

Mechanisms of Rejection: Current Perspectives

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Rejection is the major barrier to successful transplantation. The immune response to an allograft is an ongoing dialogue between the innate and adaptive immune system that if left unchecked will lead to the rejection of transplanted cells, tissues, or organs. Activation of elements of the innate immune system, triggered as a consequence of tissue injury sustained during cell isolation or organ retrieval and ischemia reperfusion, will initiate and amplify the adaptive response. T cells require a minimum of two signals for activation, antigen recognition, and costimulation. The activation requirements of naive T cells are more stringent than those of memory T cells. Memory T cells are present in the majority of transplant recipients as a result of heterologous immunity. The majority of B cells require help from T cells to initiate antibody production. Antibodies reactive to donor human leukocyte antigen molecules, minor histocompatibility antigens, endothelial cells, RBCs, or autoantigens can trigger or contribute to rejection early and late after transplantation. Antibody-mediated rejection triggered by alloantibody binding and complement activation is recognized increasingly as a significant contribution to graft loss. Even though one component of the immune system may dominate and lead to rejection being described in short hand as T cell or antibody mediated, it is usually multifactorial resulting from the integration of multiple mechanisms. Identifying the molecular pathways that trigger tissue injury, signal transduction and rejection facilitates the identification of targets for the development of immunosuppressive drugs.

Keywords: Transplantation, Allograft, T cell, B cell, Antibody, Cytotoxicity, Innate, Adaptive, Kidney, Pancreas, Liver, Heart, Islet, Intestine, Immunosuppression.

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Role of the Transplant in Initiating Rejection

The physical process of isolating and manipulating cells or removing, reimplanting, and reperfusing tissue or organs for transplantation initiates injury and stress responses, resulting in changes in gene and protein expression and folding within the donor tissue (1–3) that have a profound influence on the immunological response of the recipient (4). In the case of deceased organ donors some of these changes are also a direct consequence of brain or cardiac death. Although organ preservation, perfusion, or preconditioning strategies can ameliorate some of these events (5–7), they cannot, as yet, prevent all tissue damage and activation of the innate immune system.

Cells of the innate immune system express invariant pathogen-associated pattern recognition receptors (PRRs) that enable them to detect not only repeating structural units expressed by pathogens, referred to as pathogen-associated molecular patterns (8), but also markers of tissue injury or damage-associated molecular patterns (DAMPs) (Fig. 1). Local tissue damage and ischemia reperfusion injury generates many potential DAMPs, including reactive oxygen species, heat shock proteins, heparin sulfate, high mobility group box-1 after capture by the receptor for advanced glycation end products complex and fibrinogen, that can bind to PRRs. There are several families of PRRs including transmembrane proteins present at the cell surface such as toll-like receptors and C-type lectin receptors, intracellularly such as nucleotide-binding oligomerization domain and nucleotide-binding oligomerization domain-like receptors, and retinoic acid-inducible gene-I-like receptors, and secreted molecules including mannose binding lectin. The sensing of DAMPs by PRRs results in the potent activation of the inflammasome (9), upregulating the transcription of genes, and production of micro-RNAs (10) involved in inflammatory responses setting up amplification and feedback loops that augment the response and trigger adaptive immunity (Fig. 1). The end result is the production of inflammatory mediators including the proinflammatory cytokines, interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF), type I interferons, chemokines (chemoattractant cytokines) (11, 12), and the rapid expression of P-selectin (CD62P) by endothelial cells. These events identify the transplant as a site of injury and inflammation

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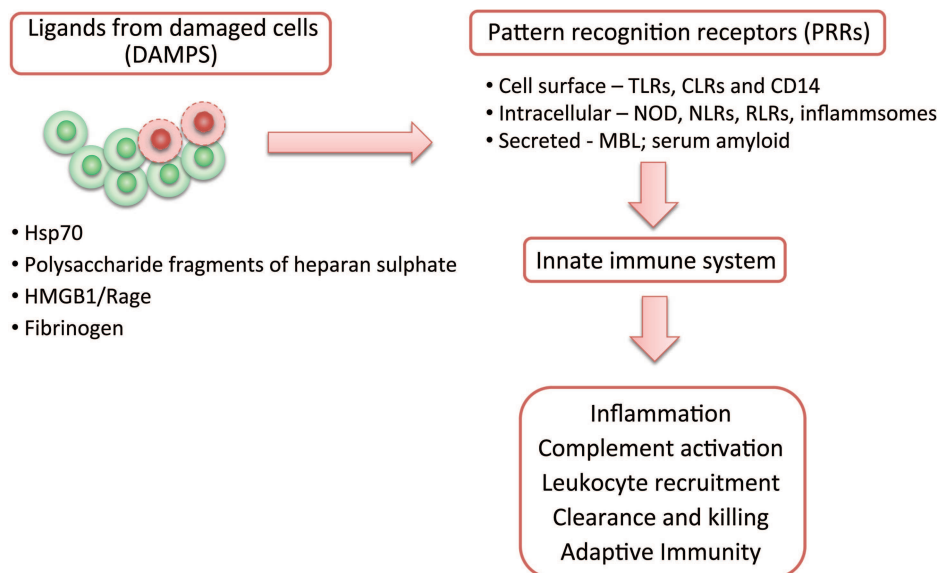


FIGURE 1. Innate immunity sets the scene for rejection. Organ retrieval and transplantation causes injury to the graft. The molecules produced by damaged cells within the allograft, known as damage-associated molecular patterns (DAMPs), are recognized by pattern recognition receptors expressed by cells of the recipient's innate immune system triggering their activation. The activated leukocytes will rapidly release inflammatory mediators and chemoattractant cytokines or chemokines, attracting other leukocytes into the graft. DAMPs may also activate complement resulting in the release of vasoactive molecules and causing further damage to the graft. Activation of innate immunity orchestrates the activation of the adaptive arm of the immune response against the transplant.

modifying the activation status, permeability, and viability of endothelial cells lining the vessels, triggering the release of soluble molecules, including antigens from the graft, inducing the production of acute phase proteins including complement factors systemically and in some cases by the organ itself, stimulating the migration of donor-derived antigen-presenting cells (APCs), dendritic cells (DCs), from the transplant to recipient lymphoid tissue (13, 14), and triggering the recruitment of inflammatory leukocytes into the graft.

Activation of the innate immune system in the early phase posttransplant is largely, a non-specific response to tissue damage and will occur, irrespective of whether there is a genetic difference between the donor and recipient (11). This is a potentially important consideration for certain types of stem-cell derived therapies, including induced pluripotent stem cells, where it is envisaged that autologous sources of cells might be used, as the initial response to the transplanted/implanted tissues may elicit some tissue damage even though the inflammatory process will eventually subside (15). Obviously, in procedures between genetically disparate individuals and when stem cells have been differentiated or modified to express new molecular entities, the activation of the innate immune system will inevitably trigger an adaptive immune response if steps are not taken to prevent it.

In this manner, the transplant itself initiates events that contribute to its own destruction. It is important to remember that innate immune response is only rarely able to reject an allograft on its own.

Allorecognition and T-Cell Activation

Animals that lack T cells are unable to reject fully mismatched transplants, whereas adoptive transfer of purified wild type T cells to these animals is able to restore allograft

rejection (16). In clinical transplantation, therapies that deplete peripheral leukocytes, including T cells, are effective in preventing and reversing episodes of acute rejection and improving long-term graft and patient outcomes (17, 18). The first step in the adaptive immune response to a transplant in a recipient who does not have preformed antibodies that can react with donor molecules is therefore T-cell recognition of alloantigen or allorecognition.

One of the reasons that transplantation induces such a dynamic immune response is the high precursor frequency of T cells able to respond to mismatched major histocompatibility complex (MHC) molecules. This high level of reactivity results from a combination of specific recognition of alloantigens or alloantigenic peptides by T cells and through cross-reactivity of T cells specific for other peptide-MHC complexes with alloantigen. Although MHC molecules are undoubtedly the most important alloantigens for triggering rejection, transplants between siblings with identical MHC molecules are still vulnerable to rejection; a phenomenon demonstrated most clearly in experimental studies (19). Rejection in this latter setting is a result of T-cell recognition of other polymorphic non-MHC molecules called minor histocompatibility antigens (miH). miH antigens are peptides derived from a wide variety of proteins from genes encoded throughout the genome, presented by host or recipient derived MHC molecules (discussed later) and are not necessarily expressed by cells of the immune system (20).

Transplantation is a unique immunological situation in which priming of recipient T cells with antigen can occur by three distinct pathways (Fig. 2a) (21). Direct allorecognition is the interaction of recipient T cells by the T-cell receptor (TCR) with intact allogeneic MHC-peptide complexes presented by donor-derived APCs, including DCs. Indirect

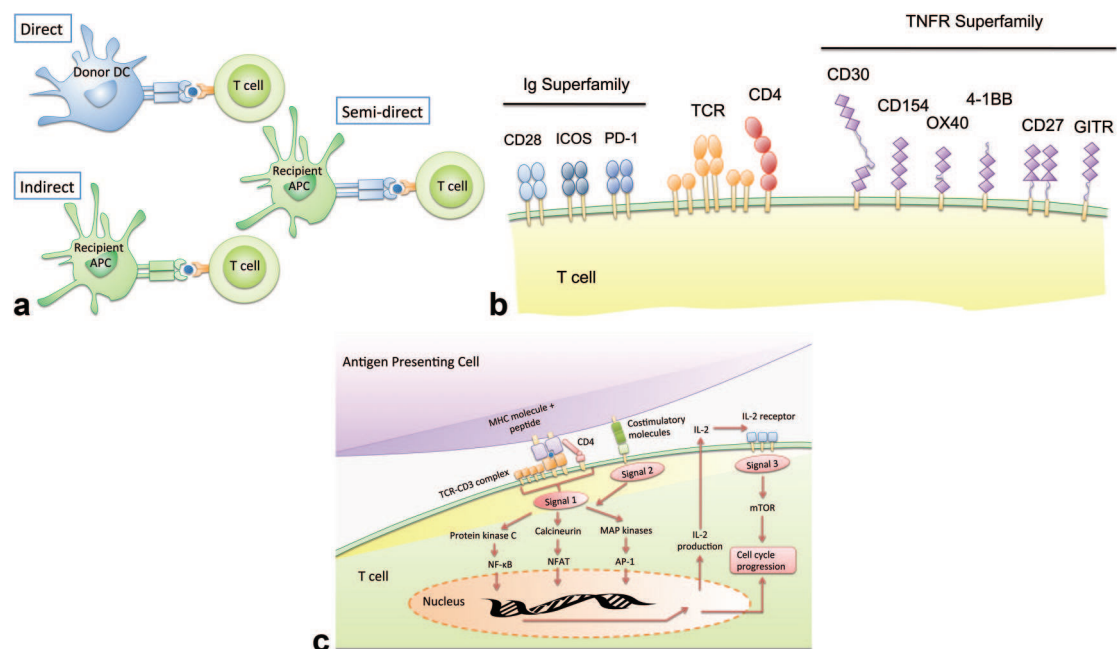


FIGURE 2. (a) How T cells recognize alloantigen—direct, indirect, and semidirect pathways of allorecognition. Direct allorecognition is the interaction of recipient T cells through the T-cell receptor (TCR) with intact allogeneic major histocompatibility complex (MHC)-peptide complexes presented by donor-derived APCs, including DCs. Indirect allorecognition occurs when peptides derived from donor MHC or minor histocompatibility antigen (miH) antigen are degraded by antigen processing pathways and presented by recipient APCs. Semi-direct allorecognition is the capture of donor MHC-peptide complexes by recipient APCs. (b) T-cell costimulatory molecules. After antigen recognition, second signals are provided to the T cell by the interaction of costimulatory molecules with their ligands on antigen presenting cells. T-cell costimulatory molecules can essentially be divided into two families: the B7 family that is best characterized by the T-cell costimulatory molecules CD28 and CD152 (CTLA-4) and the tumor necrosis factor (TNF)/TNF receptor (TNFR) family of which the prototype receptor-ligand pair is CD40 and CD154 (CD40L). (c) Supramolecular complexes and the three-signal model of T-cell activation. After antigen recognition by T cells, an immunologic synapse is formed resulting in the clustering of costimulation, signaling, and adhesion molecules to form a supramolecular activation complex (SMAC). Signal transduction pathways are triggered alternating gene transcription in the activated T cell.

allorecognition occurs when peptides derived from donor MHC or miH antigen are degraded by antigen processing pathways and presented by recipient APCs. The dominant antigenic peptides presented by the indirect pathway are the hypervariable peptide binding regions of allogeneic MHC molecules. Semi-direct allorecognition is the capture of donor MHC-peptide complexes by host APCs. The exchange of fragments of cell membrane between cells that interact with one another is a well-described phenomenon in cell biology. In the context of the immune response to an allograft, the transfer of membrane fragments from allogeneic cells expressing donor MHC molecules can result in the presentation of intact donor MHC molecules by recipient or host APCs to T cells.

Antigen presentation through the direct pathway of allorecognition plays a dominant role in initiating the adaptive immune response to an MHC-mismatched transplant. However, because there are a finite number of passenger leukocytes transferred within a transplanted organ, the role of the direct pathway in allograft rejection diminishes with time as eventually only other types of donor cells, such as endothelial cells remain in the graft to stimulate direct pathway T cells. Importantly, the indirect pathway is available for antigen presentation for as long as the graft remains in situ, and therefore becomes the dominant mode of allorecognition long term.

The significance of the semi-direct pathway of allorecognition in the context of rejection remains to be elucidated.

Costimulation

As a consequence of allorecognition, antigen-specific signals delivered to the T cell through the TCR-CD3. Signals through TCR-CD3 alone are not sufficient to fully activate naive T cells. A second essential signal is provided by the interaction of costimulatory molecules with their ligands. Costimulatory molecules can essentially be divided into two families: the B7 family which is best characterized by the T-cell costimulatory molecules CD28 and CD152 (CTLA-4) (22) and the TNF/TNF receptor (TNFR) family of which the prototype receptor-ligand pair are CD40 and CD154 (CD40L) (23) (Fig. 2b).

CD28 is constitutively expressed by T cells and binds members of the B7 family, CD80 and CD86 on APCs. CD86 is constitutively expressed by APC at low levels and rapidly up-regulated and likely to be the primary ligand for CD28, whereas CD80 is inducible and expressed later in the response. Signaling through CD28 lowers the threshold for T-cell activation, increases the stability of IL-2 mRNA and therefore expression of IL-2 and promotes T-cell proliferation and resistance to activation induced cell death by apoptosis. During an immune response, activated T cells upregulate

late expression of CD152 (CTLA-4), a molecule that has close homology to CD28, that can also bind to CD80 and CD86 but with a binding affinity 10 to 20 times greater than that of CD28. Following expression of CD152, it is able to attenuate immune responses by competing with CD28 for ligation of CD80 and CD86. The importance of CD152 as a negative regulator of immune responses was clearly demonstrated by the generation of CD152 knockout mice that when housed exposed to a wide range of environmental antigens develop a fatal disorder characterized by massive proliferation of lymphocytes.

Another effect of CD28 signaling during T-cell activation is to upregulate expression of other costimulatory molecules such as CD154 (CD40L). CD154 is the ligand for CD40 expressed by APCs, including B cells, and delivering a positive signal to the T cell, CD40-CD154 ligation activates APCs leading to increased expression of B7 family molecules and therefore amplification of T-cell activation.

As increasing numbers of novel costimulatory molecules are identified (23), it is becoming clear that the outcome of T cell-APC interaction is determined by integrating information from many pathways including the avidity of the cognate TCR-MHC-peptide interaction and the balance of positive (CD28; CD154) and negative signals (CD152, PD1) delivered by the costimulatory molecules present on the surface of the participating cells.

Signal Transduction Through TCR and Costimulatory Pathways Lead to “Signal 3”

Within the biphospholipid layer of a cell membrane cholesterol-rich regions that have been termed “lipid rafts” that contain signal transduction molecules can be identified. In resting T cells, TCRs are usually not associated with lipid rafts and are therefore unable to interact effectively with signal transduction molecules. During antigen recognition by T cells, multiple TCRs binding to MHC-peptide on the surface of the APC forming an immunological synapse resulting in the clustering of costimulation, signaling, and adhesion molecules to form a supramolecular activation complex. This triggers reorganization of the cell membrane in the vicinity of these interactions allowing TCR-CD3 complexes to integrate into lipid rafts facilitating downstream signaling by placing them near signal transduction molecules (Fig. 2c).

The intracellular signaling pathways downstream of TCR/CD3 and costimulation are complex. Briefly, TCR-MHC-peptide engagement results in the recruitment and phosphorylation of several signaling molecules. These phosphorylation events initiate a number of intracellular biochemical processes resulting in activation of the Ras- and Rac-mitogen-activated protein kinase pathways and hydrolysis of membrane phosphatidylinositol 4,5-bisphosphate to generate the secondary messengers inositol triphosphate (IP₃) and diacylglycerol (DAG). IP₃ leads to the release of stored calcium from the endoplasmic reticulum and activation of the phosphatase calcineurin, which in turn dephosphorylates the transcription factor nuclear factor of activated T cells, allowing it to translocate to the nucleus. Generation of DAG results in the activation of another transcription factor, nuclear factor- κ B, and a third transcription factor, AP-1, is generated by the mitogen-activated protein kinase cascades. The action of these transcription factors alters expression of

many genes, in particular leading to upregulation of the T-cell growth factor IL-2 and the high affinity IL-2 receptor α -chain (CD25).

Soon after activation, the generation of large amounts of IL-2 and other proliferative cytokines act in an autocrine and paracrine fashion to provide what has been described as “signal 3.” Transduction of signals delivered by IL-2 promotes cell cycle progression and initiates the clonal expansion and differentiation of activated T cells.

T-Cell Differentiation

After activation, depending on the microenvironment and additional signals the T cells receive will drive their differentiation into cells that have different cytokine signatures and functional capabilities. CD4⁺ class II-restricted T cells usually acquire helper function (Th). Different Th subsets exist each with a unique transcription factor and cytokine signatures referred to as Th1, Th2, Th17, Th9, and Tfh (follicular helper) populations (Fig. 3a). CD8⁺ class I restricted T cells are usually cytotoxic and can also be divided into subsets; Tc1 and Tc2, are the best described, although IL-17-producing CD8⁺ cells have been reported (24). Multiple factors influence T-cell differentiation after activation including the immune status of the recipient at the time of transplantation, the degree of ischemia-reperfusion injury, the degree of donor recipient mismatch, or antigen load and the immunosuppressive regimen used to prevent acute rejection. All of these elements will impact the chemokines and cytokines released by the transplanted tissues, the migration of donor-derived passenger leukocytes to the secondary lymphoid tissues, and the recruitment of recipient leukocytes and contribute to the microenvironment that exists in vivo when T cells are activated as outlined earlier. For example, DAMPS produced as a result of ischemia-reperfusion injury will trigger toll-like receptor signaling stimulating APCs to secrete IL-12 that drives the differentiation of Th1 cells that express the transcription factor Tbet and secrete IFN γ , and activating other cell populations, including NK cells (Fig. 3b). For the differentiation of Th2 cells, IL-4 is required and leads to the development of GATA-3⁺ T cells that secrete IL-4 themselves and attract eosinophils to the graft. Th2 cells have been shown to be capable of initiating rejection in their own right (25). Transforming growth factor beta (TGF- β), IL-6, IL1 β , and IL-23 have been implicated in Th17 differentiation, although the precise influence of each mediator is both species and concentration dependent (26, 27). IL-23 and IL-21 signaling upregulates *ROR γ T* that directs IL-17 transcription by the T cell. IL-17 is proinflammatory in vivo, predominantly stimulating granulopoiesis and neutrophil migration to the inflammatory site (28). There is evidence that Th17 cells and IL-17-producing CD8⁺ T cells have the capacity to play a role in rejection, particularly in the absence of a Th1 response (28–30). Th9 differentiation requires TGF- β and IL-4 to present in the microenvironment during activation. As their name suggests, Th9 cells secrete IL-9 and they recruit mast cells. Tfh cells are found in lymph nodes and are important for B-cell maturation, most likely playing a role in antibody-mediated rejection. Tfh require IL-21 for differentiation and express the transcription factor bcl-6 (Fig. 3a).

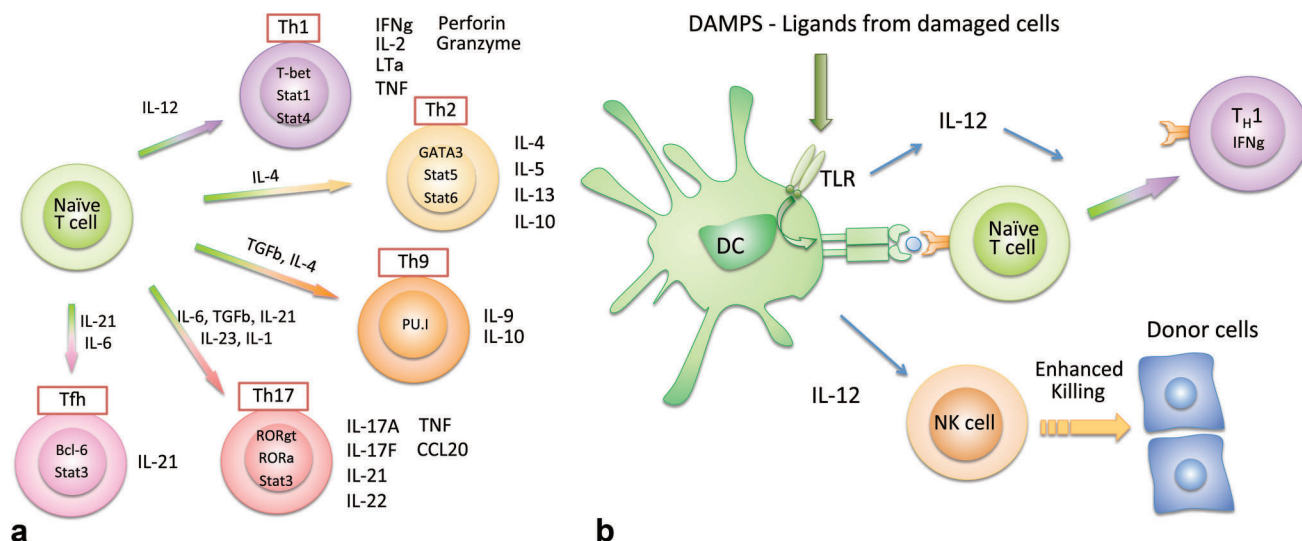


FIGURE 3. (a) Differentiation pathways for CD4⁺ T cells. The microenvironment and additional signals received by a T cell after recognition of alloantigen will drive the differentiation of the T cell into a particular pathway resulting in the differentiation of a T-cell expressing a specific transcription factor and cytokine signature that determine its functional capabilities. (b) Interplay between innate and adaptive immunity augments rejection. Ligands from damaged cells within the transplant may also engage pattern recognition receptors, for example, the Toll-like receptors (TLRs) expressed by donor-derived antigen-presenting cells present within the allograft. Triggering of antigen-presenting cell (APC) through a TLR can result in the cell producing the cytokine interleukin 12 (IL-12). When IL-12 is present at the time of allorecognition, T cells capable of recognizing donor alloantigen differentiate into Th1 cells. In addition, the presence of IL-12 will augment the activity of natural killer (NK) cells enhancing their ability to kill donor cells within the allograft.

In addition to T cells that promote immune responses, there are also populations of T cells that regulate or control immune responsiveness, so-called regulatory or suppressor T cells (Treg) (31, 32). Treg are selected in the thymus, naturally occurring, thymus-derived Treg or can be induced in the presence of antigen and a permissive microenvironment in the periphery. Treg exhibit sustained expression of the transcription factor Foxp3 (33).

B-Cell Activation and Function

B cells are generally thought of as antibody secreting cells, but in actual fact they are multifunctional as they can also act as APCs as they express MHC and costimulatory molecules including CD40 (34). B cells also express complement receptors and can therefore interact with complement coated damaged cells, facilitating antigen presentation and therefore regulation of adaptive immunity (35). As APC, B cells can interact with T cells by their TCR and costimulatory molecules, creating a cell cluster that enables cytokines secreted by the T cell to influence B-cell activation, differentiation, and antibody production. The majority of B cells is dependent on T-cell help for activation and antibody production and encounter antigen in the secondary lymphoid tissue (36). Antibody-mediated rejection is now well recognized in clinical transplantation (discussed below). Interestingly, B cells themselves may be able to contribute to the rejection process as B cells, B-cell clusters and B-cell transcripts have been found in rejecting allografts (37–41), although the presence of intragraft B cells may not always be harmful (42) and B cells present in tolerant patients and may have the ability to regulate the alloimmune response (43–45).

Mechanisms of Graft Destruction

Although the initiation of the adaptive immune response that results in allograft rejection is critically dependent on T-cell recognition of alloantigen (discussed earlier), many components of the immune system can subsequently contribute to the destruction of the transplanted tissue. Additional factors, including many already mentioned, modify the character of the immune response to an individual allograft, including the ischemia-reperfusion injury, the organ or tissue transplanted, the site of transplantation, the histocompatibility match/mismatch, the immune status of the recipient at the time of transplantation and of course, a topic not covered in this review immunosuppression.

Role for the Innate Immune System in Mediating Graft Damage

As well as creating an environment that facilitates activation of the adaptive immune system, the innate immune system represents a “preformed” set of mechanisms that can mediate some graft damage in their own right (46). Although in most cases, these mechanisms will not be sufficient to elicit rejection in the absence of adaptive immunity, they will contribute to the overall process and importantly the activity of the components of the innate immunity will be augmented in the presence of an adaptive immune response.

Initially, macrophages and other phagocytic cells when activated by recognizing DAMPs through PRRs will contribute to the local inflammatory environment. Later in the rejection process, these innate cells have the capacity to act as APCs augmenting T-cell activation, bind alloantibody secreted by activated B cells through Fc receptors expressed at

the cell surface that will further augment cellular activation or trigger antibody-dependent cellular cytotoxicity or bind immune complexes or cells coated with complement by complement receptors (CR), a process known as opsonization, resulting not only in the ingestion of damaged or necrotic donor tissue that removes antigen but also augments antigen presentation. Each of these functions acts to amplify the response or clear antigen from the system.

Activated complement components constitute a proteolytic cascade present in the plasma that generates a range of effector molecules that can damage the graft in their own right, facilitate antigen presentation and integrate the innate and adaptive immune response (47). Interestingly, some complement components are synthesized by the kidney and the liver, thus for some types of transplant the donor tissue will produce complement components locally in the graft potentially amplifying the early response to the transplant.

There are three pathways of complement activation known as the classical, alternative, and lectin pathways (Fig. 4a). Complement can be activated by a range of molecules that include some DAMPS (discussed earlier) through the alternative or lectin pathways, that is, in the absence of alloantibody enabling it to be involved early in the response to a transplant. Once alloantibody is produced and binds alloantigen at a cell surface or to form immune complexes the classical pathway is activated. This initiates a cascade that results in the activation or cleavage of C4 into two parts, C4a and C4b. C4b has the ability to bind covalently to cells or the antibody that initiated the activation in the vicinity of the activating event where it is further cleaved or degraded into small fragments including C4d. Indeed, when C4d is detected in kidney, biopsies are interpreted as indicating that rejection is antibody mediated (48, 49).

C3b and C4b can bind covalently to target cells and antibody molecules, targeting the coated targets for destruction by phagocytes that express complement receptors (CRs) for these fragments; a process known as opsonization. In addition, recognition of C3b, C4b or their fragments covalently bound to target cells by CRs on the surface of leukocytes, including B cells, facilitates antigen presentation and T-cell activation (Fig. 4b). The cleavage of C3, C4, and C5 during complement activation results in the release of soluble peptides, C3a, C4a, and C5a—anaphylotoxins—that have a range of activities that promote vasodilation and chemotaxis, thereby recruiting leukocytes to the site of activation and promoting antigen presentation, again augmenting the response to the graft. Generation of the terminal components of the complement cascade (C5b-9) results in formation of the membrane attack complex within the target cell membrane and initiation of target cell lysis. This has also been demonstrated to play an important role in ischemia-reperfusion injury.

Natural killer (NK) cells are large granular lymphocytes that are able to kill virus infected or mutated host cells in an identical manner to cytotoxic CD8⁺ lymphocytes (discussed later) and release proinflammatory mediators. They express a unique recognition system that involves activating and inhibitory receptors that enables them to detect and respond to non-self (50). The inhibitory NK cell receptors include killer-cell immunoglobulin-like receptors and NKG2A/CD94 whose ligands are self-MHC class I molecules. Thus, NK cells can recognize when self-MHC class I molecules are absent,

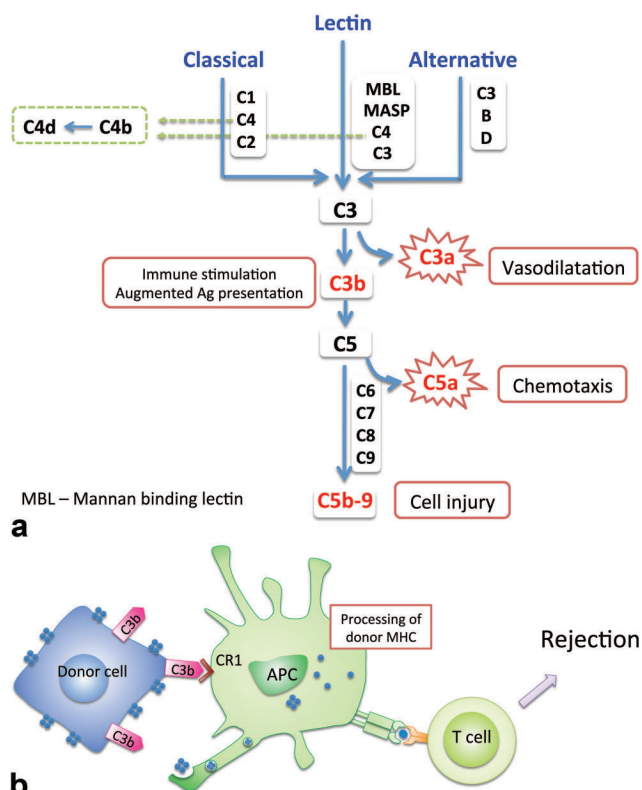


FIGURE 4. (a) Pathways of complement activation. There are three pathways of complement activation known as the classical, alternative, and lectin pathways. Complement can be activated by a range of molecules that include some damage-associated molecular pattern (DAMPs) where activation occurs the alternative or lectin pathways, that is, in the absence of alloantibody, enabling it to be involved early in the response to a transplant and by antibody after activation of the adaptive response by the classical pathway. Activation of any of the pathways triggers the cascade and results in the activation or proteolytic cleavage of complement component 3 (C3) into two pieces, C3a and C3b. C3b, such as C4b, acquires transient activity that enables it to bind covalently to cells or antibody-antigen complexes. The cleavage of C3, C4, and C5 during complement activation results in the release of soluble peptides, C3a, C4a, and C5a—anaphylotoxins—that have a range of activities that promote vasodilation and chemotaxis, thereby augmenting the immune response to the graft. (b) Complement activation enhances presentation of allopeptides to T cells by the indirect pathway of allorecognition. The covalent binding of C3b to cells within the graft allows them to be recognized by complement receptors (CR) expressed by antigen-presenting cells in the transplant recipient. In this way, donor alloantigens are targeted to the antigen-presenting cell allowing them to enter the antigen processing machinery that degrades the alloantigen into peptides. The allopeptides that are formed bind to recipient major histocompatibility complex (MHC) molecules that are transported to the cell surface enabling them to be recognized by indirect pathway T cells (shown in Fig. 2a).

so-called “missing self” triggering NK cell activation. Polymorphism of NK cell receptor targets should theoretically generate alloreactive NK cells that could contribute to tissue damage after transplantation. NK cells have been shown to be

capable of rejecting bone marrow cells that express low levels of MHC class I molecules, and NK cells with the ability to kill target cells *ex vivo* can be found in rejecting allografts, with evidence that they can play a critical role in acute and chronic rejection (46, 51).

Neutrophils are implicated in tissue injury, particularly when homeostasis is perturbed by stress or ischemia (52). Neutrophils are short-lived cells, circulating half life 6 to 8 hr and are produced at a rate of in large numbers every day—the order of up to 10^{11} cells/day. Neutrophils circulate in the blood as dormant cells, but at sites of infection or in the case of a transplant, endothelial cells capture bypassing neutrophils and guide them through the endothelial cell lining whereby they are activated. Tight regulation of neutrophils is vital as they have the ability to damage cells and are implicated in tissue injury, including damage to the graft. Neutrophils are recruited to a graft as part of the innate response early posttransplant and after T-cell activation, particularly in response to IL-17 production. Once involved, neutrophils mediate tissue injury in part by increasing by secreting chemokines CXCL1, 2, 3, and 8, binding to other cells, including endothelial cells as a consequence of adhesion molecules, β_2 integrins, interacting with endothelial cell ICAM-1, and through degranulation and secretion of heparin-binding protein. Neutrophils also generate reactive oxygen species that induce vascular leakage.

Macrophages are present in inflammatory infiltrates after transplantation as they are recruited to the graft in response to proinflammatory cytokines such as IL-1 and IL-6. Macrophages can produce both reactive oxygen species and potent degradative enzymes that have the potential to cause injury to the vascular endothelium and parenchyma. They can produce growth factors such as TGF- β , platelet-derived growth factor, and insulin-like growth factor-1 and chemokines such as MIG/CXCL9 and RANTES/CCL5. Macrophages have been shown to contribute to acute and chronic allograft rejection (53).

Leukocyte Recruitment to the Graft

The inflammatory processes at the site of transplantation result in the production of chemokines and upregulation of chemokine receptor expression by activated leukocytes, including macrophages, neutrophils, NK cells (mentioned earlier) and T cells and B cells, enabling them to migrate along the chemoattractant gradient to reach the graft tissue (54). Traffic of naive lymphocytes is usually restricted to recirculation between the blood and lymphatic systems, but, once primed in the secondary lymphoid tissues (discussed earlier), activated T and B cells can migrate into tissues, in this case the transplant.

Inflammatory signals, including cytokines, chemokines, and complement components, produced locally within the graft in the early posttransplant period affect blood vessels in the transplant causing vasodilation and endothelial activation. Activated endothelial cells rapidly externalize preformed granules called Weibel-Palade bodies that contain the adhesion molecule P-selectin. At the same time, chemokines released from the graft become tethered to the endothelium, and these alterations in endothelial surface markers advertise to passing leukocytes that an inflammatory process is occurring in the neighboring tissue. Leukocytes are usually con-

veyed within the fast laminar flow at the center of blood vessels, but once activated leukocytes reach postcapillary venules in proximity to the graft, they are able to leave this rapid flow and move toward the edge of the vessel. This occurs in response to the local chemokine gradient and is assisted by the slower blood flow in the vasodilated blood vessels near the graft. Leukocyte extravasation is a multi-step process. Initially, low affinity interactions develop between endothelial P-selectin and sialyl-Lewis^x moieties that are present on the surface of activated leukocytes. These interactions continually form and break down and the leukocyte “rolls” along the endothelial surface. If chemokines are present on the endothelial surface, conformational changes in leukocyte integrin molecules occur that allow them to bind other endothelial adhesion molecules such as ICAM-1. These higher affinity interactions cause arrest of the leukocyte on the endothelial surface allowing it to commence extravasation. Having entered the tissues, the activated leukocytes continue to migrate along chemokine gradients to invade the graft.

Cytotoxic T Cells

Naive MHC class I restricted CD8⁺ cytotoxic T cells (CTLs) are activated as a result of the formation of a three-cell cluster with the helper cell and the APC or as a result of an activated CD4⁺ T helper cell “licensing” the APC to activate CTLs. CD40/CD154 costimulatory signals play an important role in this process. Activated CTLs migrate to the graft site where they are able to identify their target cells by recognition of allogeneic class I MHC molecules. Once they have located their target cell, they release granules containing cytotoxic molecules such as perforin and granzyme B and upregulating cell surface expression of Fas ligand (FasL) and secreting soluble mediators such as TNF- α . In kidney transplant recipients experiencing rejection, increased levels of perforin and granzyme B mRNA have been found in the urine (55). Target cell killing by CTLs is achieved by the induction of apoptosis. Perforins polymerize and insert into the target cell membrane, forming a pore that facilitates the entry of granzyme B and other compounds into the cell. Granzyme B is a protease that is able to initiate apoptosis by several mechanisms including activation of caspase cascades. Binding of FasL to Fas on the target cell surface is also able to trigger apoptosis by activating caspases.

Delayed Type Hypersensitivity and Helper T-Cell-Mediated Responses

Alloantigen-specific CD4⁺ T cells (typically T-helper 1 cells) contribute to the effector phase of allograft rejection by a non-specific effector mechanism referred to as the delayed-type hypersensitivity (DTH) response.

DTH reactions are characterized by the release of multiple soluble mediators including the proinflammatory cytokines IL-1, IFN- γ , and TNF- α . Damage to the graft occurs as a result of the ensuing infiltration of activated leukocytes, including monocytes, macrophages and eosinophils and the production of non-specific mediators, such as nitric oxide, reactive oxygen species, and inflammatory arachidonic acid derivatives (prostaglandin E₂, thromboxane, and leukotrienes). This activity is triggered in an antigen-specific manner by Th cells but the effector mechanisms that lead to the destruction of the graft are non-specific. DTH reactions have

been shown to directly affect graft physiology by altering cell permeability and vascular smooth muscle tone and play a role in both acute and chronic allograft rejection (56).

Activated CD4⁺ T cells also express cytokines and costimulatory molecules that allow them to provide help for B-cell proliferation, differentiation, antibody class switching, and affinity maturation.

B Cells and Antibody-Mediated Rejection

The antigenic targets of alloantibodies are mismatched MHC molecules, but antibodies that recognize miH, endothelial cell, blood group antigens, and autoantigens also contribute to rejection (57). Antibody-mediated rejection can begin within days after transplantation but can also contribute to late graft loss (58). The mechanism of antibody-mediated damage is most likely primarily but not exclusively through complement fixation. As mentioned earlier, the introduction of histologic staining for complement 4d (C4d) in renal allograft biopsies allows indirect identification of antibody deposition and complement fixation, and peritubular C4d staining is strongly associated with early and late graft failure. If C4d binding is detected, antibody-mediated rejection is clearly indicated. However, the technique is relatively insensitive and therefore the absence of C4d does not rule out antibody as the cause of rejection. Antibodies can also elicit damage to the graft through other mechanisms. NK cells and macrophages express receptors that bind to the Fc region of antibodies that stimulates these cells to kill target cells through antibody-dependent cellular cytotoxicity providing a second mechanism by which alloantibodies can induce donor cell death.

Antibody-mediated rejection is demonstrated most dramatically if patients have preformed alloantibodies at the time of transplantation, where hyperacute rejection frequently results in graft destruction within minutes of organ reperfusion. In this situation, the antibodies (which are usually directed at allogeneic MHC molecules, ABO blood group antigens or antigens expressed on graft endothelium, that include the angiotensin type 1 receptor) cause local activation of the coagulation and complement cascades resulting in extensive thrombosis within the vascular supply to the graft culminating in infarction. Although modern crossmatch techniques have made hyperacute rejection through human leukocyte antigen reactive antibodies extremely rare, the humoral arm of the immune system is increasingly being implicated in the pathogenesis of acute rejection episodes and chronic allograft damage (40, 57).

Tertiary Lymphoid Structures

Tertiary lymphoid organs or tissues are lymphoid-like structures found at sites of chronic inflammation. They found within some organ allografts, but their role in influencing graft survival is currently unknown (59). Tertiary lymphoid organs are similar in structure to secondary lymphoid organs (34) in that they contain follicles, comprising germinal center B cells and interdigitating follicular DCs, surrounded by distinct T-cell areas intermingled with DCs and specialized endothelial cells that form high endothelial venules, but they are not encapsulated. Moreover, the pattern of lymphoid chemokine expression within them is also similar. The development of tertiary lymphoid organs within allografts has been found to require humoral immunity (60), and the presence of

intra-graft Th17 cells has been shown to induce lymphoid neogenesis within the graft and promote chronic rejection (61). However, in contrast, regulatory T cells and IL-10 secreting B cell have also been found with these intra-graft structures (41). Thus, at present, the impact of tertiary lymphoid organs on allograft rejection, or indeed survival, remains to be elucidated.

Immunological Memory in Transplantation

After primary antigen exposure, long-lived antigen-specific memory T and B cells are generated that are able to deliver a more rapid and higher magnitude immune response, if the same antigen is encountered on a subsequent occasion. Memory cells have a reduced activation threshold and are less dependent on costimulation (62). As a result they are able to upregulate effector function and cytokine secretion more rapidly than naive lymphocytes. With increasing age the proportion of memory T cells within an individual's peripheral T-cell pool increases reflecting cumulative antigen exposure and can be as high as 50% in adult humans.

Although the generation of immunological memory is beneficial for protection against infectious pathogens, in transplantation the presence of allospecific memory produces an accelerated or "second-set" rejection response. In clinical transplantation, evidence of previous sensitization to donor antigens is associated with increased risk of acute rejection episodes and premature graft failure. Memory-type responses toward alloantigens are frequently a result of exposure to alloantigens at the time of a previous blood transfusion, pregnancy, or transplant. However, it is now recognized that memory-type responses may also be generated as a consequence of antigen receptor cross-reactivity (heterologous immunity) or by homeostatic proliferation of lymphocytes after an episode of lymphopenia such as are induced in transplant recipients by administration of leukocyte-depleting agents.

Sequential viral infections in mice were found to generate populations of alloreactive memory-phenotype T cells (63). Thus, heterologous immunity will result in some recipients, maybe the majority, having populations of memory T cells that can cross react with donor alloantigen resulting in memory-phenotype responses toward the graft without previous sensitization to donor alloantigen.

The size of the peripheral T-cell pool, and the relative ratios of CD4⁺:CD8⁺ and naive:memory cells, are tightly regulated in vivo by homeostatic mechanisms. A consequence of this is that reduction of the overall T-cell population during illness or after induction therapy in transplantation induces the residual T cells to proliferate, whether cognate antigen is present. A proportion of T cells undergoing homeostatic proliferation in response to lymphopenia differentiate into a phenotype that resembles that of antigen-experienced or memory T cells, including downregulation of CD62L (L-selectin), an adhesion molecule that is expressed on naive T cells and is necessary for entry into lymph nodes by high endothelial venules, and upregulation of CD44, an adhesion molecule that binds to hyaluronic acid and enables activated or memory-phenotype T cells to leave the vascular system and enter peripheral tissues. These T cells also exhibit memory T-cell-like behavior as they are less dependent on costimulation by CD28 and as a result have a reduced activation threshold. Moreover, after activation their capacity to secrete cytokines, proliferate and manifest effector functions is enhanced compared with naive T cells.

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