cells (Figure 1B).[9,10]

T cells have evolved in higher organisms because many pathogens reside inside host cells and are thus "invisible" to antibodies (which do not penetrate cells). T cells use heterodimeric T-cell receptors (TCR) to recognize peptides expressed in the context of highly polymorphic major histocompatibility (MHC) molecules (called human leukocyte antigens [HLA] in humans). During ontogeny, most self-reactive T cells are eliminated, enriching for a repertoire capable of recognizing foreign peptides plus self MHC.

T-cell activation requires recognition of its specific peptide/MHC antigen expressed on a professional antigen presenting cell or antigen-presenting cell (APC), for example, a dendritic cell (DC), in the context of appropriate costimulatory signals. Examples of costimulatory molecules are T-cell–expressed CD28 interacting with APC-expressed CD80 or CD86, and T-cell–expressed CD154 interacting with APC-expressed CD40.[16,17] In the absence of costimulation, T cells that interact with their specific

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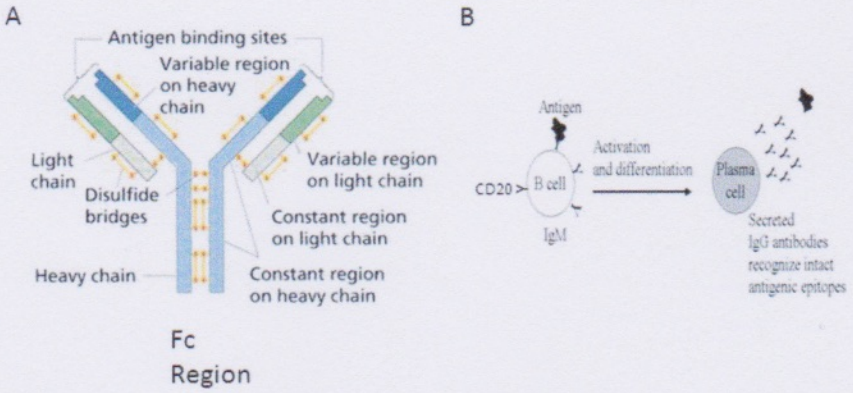

Fig 1. Y-shaped antibody structure (A) composed of highly polymorphic antigen binding regions and an Fc region mediates effector functions. Alloantibody can recognize polymorphic regions of allogeneic MHC molecules. In B-cell activation and differentiation (B), plasma cells secrete antibodies providing a potent and rapid immune response against foreign antigens. **Abbreviations:** MHC, major histocompatibility.

peptide/MHC ligands are either deleted, become anergic (unresponsive), or differentiate into protective regulatory cells. Regulatory T cells (Tregs) are T cells capable of inhibiting other cellular immune responses and are thought to be essential for prevention of autoimmune disease.[18]

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When TCR engagement is accompanied by appropriate costimulatory signals, T-cell activation occurs, initiating a series of intracellular signaling pathways that result in secretion of interleukin 2 (IL-2), and subsequent T-cell proliferation and differentiation into an effector cell (see Figure 2). Once activated, the primed effector T cells are capable of migrating to sites of inflammation (driven in part by chemoattractant chemokines) where they re-encounter their specific antigen expressed on target cells. This engages effector machinery, which includes release of proinflammatory cytokines (interferon-γ [IFN-γ] and IL-17, among others) and leads to granzyme B/perforin- or Fas-mediated cytolytic killing. The result is a coordinated destruction of the antigen-expressing cells, and, in the context of a pathogen, elimination of the agent. For both T and B cells, resolution of the immune response results in the development of highly specific memory capable of reactivating rapidly and preventing reinfection.

When T-cell receptor engagement is accompanied by appropriate costimulatory signals, T-cell activation occurs, initiating a series of intracellular signaling pathways that result in secretion of interleukin-2, and subsequent T cell proliferation and differentiation into an effector cell.

WHAT ARE ANTIGENIC TARGETS ON TRANSPLANTED ORGAN?

In theory, any antigen found on the donor but not the recipient is foreign to the recipient and thus potentially immunogenic. The majority of graft-expressed immune targets are the polymorphic HLA molecules. Class I HLA molecules (HLA A, B, C) are expressed on all nucleated cells, are heterodimers

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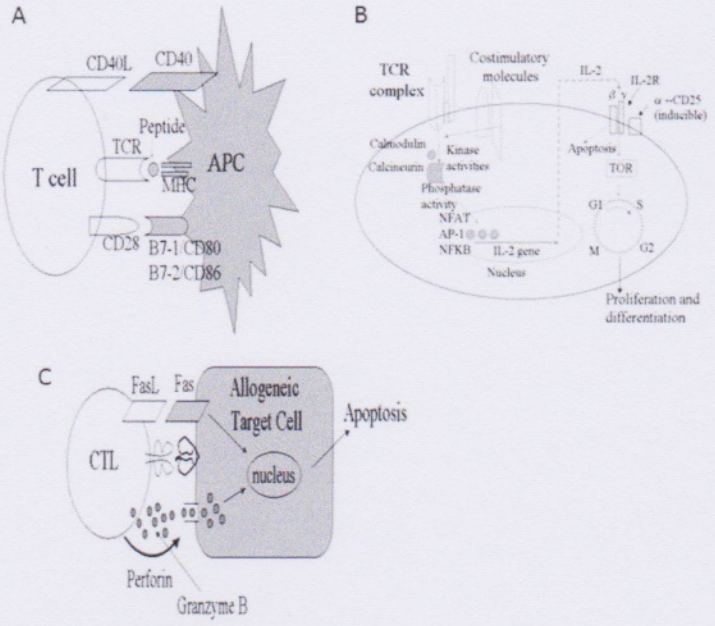

Fig 2. Costimulation is required for full activation of T cells. Schematic illustrating the best-characterized costimulatory interactions (A) between T cell-expressed CD28 and APC-expressed B7-1 or B7-2 (belatacept blocks this interaction), and T cell-expressed CD40L interacting with APC-expressed CD40. Schematic of T-cell activation (B) culminating in proliferation and differentiation of T cells with sites of action for calcineurin inhibitors, basliliximab/daclizumab, rapamycin, azathioprine, and mycophenolic acid. T-cell cytotoxic mechanisms (C) leading to graft cell death. **Abbreviations:** APC, antigen-presenting cell; IL, interleukin; MHC, major histocompatibility; APL, activating protein-1; CTL, cytotoxic lymphocyte; G1, growth phase 1; G2, growth phase 2; M, mitotic phase; NFAT, nuclear factor of activated T-cells; NFKB, nuclear factor kappa B; S, synthesis phase; TCR, T cell receptor; TOR, target of rapamycin.

consisting of a single transmembrane polypeptide chain (the α-chain) and a $\beta 2$ microglobulin, and interact with CD8 T cells. Class II HLA molecules (HLA DR, DP, DQ) are expressed on professional APCs (macrophages, DCs, and B cells) and on activated parenchymal cells; are heterodimers, consisting of 2 homologous proteins, an α and β chain; and interact with CD4+ T cells. All HLA molecules contain a binding groove containing noncovalently bound peptides. The HLA polymorphisms that differentiate the alleles found at a given locus (eg, HLA A1 versus A2) predominantly fall within the peptide binding grooves such that different alleles tend to bind to different repertoires of peptides. As a consequence, humans express a huge diversity of polymorphic HLA molecules so as to be able to recognize essentially any peptide derived from a universe of pathogens and thereby permit survival of the species (if a virus cannot be recognized by the immune system, the virus can kill the host) (Figure 3). The polymorphic nature of HLA provides large numbers of potential antigens for recognition by a recipient of an organ transplant.[19–21] Other transplant antigens include ABO blood group molecules and polymorphisms of non-HLA proteins (often referred to as minor antigens; see below).[22,23]

T CELLS AND TRANSPLANT INJURY

T cells use their TCRs to directly recognize intact donor HLA molecules expressed on donor cells (direct allorecognition). Because T cells are trained to

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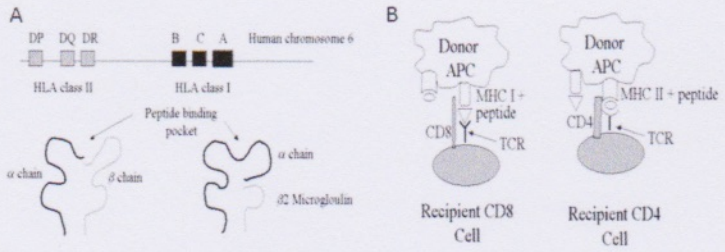

Fig 3. MHC molecules (A) are the primary targets of the alloimmune response. HLA molecules are encoded for on human chromosome 6. The class I loci (A, B, C) encode polymorphic alpha chains that combine with b2-microglobulin to produce an intact HLA class I molecule. The class II loci (DR, DP, and DQ) encode polymorphic alpha and beta chains that combine to produce intact HLA class II molecules. Alloreactive T cells (B) recognize transplant antigens on processed by class I and II HLA molecules. Recipient CD4+ cells recognize peptides processed through HLA class II molecules and CD8+ T cells recognize peptides processed through HLA class I molecules. **Abbreviations:** APC, antigen-presenting cell; HLA, human leukocyte antigen; MHC, major histocompatibility; TCR, T cell receptor.

recognize self and not foreign HLA, T cells responding through this direct pathway are likely a result of cross-reactivity. Interestingly, this cross-reactive response occurs at extremely high frequency (up to 1%–10% of the T-cell repertoire). As an explanation to account for this observation, consider that in grafts expressing different HLA molecules from the recipient, essentially every HLA/peptide complex is a structure that has never been "seen" before by the recipient's immune system. If the recipient's T cells recognize even a small proportion of these complexes through chance cross-reactivity, then there will be a high frequency of alloreactive T cells.[24–26] T cells responding through the direct pathway are thought to contribute to acute and chronic allograft injury.[27,28]

T cells can also recognize transplant antigens via the so-called indirect allorecognition pathway.

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In the indirect pathway, donor graft cells are endocytosed by recipient APCs with the result that donor-derived proteins can be processed into peptides and expressed on the surface of recipient APCs in the context of recipient MHC molecules, just as any foreign antigen would be presented.[24–26] The majority of the donor peptides are derived from the polymorphic regions of the donor MHC molecules themselves. The frequency of T cells responding through the indirect pathway is low compared with the direct pathway and accounts for approximately 5% to 10% of the total alloresponse (or <0.1% of the total T-cell repertoire).[29] Both CD4+ and CD8+ T cells can respond to indirectly presented peptides expressed by recipient MHC II or MHC I molecules, respectively. In contrast to the direct pathway, T cells responding through the indirect pathway do not recognize any antigen on the graft itself; they recognize donor-derived peptide antigens presented on recipient MHC. Despite this, indirectly primed T cells participate in the rejection process and may be preferentially important in the development of chronic allograft dysfunction.[28] As recipient APCs migrate out of the donor organ over time, they are replaced by infiltrating donor APCs, thus setting up a situation in which indirect recognition may be the dominant effector pathway within the transplanted organ (Figure 4).[30]

Minor histocompatibility (mH) antigens are also targets of the transplant reactive immune response. The importance of such mH is apparent in the case of a kidney transplant from one HLA-matched sibling (nontwin) to another (no HLA disparities). Even in the absence of HLA disparities, the recipient will rapidly reject the graft without immunosuppression because of mH differences. Minor antigens are molecularly defined as non-MHC, donor-derived peptides, expressed in the context of MHC molecules common to the recipient and the donor, that are sufficiently immunogenic to induce graft rejection. One illustrative example is the male antigen H-Y, which drives rejection of male mouse skin grafts by otherwise identical female recipients. A number of minor antigens, including H-Y antigens, can contribute to transplant injury in humans (Figure 5).[22]

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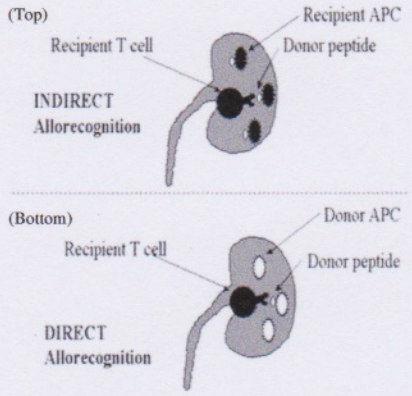

Fig 4. Alloreactive T cells recognize transplant antigens through 2 distinct pathways. A. Recipient T cells (Top) recognize and respond to donor-derived peptides processed and presented by recipient APCs on MHC molecules through the indirect pathway of allorecognition. Recipient CD4+ and CD8+ T cells (Bottom) recognize and respond to donor MHC: donor peptide complexes on graft-derived cells through the direct pathway of allorecognition. **Abbreviations:** APC, antigen-presenting cell; MHC, major histocompatibility.

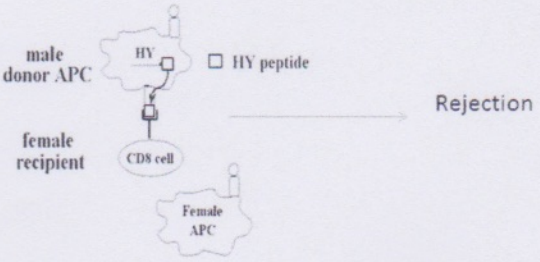

Fig 5. Schematic representation of a minor transplantation antigen derived from the male antigen H-Y. T cells from female recipients of identical transplants derived from male donors respond to a MHC class I restricted, H-Y peptide found in donor cells but not in recipient cells. The peptide is presented in the context of MHC molecules shared between the donor and the recipient; it is only the peptide that is different between the 2 individuals. **Abbreviations:** APC, antigen-presenting cell; MHC, major histocompatibility.

T-CELL ACTIVATION AND TARGETS OF IMMUNOSUPPRESSIVE THERAPY

As described above for T cells reactive to foreign antigens, naïve alloreactive T cells require costimulatory signals to undergo activation.[16,17] The recognition that costimulation is required for T-cell activation led to the development of therapeutic agents capable of blocking costimulatory signals as means for inhibiting pathogenic T-cell immunity. One such potent immunosuppressive reagent, belatacept, blocks the interaction between T-cell–expressed CD28 and APC-expressed CD80 (Figure 2A). Belatacept was approved by the US Food and Drug Administration in 2011 as an effective immunosuppressant for kidney transplant recipients that has the potential to avoid some of the side effects attributed standard immunosuppressants used clinically (including cyclosporine and tacrolimus).[31] A number of additional costimulatory pathways, including CD40/CD154, ICOS-B7RP-1, CD134/CD134L, CD70/CD70L, and PD1/PD1L, contribute to activation of alloreactive T cells in mice, although their specific roles in humans remain unclear and therapies that effectively target these molecules have not yet reached the clinic.[16]

As described above for T cells reactive to foreign antigens, naïve alloreactive T cells require costimulatory signals to undergo activation.

If a T cell receives both a signal through the TCR and a second costimulatory signal, then a number of intracellular activation steps ensue. A calcium flux activates the intracellular molecule calmodulin and allows it to bind to a calcium binding protein called calcineurin, the target of the immunosuppressant drugs cyclosporine A and tacrolimus. This activates a phosphatase followed by a number of downstream reactions that leads to binding of the transcription activating factor NFAT (among others) to the IL-2 promoter. As a consequence, release of this potent T-cell growth factor occurs. Full T-cell activation also leads to up-regulation and expression of the high-affinity α chain of the IL-2 receptor (CD25) on the surface of the T cell (the target of anti-CD25 monoclonal antibodies such as basiliximab and daclizumab). The synthesized and released IL-2 acts in an autocrine and paracrine manner and binds to the up-regulated IL-2R and is one of the main targets of corticosteroid therapy. Corticosteroids inhibit the expression and transcription of several cytokine genes including IL-2 (as well as IL-1, IL-6, IFN-γ, and tumor necrosis factor α [TNF-α]), thereby blocking T-cell proliferation and T-cell–dependent immunity.[32–35] Otherwise, signaling through the IL-2R initiates another cascade mediated in part through a protein called mTOR (mammalian target of rapamycin; the therapeutic target of drug sirolimus). This results in translation of a number of new proteins and allows the cell to progress from the G1 phase to the S phase of the cell cycle, resulting in proliferation (the immunosuppressants azathioprine and mycophenolic acid are

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inhibitors of DNA synthesis and thus inhibit T-cell activation at this stage) (Figure 2B).

T-cell activation precipitates differentiation as manifested by the ability to mediate effector functions (eg, secrete cytokines and kill) and by altering a variety of cell surface molecules, including L-selectin (the lymph node homing receptor) and chemokine receptors, which allows the cells to leave lymphoid organs and circulate widely in the periphery. Chemokines are a family of small cytokines with chemoattractant properties that are produced by donor graft cells and recipient immune cells. Chemokines function mainly as chemoattractants for leukocytes, recruiting monocytes, neutrophils, and other effector cells from the blood to sites of infection or damage. They can be released by many different cell types and serve to guide cells involved in innate immunity and also the lymphocytes of the adaptive immune system. Chemokine receptors expressed on activated T cells interact with a wide array of chemokine molecules to help facilitate recruitment to the site of inflammation, including allografts. Following interaction with their specific ligands, chemokine receptors trigger a flux in intracellular calcium (Ca2+) ions, which generates a chemotactic response of that cell, thus guiding the cell to a desired location within the organism. A number of pharmacological inhibitors of chemokine/chemokine receptors have been developed in an effort to prevent T-cell migration toward sites of inflammation, but none have yet proven to be efficacious in preventing transplant rejection in humans.[36–38]

Once an effector T cell has been attracted to the inflamed graft, it will re-encounter its specific alloantigen on graft cells and initiate effector mechanisms leading to cytolysis. Major mediators of graft cell cytotoxicity are perforin/granzyme B and FasL/Fas. T-cell–expressed FasL binds to target cell-expressed Fas, initiating apoptosis. Perforin release from effector T cells initiates pore formation, thereby permitting T-cell–derived granzyme B to enter the cytosol where it activate caspases, resulting in cell death. Research studies have shown that quantifying gene expression for perforin and granzyme B in urinary cells of kidney transplant recipients has the potential to noninvasively diagnose rejection (Figure 2C).[39]

Much of the above discussion has focused on alloreactive T cells derived from the pool of naïve (not previously activated) T cells. Research over the past 15 years has shown that a significant proportion of the alloreactive T-cell repertoire in humans are memory cells.[40,41] Although such T-cell memory can occur as a result of a previous transplant, alloreactive T-cell memory can be induced by exposure to transplant antigens via blood transfusion or pregnancy and may occur as a cross-reactive response to environmental antigens (eg, infections).[42] Regardless of their origin, alloreactive memory cells have lower/different costimulatory requirements than naïve cells, are resistant to most immunosuppressant medications, and can rapidly engage effector machinery without a differentiation step.[43] These features provide memory cells with an important ability to prevent reinfection but are detriments in the context of transplantation. In support of this statement, research studies indicate that measurements of donor reactive memory cells performed prior to transplant strongly and directly correlate with worse posttransplant outcomes.[44–46] This recognition that measurements of donor reactive memory could be used to assess risk of incipient posttransplant injury has the potential to guide therapeutic decision-making aimed at prolonging graft survival, but it will require development of therapies capable of inhibiting memory T cells.

Research over the past 15 years has shown that a significant proportion of the alloreactive T-cell repertoire in humans are memory cells. Regardless of their origin, alloreactive memory cells have lower/different costimulatory requirements than naïve cells, are resistant to most immunosuppressant medications, and can rapidly engage effector machinery without a differentiation step.

ANTIBODIES AND TRANSPLANT INJURY

Antibodies reactive to transplant antigens recognize 3-dimensional epitopes found on donor cells that are distinct from the host (foreign). Antibodies reactive to donor HLA molecules (alloantibodies) and to ABO antigens can be present prior to transplantation as a result of pregnancy, blood transfusion, previous transplant, or chance cross-reactivity with environmental antigens.[47–49] Anti-HLA antibodies can also develop posttransplantation as a consequence of inadequate immunosuppression and are correlated with graft injury.[50,51] Regardless of their origin, transplant reactive antibodies bind to their targets expressed on graft cells. Because endothelial cells

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lining the vasculature feeding the graft are often the initial site of exposure to the recipient's serum, endothelial cells are important targets of antibody-initiated injury.

Antibodies reactive to donor human leukocyte antigen molecules (alloantibodies) and to ABO antigens can be present prior to transplantation as a result of pregnancy, blood transfusion, previous transplant, or chance cross-reactivity with environmental antigens. Anti-human leukocyte antigen antibodies can also develop posttransplantation as a consequence of inadequate immunosuppression and are correlated with graft injury.

Once an antibody binds a donor cell, it initiates inflammation by activating the complement cascade, producing chemoattractant and proinflammatory anaphylatoxins, C3a and C5a, as well as resulting in formation of the C5b-9 membrane attack complex, which activates and injures endothelial cells. Simultaneously, antibody binding to FcRs expressed on neutrophils, macrophages, and NK cells can result in release of oxygen radicals and cytokines, among other mediators that amplify inflammation. The result is often vascular thrombosis and vasculitis. Low titers of alloantibodies can precipitate acute vascular rejection and/or chronic vascular injury and graft fibrosis following transplantation. Evidence of complement activation in the tissue (C4d staining) is used clinically to support a diagnosis of antibody-mediated injury.[52] The recognition that complement, and particularly C5b-9, is a key effector mechanism underlying antibody-mediated injury led to studies testing the efficacy of an anti-C5 mAb to block/reverse antibody-mediated rejection.[53–55] Preliminary results are promising, although larger studies are needed.

If preformed antibodies are present at sufficiently high concentrations, antibody-mediated hyperacute rejection that leads to immediate graft thrombosis can follow the transplantation procedure.[56] The recognition of this has driven transplant physicians to avoid transplanting organs into candidates with antibodies reactive to donor HLA molecules.

A number of testing strategies are used to identify such antibodies. HLA typing for class I and II antigens and blood-group typing are performed in the donor and the recipient prior to transplantation so as to identify the potential immunogenic targets. The presence of preformed antibodies that are reactive to donor HLA molecules is tested in 2 separate ways. The panel reactive antibody (PRA) test is used to identify any HLA-reactive antibodies in the recipient and is performed while the patient is on the transplant waiting list. In one form of this test, serum from a transplant candidate is tested for its ability to bind to, and lyse, a panel of donor cells expressing varied HLA molecules. A newer method uses HLA-coated beads, rather than a panel of HLA typed cells. If a transplant candidate's serum binds to a proportion of the HLA-coated beads (as detected by a flow cytometry readout), the test is interpreted as positive for donor-reactive antibodies. The purpose of both of these methods is to determine how likely it is that a given transplant candidate will have antibodies against an organ that he or she is offered. The advantage of the newer testing is that it better defines that any reactivity against a cell is actually reactive to an HLA molecule (rather than nonspecifically binding to the cell). In addition, commercially available beads coated with single HLA allelic variants (eg, B7 or A2) can be used to define the specific reactivity of the patients. If a patient is known to have serum antibodies reactive to HLA A2, then a physician may decide that any A2-expressing donor organ should be avoided in this individual. Regardless of how it is done, the PRA test is used as a screening risk-assessment tool. Patients with high PRA results (many anti-HLA antibodies) remain on the waiting list longer because it is more likely that they will have antibodies reactive to a potential donor.[48]

Crossmatch testing is performed just prior to kidney transplant surgery to determine if the recipient has antibodies reactive to that donor organ. Recipient serum is tested against cells procured from the donor. If the donor cells are lysed (or if antibody binding is detected by flow cytometry), then the test is interpreted as positive and most centers will not proceed with transplant. Crossmatch testing often cannot be performed prior to heart and lung transplants because of time constraints, and many transplant centers ignore preformed antibodies when transplanting these organs. Increasing evidence indicates that preformed antibodies to HLA molecules do adversely affect heart and lung transplant outcomes. As a result, serum reactivity to HLA single-antigen beads is being used as a "virtual" crossmatch to define serum reactivity to each HLA allele while the patient is waiting for an organ. If the organ

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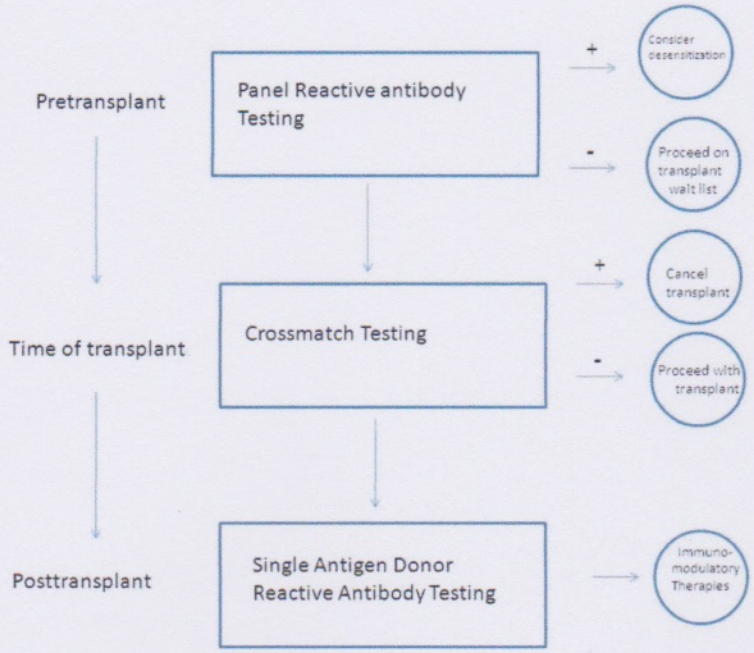

Fig 6. Transplant clinical immunologic testing flowchart. The 3 time points for immunologic testing are shown. Pretransplant PRA testing stratifies patients to determine how likely a patient will have antibodies to an offered organ. Crossmatch testing determines the risk of hyperacute rejection. Single-antigen testing is used to determine donor specificity of antibodies and helps determine immunomodulatory therapy. **Abbreviations:** PRA, panel reactive antibody.

expresses the HLA allele to which their serum reacts, the transplant is not performed and they remain on the waiting list until an acceptable organ becomes available (Figure 6).[57]

Transplant candidates with high PRA values (strong and varied anti-HLA antibodies) are unlikely to find an acceptable donor within a reasonable time and are thus at higher risk of death while waiting for a transplant. As a result, physicians have attempted to reduce the antibody titers in these patients using desensitization protocols so as to increase the likelihood of finding an acceptable organ. These protocols predominantly used intravenous immunoglobulin (IVIG) and plasmapheresis, although there is no currently accepted standard of practice. Intravenous immunoglobulin is derived from pooled donor serum and has been used in a variety of inflammatory disorders. The therapeutic benefits of IVIG have been attributed to the binding of Fcγ receptors on immune cells by the Fc portion of immunoglobulin G, which modulates an immune response. Other potential mechanisms of IVIG include the absorption of active complement components and the induction of anti-idiotypic antibodies.[58,59] Desensitization using plasmapheresis can remove antibodies at least transiently, leading to negative crossmatches and permitting transplantation in some of these higher-risk patients.[60–63] The efficacy of desensitization is suboptimal, as are outcomes following transplant in these patients, making this an active area of research.

Because antibodies are produced by B cells and plasma cells, another therapeutic strategy to limit antibody-mediated injury is to eliminate alloreactive B cells using therapeutic agents other than the plasmapheresis procedure. Mature B cells and memory B cells express the cell surface molecule CD20. Rituximab is an FDA-approved antibody specific for CD20 that is capable of depleting B cells and is being tested for its efficacy to prevent production and or eliminate alloantibodies in transplantation.[64] As noted earlier, BAFF and APRIL are cytokines critical for B-cell survival.[15] These molecules are targets of newly developed therapeutic agents tested as inhibitors of antibody formation in transplantation and autoimmunity.[65] Plasma cells, the predominant producers of antibodies, do not express CD20 and are BAFF/APRIL-independent. As a consequence, plasma cells are resistant to therapies targeting these molecules. Newer therapeutic strategies capable of depleting antibody-secreting plasma cells, including

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the proteasome inhibitor bortezomib used to treat multiple myeloma, are being tested in transplant settings, but none are yet approved for use in transplantation.[66]

IMMUNOLOGIC TOLERANCE

The ultimate goal of transplantation is to induce immune tolerance, where the immune system does not respond to the transplant but is otherwise fully functional in the absence of immunosuppression. Spontaneous allograft tolerance, often noted in situations where patients unilaterally stop taking their immunosuppressants, can occur many years after liver transplantation and has been described in occasional kidney transplant recipients, but these latter cases in particular are rare. Ongoing studies are attempting to identify molecular signatures capable of predicting tolerance, but until such markers are available, the risks of graft loss far outweigh the potential gains of removing immunosuppressants. Stopping immunosuppressant medication in otherwise very stable transplant recipients is highly discouraged.

Researchers are also attempting to actively induce transplant tolerance. One approach is to eliminate the recipient's immune system and re-educate it in the presence of a transplant with the idea that this will "train" the new immune system that the organ is not foreign. Immunoablation with radiation and chemotherapy, followed by transplantation of donor bone marrow and a donor organ, has successfully induced tolerance in animal models.[67,68] A similar strategy has been attempted in humans with kidney failure from multiple myeloma, where a bone marrow transplant and a kidney transplant are both clinically indicated.[69] Small numbers of patients have been successfully weaned of immunosuppressants following such therapy, but larger numbers of patients and longer follow-up are required before the approach can be used more broadly. A cutting-edge alternative strategy for tolerance induction being tested by several research groups involves isolating regulatory T cells from transplant candidates, expanding them ex vivo in specialized facilities, and transfusing them back into the patient with donor organs in an attempt to induce tolerance.[70,71] Results of initial studies using this approach should be available within the next several years.

CONCLUSION

Organ transplantation results in a potent immune response. Understanding the complexities of this response has important diagnostic and therapeutic consequences. Current application of our immunology knowledge has permitted development of drugs that effectively control this response leading to reduced acute rejection rates. It is hoped that expansion of our knowledge of these mechanisms will allow us to identify new agents aimed at prolonging allograft survival. Present observations will lay the foundation for noninvasive diagnosis of immune reactivity and predict posttransplant outcome for patients to guide individual therapy. And, ultimately conquering of this organ-specific immune response will lead to the fulfillment of the ultimate goal of transplantation: the induction of tolerance.

DISCLOSURES

Potential conflict of interest: Nothing to report.

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