
Immunobiology of Transplantation

I. Esme Dijke

Abbreviations

ABOi	ABO incompatible	HSCT	Hematopoietic stem cell transplantation
ADCC	Antibody-dependent cellular cytotoxicity	ICOS	Inducible T-cell co-stimulator
aGvHD	Acute graft-versus-host disease	IFN	Interferon
AMR	Antibody-mediated rejection	Ig	Immunoglobulin
APC	Antigen-presenting cell	iNKT-cell	Invariant natural killer T-cell
ASC	Antibody-secreting cell	iNOS	Inducible nitric oxide synthase
BAFF	B-cell-activating factor	I/R	Ischemia/reperfusion
BCL	B-cell lymphoma	IL	Interleukin
BCR	B-cell receptor	KIR	Killer cell immunoglobulin-like
Breg	Regulatory B-cell	MBL	Mannose-binding lectin
cGvHD	Chronic graft-versus-host disease	mDC	Myeloid DC
CTLA	Cytotoxic T-lymphocyte-associated antigen	MDSC	Myeloid-derived suppressor cell
DAMP	Damage-associated molecular pattern	MHC	Major histocompatibility complex
DC	Dendritic cell	MICA/MICB	Major histocompatibility complex class I-related chain A and B
DN	Double negative	Mregs	Regulatory macrophages
DSA	Donor-specific antibody	mTEC	Medullary thymic epithelial cell
Fab	Antigen-binding fragment	mTOR	Mammalian target of rapamycin
Fc	Crystallizable fragment	MyD88	Myeloid differentiation factor 88
FDC	Follicular dendritic cell	MZ	Marginal zone
FOXP3	Forkhead box P3	NK-cell	Natural killer cell
GC	Germinal center	NKG	Natural killer group
GvHD	Graft-versus-host disease	NKT-cell	Natural killer T-cell
GvL	Graft versus leukemia	PAMP	Pathogen-associated molecular pattern
HLA	Human leukocyte antigen	PD(-L)	Programmed cell death protein (-ligand)
		pDC	Plasmacytoid DC
		PDGF	Platelet-derived growth factor
		PRR	Pattern recognition receptor
		RNS	Reactive nitrogen species
		ROS	Reactive oxygen species
		SHM	Somatic hypermutation

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STAT	Signal transducer and activator of transcription
Tcm-cell	Central memory T-cell
TCR	T-cell receptor
TD	T-cell dependent
Tem-cell	Effector memory T-cell
Tfh	Follicular T helper
TGF	Transforming growth factor
Th	T helper
TI	T-cell independent
TIM	T-cell immunoglobulin and mucin domain
TLR	Toll-like receptor
TNF	Tumor necrosis factor
Treg	Regulatory T-cell

Introduction

Transplantation, defined as the act of transferring cells, tissues, or organs from one site to another, has emerged as an effective therapy for the treatment of a variety of medical conditions, including end-stage organ failure, chronic diseases, and malignancies. A major barrier in providing this therapy effectively as a routine medical treatment is the body's immune system. This system has evolved into an extraordinary network with intricate and dynamic mechanisms to protect the host from invading pathogens. The "default setting" of a functional immune system is to eliminate everything that is foreign (non-self) or malignant while being unresponsive to self and benign. Transplantation within the same body (autograft) generally leads to few immunologic events, because the transplanted cells or tissues are recognized as self. Transplantation from one body to another body of the same species (allograft) or different species (xenograft), however, typically results in attack of the transplanted graft by the host's immune system (rejection) or, as is the case with HSCT, attack of the host's body by the transplanted immunocompetent cells (graft-versus-host disease [GvHD]), because it is recognized as foreign. In addition, the physical process of cell or organ procurement and transplantation can induce thermal and metabolic stress responses

in the graft, which result in cell damage and death. The subsequent release of cellular components can activate the immune system, affecting graft function and survival. Successful transplantation includes strategies to bypass or override the "default setting" of the immune system in order for graft acceptance to occur. Elucidating the immunologic mechanisms that are involved in transplant rejection and graft dysfunction is key to understanding clinical and pathological features of these processes, for developing strategies to augment donor/recipient compatibility, to designing new immunosuppressive treatments, and to promoting acceptance of the allograft.

Transplantation Antigens

Antigens are molecules that serve as targets for receptors of the immune system. In theory, any antigen expressed by the transplanted graft that is not expressed in the host can be identified as foreign by the host's immune system and is thus potentially immunogenic. Antigens that are involved in graft rejection include major histocompatibility complex (MHC) molecules, ABO blood group antigens, and minor histocompatibility antigens.

MHC Molecules

MHC molecules, also termed human leukocyte antigens (HLA) in humans, are cell surface proteins that present peptide fragments of endogenous and exogenous antigens to T-cells. The HLA complex is encoded on the short arm of chromosome 6 and contains more than 220 genes with diverse functions (Fig. 1). Due to the close proximity of MHC loci, HLA genes are almost always inherited together without crossover events. This "set" of HLA genes inherited from one parent is called a haplotype. Because chromosome 6 is an autosome (a chromosome with two pairs), each individual has two HLA haplotypes, one from each biological parent. There is 25 % probability that biological siblings have identical HLA haplotypes.

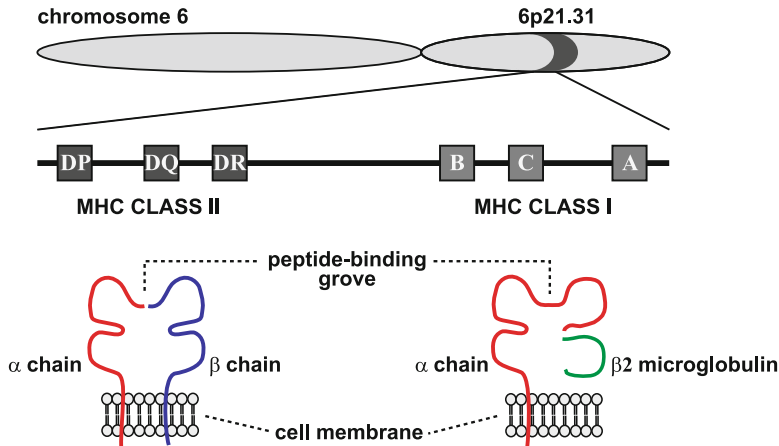


Fig. 1 *MHC molecules.* Genes of the MHC complex (also termed HLA complex in humans) are located on the short arm of chromosome 6. Class I loci (HLA-A, HLA-B, and HLA-C) encode polymorphic transmembrane α -heavy chains that combined with a non-polymorphic β 2-microglobulin produce MHC class I molecules. Class I is constitutively expressed on all nucleated cells as well as platelets and generally presents small endogenous antigens to CD8⁺ T-cells. Class II loci (HLA-DP, HLA-DQ,

and HLA-DR) encode polymorphic transmembrane α - and β -chains that form heterodimers to produce MHC class II molecules. Class II molecules are constitutively expressed on APCs, and their expression is upregulated on activated T-cells and on epithelial and vascular endothelial cells after exposure to inflammatory cytokines. These molecules present antigens derived from extracellular proteins to CD4⁺ T-cells. Abbreviations: *MHC* major histocompatibility complex

MHC molecules with clinical relevance to transplantation are divided into class I and class II antigens. **Class I HLA molecules** are composed of a polymorphic transmembrane α -heavy chain encoded by genes of the HLA-A, HLA-B, or HLA-C loci and a non-polymorphic β 2-microglobulin (Fig. 1). These molecules are constitutively expressed on all nucleated cells and generally present small endogenous proteins, such as viruses or self-protein fragments, to CD8⁺ T-cells [1]. Class I HLA is also expressed on platelets. **Class II HLA molecules** are heterodimers composed of polymorphic transmembrane α - and β -chains encoded by genes in the HLA-D region (Fig. 1). In contrast to class I molecules, class II molecules are only constitutively expressed on professional antigen-presenting cells (APCs), such as dendritic cells (DCs), macrophages, and B-cells. Their expression is upregulated on T-cells upon activation and on epithelial and vascular endothelial cells after exposure to inflammatory cytokines, small proteins produced by various immune cells that affect the behavior of cells [1]. Class II molecules present antigens derived from exogenous proteins to CD4⁺ T-cells.

Since the biological function of MHC molecules is to present antigens, a large variety in these molecules is required to be able to bind the vast number of foreign peptides derived from pathogens or malignant cells. Polymorphisms in MHC genes allow for a huge diversity in alleles that bind to different peptides. To date, more than 9700 class I alleles and 3200 class II alleles have been identified [2]. This polymorphic nature of MHC genes provides a huge array of antigens that can be recognized as foreign by the immune system of a transplant recipient.

ABO Blood Group Antigens

ABO blood group antigens are carbohydrate structures that are expressed not only on red blood cells but also on many tissues of embryonic mesodermal origin, including vascular endothelial cells. Expression of only the H chain defines individuals as blood group O, while addition of the A or B terminal trisaccharide epitopes, or both, defines individuals as blood group A, B, or AB, respectively. Individuals generally

produce “natural” antibodies (i.e., without previous exposure to the antigens) to non-self ABO antigens, presumably due to immunologic cross-reaction to similar epitopes on gut flora [3, 4]. Transplantation of an ABO-incompatible (ABOi) allograft carries a high risk of hyperacute rejection due to the binding of these preformed natural antibodies to non-self ABO antigens on the graft endothelium. Desensitization protocols to remove preformed antibodies from the recipient’s circulation, such as splenectomy, plasmapheresis, and immunosuppressive agents directed at B-cells, have been used to cross the ABO barrier in kidney and liver transplantation [5, 6], but ABO antibodies may return due to B-cell memory and long-lived plasma cells. Because of a delayed production of ABO antibodies during normal infancy, however, ABOi heart and liver transplantation can be performed safely in infants without the need for aggressive interventions [7, 8]. In contrast to ABO antigens, the rhesus factor and other red cell antigens are not expressed on the endothelium and are therefore less relevant to organ transplantation.

Autoantigens and Minor Histocompatibility Antigens

Although HLA and ABO antigens are the major immunologic barriers to transplantation, they are not the only antigens that can induce an immune response to the donor graft. Peptides derived from endogenous antigens can also contribute to the pathogenesis of acute and chronic rejection in transplantation. Many of these cellular antigens remain poorly defined, but the majority are autoantigens, including vimentin and cardiac myosin in the heart [9, 10], collagen V and K- α 1 tubulin in the lung [11, 12], and agrin and angiotensin II receptor type 1 in kidney transplantation [13, 14]. Minor histocompatibility antigens are immunogenic peptides derived from polymorphic self-proteins. Male-specific minor histocompatibility (H-Y) antigens may induce an immune response when a male organ is transplanted into a female recipient. Development of H-Y antibodies is associated with acute rejection in kidney trans-

plantation [15]. Furthermore, MHC class I-related chain A and B (MICA and MICB) antigens are ligands for the activating natural killer group 2 member D (NKG2D) receptor on natural killer (NK) cells and some T-cells. These antigens are expressed in low levels on a variety of cells, including monocytes, epithelial cells, and endothelial cells, but can be upregulated in response to cellular stress such as ischemia/reperfusion (I/R) injury. Antibodies against MICA and/or MICB have been associated with allograft rejection in kidney and heart transplant recipients [16–18].

Adaptive Immunity in Transplantation

T-Cell-Mediated Immunity

T-cells, lymphocytes that are defined by the expression of T-cell receptors (TCRs) on their surface, play a key role in allograft rejection. T-cells develop in the thymus from bone marrow-derived hematopoietic progenitors, where they are educated to recognize foreign antigens presented in the context of self-MHC molecules. T-cells that express the co-receptor CD4 are restricted to the recognition of MHC class II molecules, whereas CD8-expressing T-cells are restricted to MHC class I molecules. Once emigrated from the thymus, T-cells continuously circulate through the peripheral secondary lymphoid organs and “screen” APCs to find their cognate antigen. Recognition of foreign antigens of the transplanted graft is referred to as allorecognition. Without some form of immune modulation, allorecognition by T-cells will result in T-cell activation, differentiation into effector cells, migration to the allograft, and consequent destruction of the graft.

T-Cell Allorecognition

Foreign antigens are recognized by T-cells via the TCR complex, which generally consists of a heterodimeric TCR protein formed by highly variable α - and β -chains and CD3 molecules (γ , δ , ϵ , ζ) that mediate intracellular signaling (Fig. 2a).

A small subset of T-cells expresses a heterodimeric TCR protein that is formed by γ - and δ -chains; these T-cells are referred to as $\gamma\delta$ T-cells. The TCR does not directly bind antigens and recognize them as foreign. Rather, it recognizes a peptide fragment derived from an antigen bound in the peptide-binding groove of a MHC molecule expressed on APCs. Epitopes that are recognized by TCRs are often buried within the antigen and therefore antigens must be processed first by APCs before they are presented in MHC molecules to T-cells.

In organ transplantation, allorecognition by host TCRs can occur via one of three pathways (Fig. 2b): (1) the **direct** pathway, whereby TCRs recognize intact donor MHC molecules expressed on graft parenchymal cells or on donor APCs transplanted with the graft; (2) the **indirect** pathway, in which antigens of the graft are endocytosed and processed by host APCs and presented as peptides in the context of self-MHC molecules; and (3) the **semi-direct** pathway, in which host APCs acquire and present intact donor MHC molecules to the host T-cells [19]. The latter may

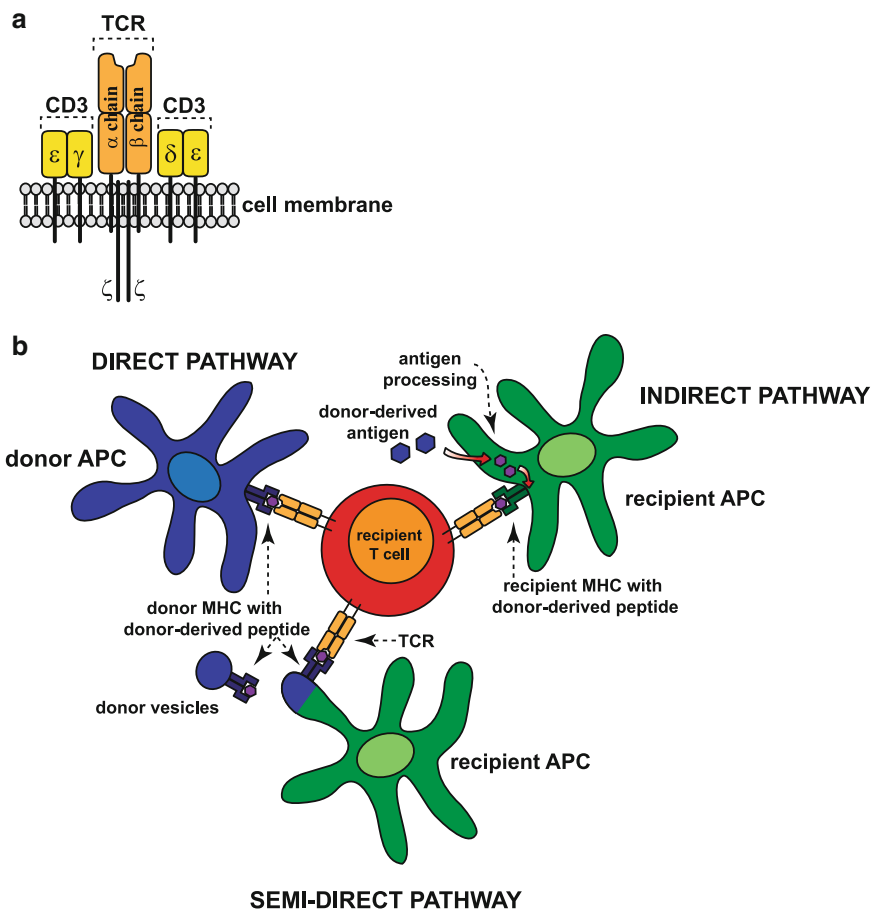


Fig. 2 T-cell allorecognition. (a) T-cells recognize foreign antigens via the TCR complex. This complex is composed of a heterodimeric TCR protein that is formed by a highly variable α - and β -chain and CD3 molecules (CD3- γ , CD3- δ , CD3- ϵ , CD3- ζ) that are assembled with the TCR heterodimer to provide intracellular signaling. (b) Allorecognition by TCRs on recipient T-cells can occur via three different pathways: (1) the direct pathway,

whereby TCRs recognize intact donor MHC molecules on donor cells; (2) the indirect pathway, where donor antigens are endocytosed and processed by recipient APCs and presented as peptides in the context of self-MHC molecules; and (3) the semi-direct pathway, in which recipient APCs acquire and present intact donor MHC molecules to the recipient T-cells. *APC* antigen-presenting cell, *MHC* major histocompatibility complex, *TCR* T-cell receptor

occur as a result of cell-cell contact between host and donor cells or by release and uptake of small donor vesicles (exosomes) by the host APCs [20]. The direct pathway can mediate a strong immune response in the immediate post-transplant period and contributes to acute rejection [21, 22]. Since T-cells are educated in the thymus to be restricted to self-MHC and not foreign MHC molecules, recognition of alloantigens via this pathway is likely due to cross-reactivity. Interestingly, whereas only approximately 1 in 10^6 T-cells may recognize foreign antigens presented by self-MHC, it is estimated in mouse model studies that a much higher frequency of T-cells can directly recognize allogeneic MHC-peptide complexes (~1–10% of the T-cell repertoire) [23]. This high frequency may be due to the high level of diversity in MHC alleles allowing recognition of a multitude of peptides; every donor MHC-peptide complex may essentially be a foreign structure to the host's immune system. In contrast, the frequency of T-cells responding via the indirect pathway is lower; studies in animal models have shown that approximately 3–10% of the total alloreactive T-cell pool recognizes donor peptides presented by self-MHC (<0.1% of the T-cell repertoire) [21, 24]. This pathway is considered to play a predominant role in the development of chronic rejection, but may also contribute to acute rejection [21, 22, 25].

T-Cell Activation

Once the TCR binds successfully to its specific MHC-peptide complex, T-cell activation is initiated. The co-receptors CD4 and CD8, specific for MHC class II and class I, respectively, increase the binding affinity between the TCR and MHC-peptide complex, thereby facilitating prolonged cell-cell interaction. For naïve T-cells, signaling via the TCR (**signal 1**) alone is not sufficient and a second signal is needed to complete activation. This non-antigen-specific signal (**signal 2**) is provided by interactions of co-stimulatory receptors on T-cells with their ligands expressed on APCs or upregulated on some parenchymal cells such as endothelial cells during inflammatory conditions (Fig. 3). In the absence of co-stimulation, the T-cell may become anergic (a state of immune unresponsiveness) or undergo apoptosis.

Numerous co-stimulatory pathways have been identified, but the CD28-B7 pathway is the best studied and likely most crucial for activation of naïve T-cells [26]. CD28 is expressed on T-cells and has two ligands that are expressed by APCs: B7-1 (CD80) and B7-2 (CD86). Following activation, additional co-stimulatory molecules, including members of the immunoglobulin (Ig) family (e.g., inducible T-cell co-stimulator (ICOS)), the tumor necrosis factor (TNF)/TNF receptor family (e.g., CD154, 4-1BB, and OX40), and the T-cell Ig and mucin domain (TIM) family, are upregulated on T-cells to promote proliferation, differentiation, and/or survival [26, 27]. Many of these co-stimulatory molecules remain expressed after naïve T-cells differentiate into effector cells and memory cells, lowering the threshold for further activation. In addition to the upregulation of co-stimulatory molecules, activated T-cells also upregulate the expression of co-inhibitory molecules, which downregulate TCR signaling and suppress T-cell activation in order to restrain T-cell responses. These inhibitory molecules are members of the Ig family and include cytotoxic T-lymphocyte-associated antigen (CTLA)-4 (homologous to CD28 and binds to B7-1 and B7-2 with higher affinity than CD28), programmed cell death protein (PD)-1, B- and T-lymphocyte attenuator (BTLA), and CD160 [26].

When a T-cell receives both signals 1 and 2, various intracellular signal transduction pathways are activated: the calcium-calcineurin pathway, the RAS-mitogen-activated protein (MAP) kinase pathway, and the inhibitor of nuclear factor κ B kinase (IKK)-nuclear factor κ B (NF- κ B) pathway (Fig. 3). These pathways involve a chain of reactions transmitting signals to transcription factors that regulate gene expression. Subsequently, other molecules are expressed on the cell surface, including cytokine receptors, such as the interleukin (IL)-2 receptor α -chain (CD25), and chemokine receptors. In addition, cytokines are produced that promote T-cell proliferation (e.g., IL-2 and IL-15). The binding of these cytokines to their receptors on T-cells delivers a third signal (**signal 3**) that activates the mammalian target of rapamycin (mTOR) pathway, triggering T-cell cycling and proliferation (Fig. 3).

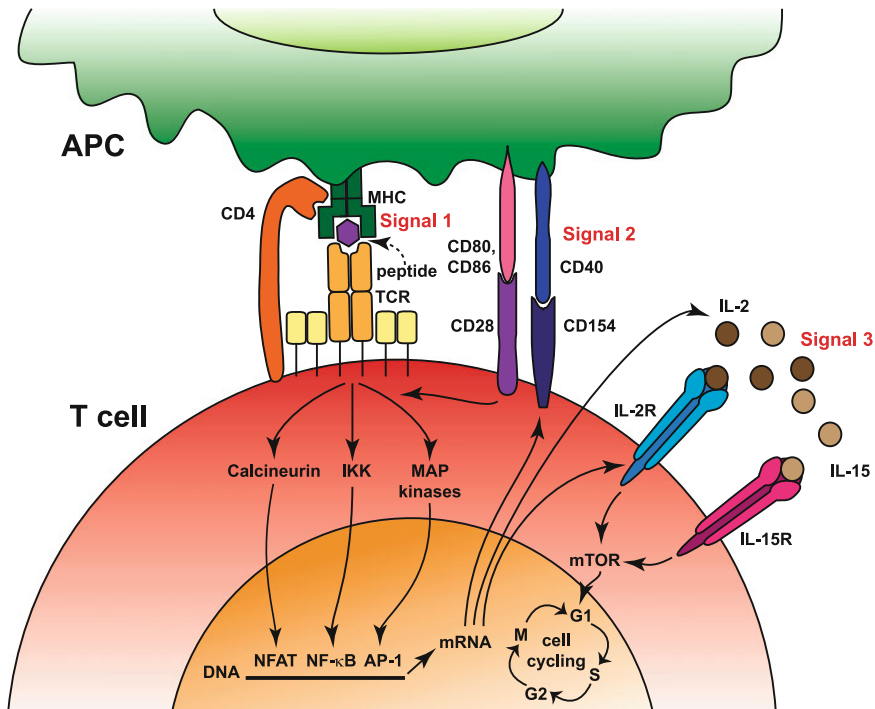


Fig. 3 *T-cell activation.* Activation of naive T-cells involves three signals: *Signal 1*=binding of the TCR to their specific MHC-peptide complex. Co-receptors (CD4 or CD8) increase binding affinity, thereby facilitating prolonged cell-cell interaction. *Signal 2*=binding of co-stimulatory receptors on the T-cell with their ligands expressed on APCs or upregulated on some parenchymal cells, such as endothelial cells, during inflammation. Signals 1 and 2 together activate various intracellular signal transduction pathways which transmit signals to transcription factors that regulate gene expression. Subsequently, other molecules, such as co-stimulatory

molecules and cytokine receptors, are upregulated on the cell surface and cytokines are produced. *Signal 3*=binding of cytokines to their cytokine receptors. This binding activates the mTOR pathway, triggering cell cycling and proliferation. *AP-1* activating protein-1, *APC* antigen-presenting cell, *G1/G2* gap (growth) phase $\frac{1}{2}$, *IKK* inhibitor of nuclear factor κ B kinase, *IL-x(R)* interleukin-x (receptor), *M* mitotic phase, *MAP* mitogen-activated protein, *MHC* major histocompatibility complex, *mTOR* mammalian target of rapamycin *NF* nuclear factor, *NFAT* nuclear factor of activated T-cells, *S* synthesis phase, *TCR* T-cell receptor

Since T-cell activation is one of the key processes of allograft rejection, various components of this process are used as targets for immunosuppressive therapy. Cyclosporine A and tacrolimus block the calcium-calcineurin pathway by inhibiting calcineurin phosphatase. Anti-CD25 monoclonal antibodies (basiliximab and daclizumab) target the IL-2 receptor. The mTOR inhibitors (sirolimus and everolimus) and antiproliferative agents (azathioprine and mycophenolic acid) suppress the mTOR pathway and T-cell cycling, respectively, inhibiting proliferation of T-cells. Various therapeutic agents that can block co-stimulatory pathways, such as the CD28-B7, CD40-CD154, or LFA-1-ICAM pathway, are being explored, but most of these blockers are not

yet used in clinical practice. To date, only belatacept, a CTLA-4-Ig fusion protein, has been approved by the US Food and Drug Administration for the treatment of kidney transplant recipients [28]. Immunosuppressive agents and their mechanisms are discussed in greater depth in chapter “Immunosuppressive Drugs in Solid Organ Transplantation”.

T-Cell Differentiation

Activation of T-cells is followed by differentiation into effector T-cells. Effector CD4⁺ T-cells provide “help” to other immune cells by coordinating and amplifying or modulating the immune response through the production of cytokines; these cells are often referred to as T helper (Th)

cells. Effector CD8⁺ T-cells play an important role in the elimination of cells that express foreign antigens and are therefore called cytotoxic T-cells. This division of labor between CD4⁺ and CD8⁺ T-cells, however, is not mutually exclusive: CD4⁺ T-cells can exhibit cytolytic functions and CD8⁺ T-cells secrete many of the cytokines produced by CD4⁺ T-cells. T-cell differentiation into different Th lineages is dependent on various factors, including cytokine milieu, the type of APC, the concentration of antigen, and co-stimulatory molecule signaling. Cytokines that initiate differentiation are mainly secreted by DCs and other innate immune cells, but some of the cytokines are produced by the differentiated cells themselves, creating a positive feedback loop. Downstream of the cytokine signaling, transcription factors are induced that activate genes that promote development of a particular cell subset and suppress differentiation into other subsets. It is important to remember, however, that T-cell differentiation is not terminal: all Th cell subsets have the capacity for plasticity and can switch into other phenotypes under certain conditions [29, 30].

Until the last decade, Th cells were typically divided into two major subsets: Th1 and Th2 cells (Fig. 4). **Th1 cells** play an important role in the elimination of intracellular pathogens and malignant cells. These cells develop in the presence of IL-12 and interferon (IFN)- γ and their key transcription factors are T-box transcription factor (T-bet), signal transducer and activator of transcription (STAT)1, and STAT4 [31]. Th1 cells have been implicated as key mediators of allograft rejection. Through the secretion of pro-inflammatory cytokines, such as IFN- γ and TNF- α , these cells activate macrophages, NK-cells, and cytotoxic T-cells. High mRNA and protein expression levels of IFN- γ and/or TNF- α have been associated with acute rejection in kidney, heart, and lung transplantation [32–35]. Th1 cells also produce IL-2, an important T-cell growth factor for both Th cells and cytotoxic T-cells. Furthermore, alloreactive Th1 cells themselves can directly eliminate target cells, including donor cells, through cytotoxic pathways [36].

Th2 cells are involved in the elimination of extracellular pathogens and induction of the humoral response as well as development and maintenance of allergy responses. IL-4 and IL-2 are critical cytokines for Th2 differentiation. IL-4 induces the transcription factor STAT6, which upregulates expression of the master regulator GATA3 [31]. Key effector cytokines produced by Th2 cells are IL-4, IL-5, IL-9, IL-10, and IL-13. Because Th2 cytokines have an antagonizing effect on Th1 cells, it has long been postulated that Th2 cells can modulate alloimmune responses and prevent allograft rejection. Although there are some data that support this view [37, 38], other data suggest that Th2 cells can also mediate graft rejection. In a mouse transplant model, Th2 cells rejected islet grafts with similar efficiency as Th1 cells [39]. Th2 cytokines have been associated with chronic rejection in clinical transplantation [40, 41]. Furthermore, Th2 cytokines activate eosinophils, which have been shown to play a role in allograft rejection [42].

In the last decade, other Th cell subsets have been described that have a cytokine profile that is distinct from the classical Th1 and Th2 subsets (Fig. 4). **Th17 cells**, named for their production of the pro-inflammatory cytokine IL-17, are involved in the elimination of extracellular pathogens. In addition, these cells orchestrate the pathogenesis of inflammatory and autoimmune disorders [43]. The development of Th17 cells can be divided into three phases: the differentiation phase mediated by transforming growth factor (TGF)- β and IL-6, the self-amplification phase by IL-21, and the stabilizing phase by IL-23. The key transcription factors are retinoic acid receptor-related orphan receptor gamma-T (ROR γ t) and STAT3 [31]. IL-17 contributes to inflammation by promoting the release of the chemokine IL-8 by epithelial and endothelial cells, which recruits neutrophils to the site of inflammation [44]. In addition to IL-17, Th17 cells produce IL-21, which activates T-cells and NK-cells, and IL-22, involved in host defense at mucosal surfaces as well as in tissue repair [45, 46]. Many studies in experimental transplant models have confirmed the contribution of Th17

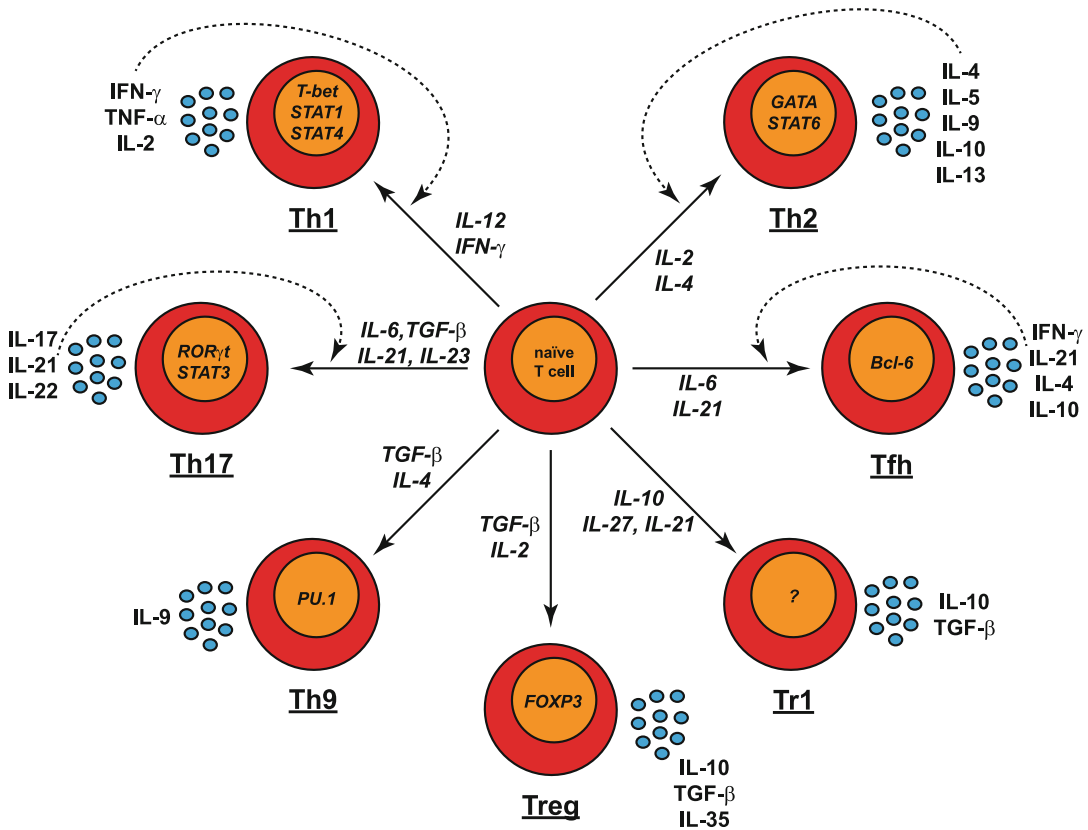


Fig. 4 Differentiation of naïve T-cells into Th cell subsets. A distinct cytokine environment drives the differentiation of activated CD4+ T-cells into different Th lineages through the induction of specific transcription factors (solid arrows). Key transcription factors are depicted in the cells. Th cells produce a variety of cytokines that contribute to the coordination and amplification or modulation of an immune response. Some cytokines can

self-amplify the differentiation process (dotted arrows). *BCL* B-cell lymphoma, *FOXP3* forkhead box P3, *IFN* interferon, *IL* interleukin, *ROR γ t* retinoic acid receptor-related orphan receptor gamma-T, *STAT* signal transducer and activator of transcription, *T-bet* T-box transcription factor, *Tfh* follicular T helper, *TGF* transforming growth factor, *Th* T helper, *TNF* tumor necrosis factor

cells to allograft rejection [47]. In addition, IL-17 has been implicated in the development of immune responses to autoantigens after lung transplantation [48, 49]. In humans, IL-17 mRNA expression has been detected in allografts undergoing acute or chronic rejection, and IL-17 mRNA has been found in the urinary sediment of patients with subclinical borderline rejection [50–52].

In the absence of TGF- β , IL-6 and IL-21 mediate the differentiation of follicular T helper (**Tfh**) cells [53]. These cells are mainly localized in follicles of secondary (or peripheral) lymphoid

organs and are critical for the formation and maintenance of germinal centers (GCs) and B-cell differentiation into long-lived plasma cells and memory cells. The key transcription factor for Tfh cells is B-cell lymphoma (BCL)-6. Although data about the role of Tfh cells in allograft rejection are still limited, some studies have indicated that these cells contribute to the development of humoral immunity against the allograft [54, 55].

A recently defined subset is the **Th9 cell** subset, characterized by the production of IL-9 [56]. These cells develop in the presence of TGF- β and

IL-4. To date, little is known about Th9 cells in humans, but experimental mouse models have shown that these cells can regulate allergic inflammation, autoimmune disease, and antitumor immunity [57]. Their role in transplantation remains to be elucidated.

T-cell activation under specific cytokine and co-stimulatory conditions can also drive T-cell differentiation into T-cell subsets with regulatory properties (Fig. 4). TGF- β is one of the key cytokines for the development of peripheral-derived regulatory T-cells (**Tregs**) [58]. Transcription factors Smad2 and Smad3 downstream of TGF- β signaling induce the expression of forkhead box P3 (FOXP3), the key transcription factor for Tregs. The co-inhibitory molecules CTLA-4 and PD-ligand (PD-L1) 1 as well as the vitamin A metabolite retinoic acid facilitate and enhance FOXP3 expression [59–61], which is essential for Treg function and maintenance. In addition, IL-2 is pivotal for Treg survival, proliferation, and stability. Similar to their counterpart, i.e., thymus-derived FOXP3+ Tregs that emigrate from the thymus, peripheral-derived FOXP3+ Tregs use various suppressive mechanisms to regulate immune responses, including cell-cell contact-mediated suppression by the expression of inhibitory molecules, metabolic disruption of effector T-cells, and secretion of immunoregulatory cytokines, such as IL-10, TGF- β , and IL-35 [58]. Another Treg subset that has been described to develop from naïve T-cells in the periphery is the so-called **Tr1 cell** subset. These cells can develop in the presence of IL-10 and IL-27 [62, 63]. IL-27 enhances expression of the transcription factor c-maf, IL-21 receptor, and the co-inhibitory molecule ICOS [64]. IL-21 is crucial for the amplification phase of Tr1 cells. The regulatory function of Tr1 cells can be attributed to the secretion of IL-10 and TGF- β as well as their capability to kill target cells [65]. Both peripheral-derived FOXP3+ Tregs and Tr1 cells play an important role in the downregulation of immunity. The potential role of Tregs in prevention of allograft rejection and induction of transplant tolerance is further discussed in section “Immune Regulation.”

T-Cell Migration and Allograft Destruction

Upon activation, effector T-cells upregulate the expression of specific chemokine receptors and other cell surface molecules, such as adhesion molecules, that will allow them to migrate from lymphoid organs to the site of inflammation or, in case of transplantation, to the allograft. Chemokines are small cytokines with chemoattractant properties that guide immune cells; homeostatic chemokines control the migration of cells during normal processes of homeostasis, such as tissue development and maintenance, whereas pro-inflammatory chemokines recruit cells to the site of inflammation. Although the exact chemokines and chemokine receptors that recruit T-cells to the allograft have not been fully elucidated, expression of the chemokines CCL3 (MIP-1- α) and CCL5 (RANTES) and their receptor CCR5 as well as CXCL9 (MIG), CXCL10 (IP-10), and CXCL11 (I-TAC) and their receptor CXCR3 has been associated with rejection in kidney, heart, and lung transplant recipients [66–68]. Various therapeutic antagonists that target chemokine/chemokine receptors are being explored for the prevention of T-cell migration to the allograft; however, none are currently being used in clinical care [69].

Once effector T-cells are recruited to the graft, they will reencounter their specific alloantigens and initiate mechanisms leading to graft destruction. CD4+ and CD8+ T-cells can respectively recognize MHC class II and I molecules expressed on graft cells. It has been shown that both alloreactive CD8+ T-cells and CD4+ T-cells are capable of acutely rejecting cardiac allografts via the direct allorecognition pathway [70, 71]. Effector T-cells can eliminate target cells via two major cytotoxic pathways: the Fas/FasL (CD95/CD95L) pathway and the granzyme/perforin pathway (Fig. 5). Engagement of FasL expressed on activated effector T-cells to Fas on target cells results in apoptotic cell death through caspase activation. Perforin and granzymes are cytotoxic molecules that are secreted by effector T-cells. Perforin inserts itself into the cell membrane of the target cell, forming a pore through which granzymes can enter the cell. Granzymes are ser-

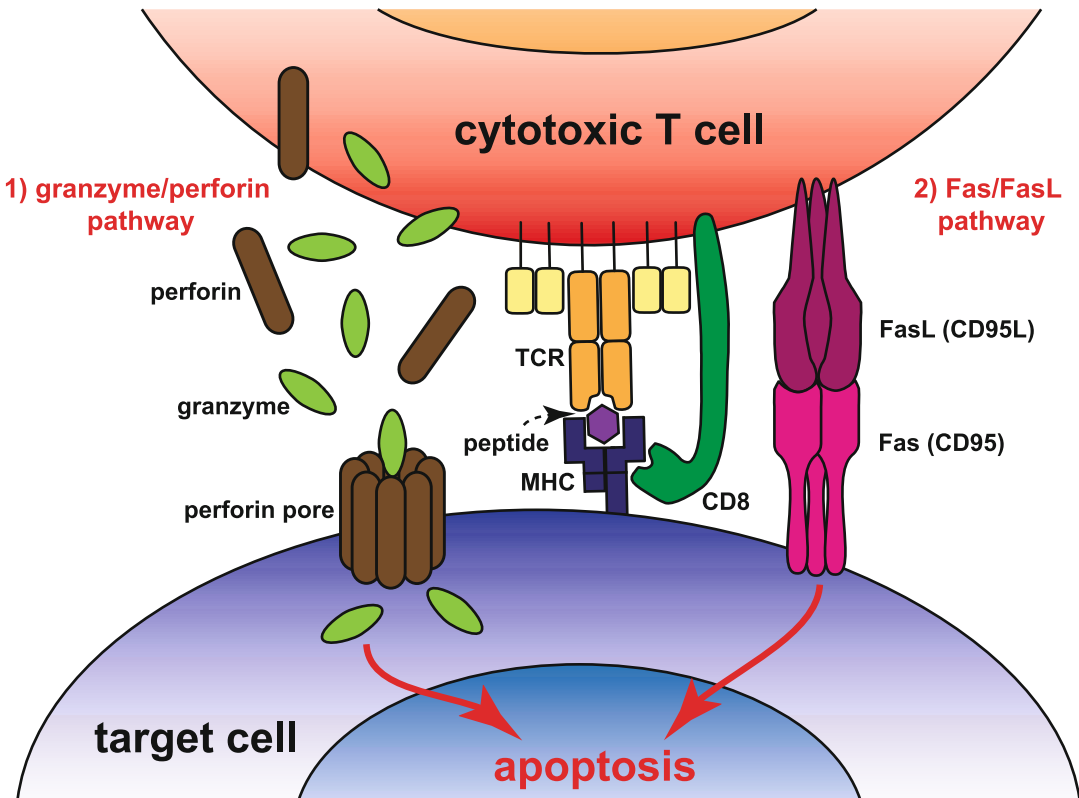


Fig. 5 *Cytotoxic pathways.* Effector T-cells can kill target cells via two cytotoxic pathways: (1) Through the secretion of cytotoxic molecules perforin and granzymes (granzyme/perforin pathway). Perforin forms pores in the cell membrane of the target cells through which granzymes can enter the target cell. Granzymes are serine proteases

that induce apoptosis through caspase-dependent and caspase-independent pathways. (2) Through the expression of FasL. FasL binds to Fas expressed on the target cell (Fas/FasL pathway), leading to apoptotic cell death through caspase activation. *MHC* major histocompatibility complex, *TCR* T-cell receptor

ine proteases that induce cell death in the target cell through caspase-dependent and caspase-independent pathways. Although both CD4+ and CD8+ cells are capable of utilizing both cytotoxic pathways, it has been suggested that CD4+ Th1 cells primarily act via the Fas/FasL pathway, whereas cytotoxic CD8+ T-cells act via the granzyme/perforin pathway. Both pathways have been implicated in allograft rejection in experimental transplant studies [36, 72]. Clinical studies have shown that abundant granzyme A, granzyme B, and perforin mRNA and/or protein expression can be detected in biopsies as well as urine samples in rejecting allografts [72]. In addition, upregulated Fas/FasL expression is associated with rejection [73, 74]. Other processes by

which effector T-cells mediate rejection in the allograft include the release of pro-inflammatory cytokines, which upregulate expression of MHC molecules on the graft resulting in increased allo-antigen presentation, as well as through stimulation of innate immune cells, such as neutrophils, eosinophils, macrophages, and NK-cells, to kill graft cells. If not modulated, these processes will ultimately destroy the graft.

B-Cell-Mediated Immunity

In the early years of transplantation, allograft rejection was believed to be mainly a T-cell-mediated immune response; B-cells, lymphocytes defined by

the expression of clonally diverse B-cell receptors (BCRs) on their surface, were viewed as somewhat secondary players. In the last decade, however, it has become evident that B-cells and their production of antibodies directed at graft antigens are key components in allograft rejection and subsequent graft loss. B-cells develop in the fetal liver or bone marrow and either mature at these sites or emigrate as immature B-cells (transitional B-cells) to further mature in the periphery. Survival of peripheral B-cells is dependent on continuous signaling through the BCR and the presence of survival factors, such as the B-cell-activating factor (BAFF, also known as B-lymphocyte stimulator (BlyS)). The majority of naïve mature B-cells recirculate through the B-cell areas of secondary lymphoid organs to find their cognate antigen and to interact with other immune cells, such as T-cells and DCs. Once B-cells become activated through antigen recognition or other stimulants, they can differentiate into short-lived antibody-secreting cells (ASCs), or proliferate, undergo somatic hypermutations (SHMs) in their BCR and isotype switching to develop into long-lived antibody-secreting plasma cells or memory B-cells. Besides the production of donor-specific antibodies (DSAs), B-cells may contribute to allograft rejection through antigen presentation to T-cells and cytokine production.

Allorecognition: The B-Cell Receptor and Antibodies

B-cells recognize foreign antigens via their BCR complex, which consists of an Ig molecule formed by two membrane-bound heavy chains and two smaller light chains bound by disulfide bonds, and an Ig- α /Ig- β heterodimer (CD79) that mediates intracellular signaling (Fig. 6a). Both the heavy chain and the light chain are composed of a highly variable antigen-binding region and a constant region. Heavy chains can be subdivided in five different classes or isotypes: α (IgA), δ (IgD), ϵ (IgE), γ (IgG), and μ (IgM). Both IgD and IgM receptors are expressed on the surface of naïve mature B-cells, whereas IgA, IgE, and IgG are expressed on B-cells that underwent class switching upon activation. Light chains can be subdivided into κ or λ chains,

which do not switch upon B-cell activation and differentiation.

Antibodies are the soluble form of Ig molecules and are identical to the BCR, but without the transmembrane region (Fig. 6b). Antibodies are composed of two antigen-binding fragments (Fab) and a crystallizable fragment (Fc). The Fab region, which is composed of the complete light chain and the variable antigen-binding domain with one constant domain of the heavy chain, is important for antigen recognition. The Fc region contains two or three constant domains (depending on the isotype) of both heavy chains which can interact with various immune cells and effector molecules. Whereas all isotypes of the Ig molecule are monomers on the cell surface, some soluble isotype molecules can form dimers (IgA) or pentamers (IgM) (Fig. 6c). The function of antibodies is to fix complement and to activate innate effector cells via binding to Fc receptors on the surface of these cells.

In contrast to TCRs, BCRs are able to recognize antigens in their native or unprocessed form. These antigens are not limited to proteins, but can be different molecular compositions, such as carbohydrates, lipids, and nucleic acids. Thus, whereas allogeneic T-cells only respond to peptides derived from graft proteins, B-cells may respond to other antigenic structures of the allograft, such as ABO blood group carbohydrate antigens. Furthermore, BCRs do not need their cognate antigen to be presented by MHC molecules for recognition; BCRs can engage both soluble antigens and membrane-associated antigens on the surface of APCs [75]. Soluble lymph-borne antigens may diffuse directly into the follicles of lymphoid organs where they can be acquired by antigen-specific B-cells [76]. DCs and macrophages can present antigens to B-cells through cell surface molecules that participate in the retention and presentation of antigens in their unprocessed form, including Fc receptors, complement receptors, pattern recognition receptors, and/or scavenger receptors [75, 77, 78]. Furthermore, B-cells can recognize antigens bound to antibodies, so-called immune complexes, which are retained in the follicles by follicular DCs (FDCs) via complement receptors

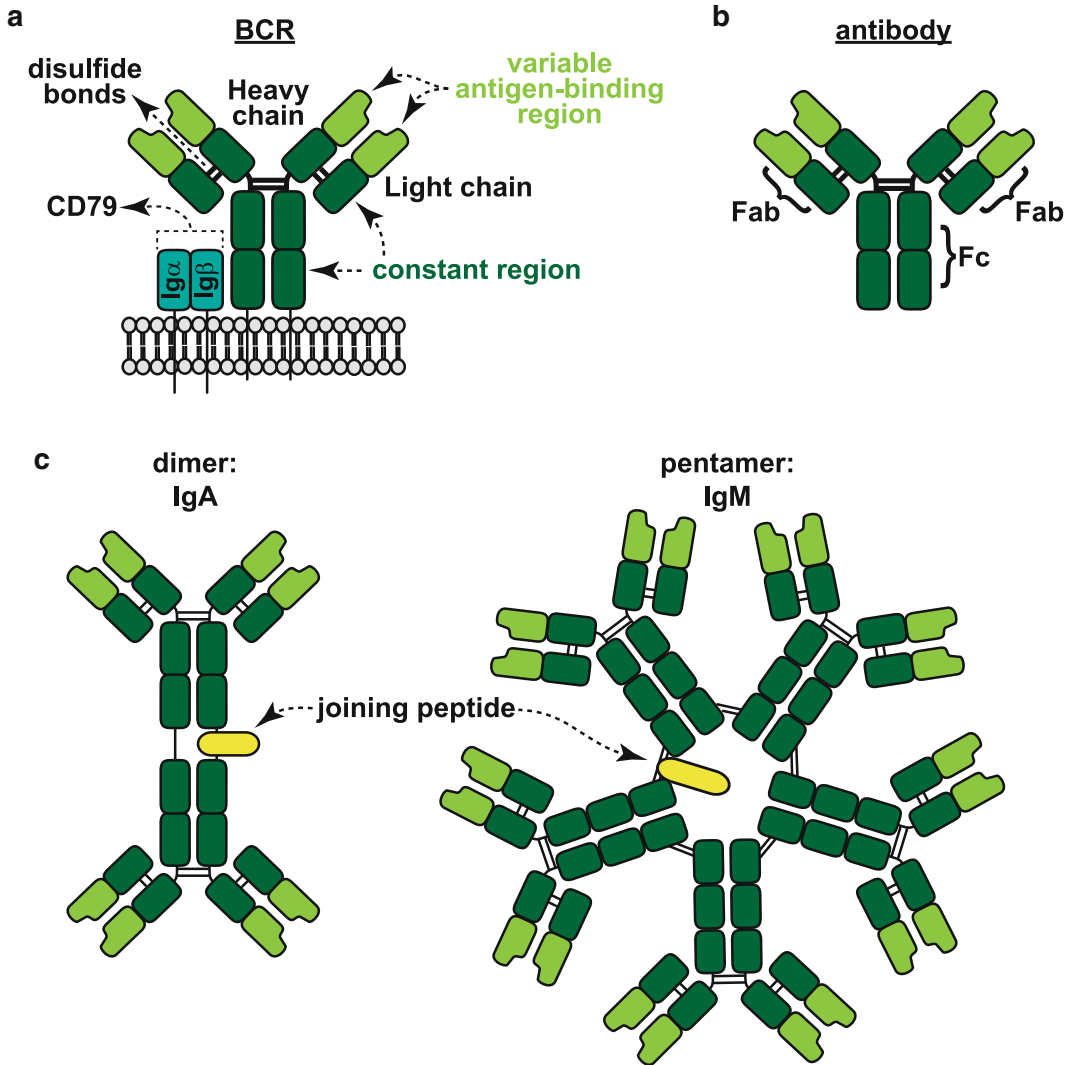


Fig. 6 *B-cell receptor and antibodies.* (a) The BCR is composed of an Ig molecule formed by two membrane-bound heavy chains and two smaller light chains bound by disulfide bonds, and an Ig- α /Ig- β heterodimer (CD79) that mediates intracellular signaling. Each heavy chain and light chain contains a highly variable antigen-binding region that recognizes foreign antigens and a constant region. (b) Antibodies are the soluble form of Ig molecules and are identical to the BCR, with the exception of the transmembrane region. Antibodies can be divided into

three fragments: two Fab regions, which are identical to each other and consist of the complete light chain and the variable antigen-binding domain with one constant domain of the heavy chain, and a Fc region, composed of two or three constant domains (depending on the isotype of the antibody) of both heavy chains. (c) IgA and IgM antibodies can bind together via disulfide bonds and a joining peptide to form dimers and pentamers, respectively. *BCR* B-cell receptor, *Fab* antigen-binding fragment, *Fc* crystallizable fragment, *Ig* immunoglobulin

1 and 2 (also known as CD35 and CD21, respectively) or Fc receptors [79].

B-Cell Activation

B-cells may be activated by **T-cell-independent (TI)** or **T-cell-dependent (TD)** pathways (Fig. 7). TI activation of B-cells may occur in a non-antigen-specific manner via Toll-like receptor (TLR) stimulation (**TI type 1 B-cell activation**). TLRs bind to pathogen-associated molecular patterns (PAMPs), molecules expressed by pathogens, and damage-associated molecular patterns (DAMPs), molecules derived from cells. These receptors, discussed in more detail in section “Toll-Like Receptors,” play a key role in the activation of innate immune cells,

but are also expressed on B-cells, providing a mechanism for the regulation of adaptive immune responses via innate signals [80]. In the transplant setting, binding of damage-induced endogenous or exogenous ligands to TLRs may lead to TI-1 B-cell activation [81].

TI B-cell activation may also occur in an antigen-specific manner through cross-linking of BCRs on the cell surface by antigens bearing repetitive epitopes, like bacterial capsular polysaccharides (**TI type 2 B-cell activation**). Although this activation does not require MHC-restricted T-cell help, it has been proposed that a second signal is needed for the differentiation into ASCs [82]. This signal may be provided by engagement of TLRs or by cytokines produced

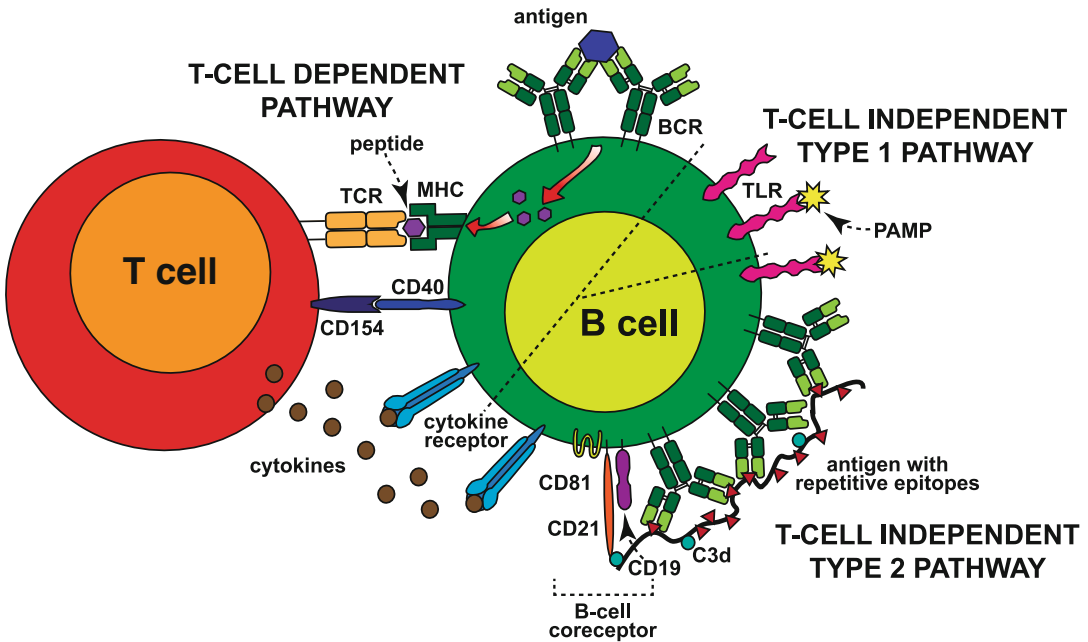


Fig. 7 *B-cell activation pathways.* Activation of B-cells may occur via three distinct pathways: (1) A T-cell-dependent pathway, where the B-cell requires the help of T-cells to become activated. B-cells are able to endocytose and process antigens that bind to the BCR into peptides and present these in the context of MHC class II molecules to CD4⁺ T-cells, thereby activating these cells. T-cells subsequently upregulate the expression of co-stimulatory molecules such as CD154 that binds to CD40 on B-cells, as well as secretes cytokines, resulting in the activation of B-cells. (2) A non-antigen-specific T-cell-independent pathway, where PAMPs bind to TLRs expressed on B-cells. (3) An antigen-specific T-cell-independent path-

way, where BCRs are cross-linked by antigens bearing repetitive epitopes, like bacterial capsular polysaccharides. Although this pathway does not require MHC-restricted T-cell help, a second signal may be needed for differentiation of B-cells into ASCs. This signal may be provided by engagement of TLRs or by cytokines produced by other immune cells, such as T-cells. In addition, binding of the complement degradation product C3d to CD21, which together with CD19 and CD81 forms the B-cell co-receptor, can lower the threshold for B-cell activation. *BCR* B-cell receptor, *MHC* major histocompatibility complex, *PAMP* pathogen-associated molecular pattern, *TCR* T-cell receptor, *TLR* Toll-like receptor

by T-cells or NK-cells. Furthermore, complement can augment TI-2 B-cell activation through binding of complement product C3d (degradation product of C3) to CD21, which together with CD19 and CD81 forms the B-cell co-receptor complex [83]. Antibodies directed against ABO blood group antigens are considered to be produced through TI-2 B-cell responses [84].

B-cell activation by antigens derived from proteins, such as MHC molecules, typically requires the help of T-cells and is therefore referred to as a TD immune response. Upon binding to the BCR, B-cells are able to endocytose and process antigens into peptides and present these in the context of MHC class II molecules to CD4+ T-cells. This antigen-specific B-cell-T-cell contact activates the T-cell, which in turn upregulates the expression of co-stimulatory molecules such as CD154 that binds to CD40 on the B-cells, as well as secretes cytokines, resulting in the activation of the B-cell.

B-Cell Differentiation

Upon TD B-cell activation, B-cells either differentiate into extrafollicular short-lived ASCs that are pivotal for rapid antibody production and early immune protection or migrate to the follicles of the secondary lymphoid organs for the formation of GCs. The GC is a highly specialized and dynamic microenvironment where activated follicular B-cells proliferate, differentiate, diversify their BCR, and change their Ig molecule isotype [85]. The formation of these centers depends on the co-migration and co-localization of B-cells, Tfh cells, and FDC. The first days after migration into GC, activated B-cells mainly undergo clonal expansion with little diversification in their BCR. After a period of B-cell proliferation, the GC is organized into two different “zones”: **dark zones and light zones** [86]. In the **dark zone**, B-cells interact with Tfh cells, proliferate, and undergo isotype class switching and SHM. Isotype class switching, also known as class-switch recombination, is the process in which the constant region of the heavy chain of the Ig molecule is changed, generating different classes of antibodies such as IgG (with subclasses IgG1, IgG2a, IgG2b, IgG2c, and IgG3 in mice

and IgG1, IgG2, IgG3, and IgG4 in humans), IgA, and IgE. SHM is a process in which point mutations are introduced in the DNA encoding the variable segments of the Ig genes, resulting in diversity of the BCR of the B-cell clones. These mutations are considered to be random and thus the BCR may have an unchanged, lower or higher affinity for its cognate antigen after SHM. B-cell clones migrate to the **light zones** of GCs to interact with antigen-presenting FDC and “test” the affinity of their mutated BCRs. The survival of these B-cells is considered to be dependent on antigen stimulation; B-cells whose SHM results in BCRs with higher affinity will have a survival advantage. The process of SHM and clonal selection is defined as affinity maturation. These B-cells further differentiate into ASCs or memory B-cells.

Besides follicular B-cells, other B-cell subsets with specific immune properties have been described, such as marginal zone (MZ) B-cells, B-1 cells, and regulatory B-cells (Bregs). **MZ B-cells** are innate-like cells that provide a first line of defense, mediating TI immune responses to antigens of blood-borne pathogens [87]. These cells mainly reside in the MZ of the spleen and in secondary lymphoid tissues with MZ-like regions. Besides their role in TI immune responses, MZ B-cells also participate in TD responses by delivering antigens bound in immune complexes to FDC via complement receptors CD21 and CD35 and by activating T-cells through their expression of MHC class II and co-stimulatory molecules [88, 89]. These cells also highly express CD1d, which is involved in the presentation of lipid antigens to a distinct subset of T-cells, the so-called natural killer T (NKT)-cells [90]. The presence of somatic mutations in the BCR of MZ B-cells in humans indicates that these cells may undergo affinity maturation, leading these cells to be called IgM+ memory B-cells [91].

B-1 cells have distinctive phenotypic and functional characteristics compared to follicular and MZ B-cells, including the capacity for self-renewal, preferred localization in peritoneal and pleural cavities, and production of natural antibodies. B-1 cells can be subdivided into two

subsets: B-1a (CD5+) cells and B-1b (CD5-) cells. B-1a cells are the major producers of natural antibodies providing innate-like immune protection against bacteria, while B-1b cells are involved in adaptive immune responses to polysaccharides and other TI-2 antigens [92]. These cells have mainly been described in rodents; there have been attempts to identify B-1-like cells in humans [93], but findings have been controversial [94].

Based on recent findings, it is now appreciated that B-cells can also differentiate into cells with immunoregulatory properties [95]. The suppressive function of **Bregs** is mainly attributed to their production of immunoregulatory cytokines IL-10, IL-35, and TGF- β . Bregs suppress the differentiation of T-cells into Th1 and Th17 subsets directly or indirectly by suppressing DC function and promote the induction of peripheral-derived Tregs [96, 97]. Multiple phenotypic characteristics have been ascribed to Bregs [98], but so far there is little evidence that these cells belong to a distinct B-cell lineage. Rather, it appears that any B-cell may differentiate into a Breg in response to environmental stimuli [95]. Furthermore, various studies suggest that this regulatory phenotype may be transient and that Bregs have the capacity to differentiate into ASCs [97, 99]. The potential role of Bregs in prevention of allograft rejection and induction of transplant tolerance is further discussed in section "Immune Regulation."

Antibody-Secreting Cells

ASCs are responsible for the production and secretion of antibodies, key components in humoral immunity. ASCs can be divided into plasmablasts and plasma cells. **Plasmablasts**, which are the precursors of plasma cells, are proliferating ASCs that are often found in the circulation, migrating from the secondary lymphoid tissues to other tissues such as the bone marrow. **Plasma cells** are terminally differentiated nondividing ASCs. Since these cells are committed to the production of antibodies and no longer need to bind or present antigens, their phenotype is distinct from B-cells: plasma cells no longer express MHC class II and other B-cell markers such as CD20 and CD21. In addition, BCR expression is

generally thought to be lost on plasma cells, although it was recently demonstrated that at least some IgM+ and IgA+ plasma cells maintain BCR expression [100]. Plasma cells express the proteoglycan syndecan-1 (CD138), a receptor for various growth factors, the adhesion molecule very-late antigen-4 (VLA-4), and the chemokine receptor CXCR4 [101]. Plasma cells typically do not migrate and are therefore seldom found in the circulation; they rather become residents for life in their organ of choice. Plasma cells that develop in the immediate immune response are generally short-lived and die within a few days through apoptosis [102]. These cells remain in the secondary lymphoid tissues and secrete non-mutated antibodies with low affinity. Antibody production peaks at about one week after immunization, providing early immune protection. Long-lived plasma cells are considered to arise from B-cells that have undergone affinity maturation and thus produce high-affinity antibodies [103]. The majority of long-lived plasma cells resides in the bone marrow and can survive for up to months or even years, depending on the availability of survival factors [104, 105]. These survival factors include a combination of various cytokines, such as IL-5, IL-6, TNF- α , and CXCL12, all produced by bone marrow stromal cells [106]. In addition, BAFF and a proliferation-inducing ligand (APRIL) may support the long-term survival of plasma cells [107].

B-Cell-Mediated Graft Destruction

Secreted antibodies circulate through the periphery to search for foreign antigens. Binding of the antibody to its cognate antigen may neutralize the target directly or attract other components and cells to eliminate the target through several mechanisms, including complement activation via fixation of complement components such as C1q and the induction of cell-mediated cytotoxicity by attracting Fc receptor-expressing innate effector cells [108, 109]. In transplantation, antibodies directed at MHC molecules, ABO blood group antigens, minor histocompatibility antigens, and autoantigens such as endothelial cell antigens are able to mediate graft destruction. The detrimental effect of these antibodies is most

dramatic in patients who have developed DSAs before transplantation, where graft destruction generally occurs within hours after transplantation (hyperacute rejection). Improved screening techniques and the introduction of interventions that reduce antibody titers (plasmapheresis, immunoadsorption columns) or inhibit antibody function (intravenous immunoglobulin (IVIg) and eculizumab) have made hyperacute antibody-mediated rejection (AMR) rare. Nevertheless, the destructive role of antibodies in the pathogenesis of acute rejection and chronic allograft injury is increasingly being recognized [110–113]. The major mechanism involved is most likely, although not exclusively, the activation of complement pathways, which is discussed in section “Complement System.” Antibody deposition and complement fixation can be identified by **immunohistochemical staining for C4d**, a degradation product of C4. Detection of C4d has been associated with acute and chronic AMR [114–116]; however, the absence of C4d does not rule out antibodies as mediators of allograft rejection. Antibodies bound to antigens on the graft may also activate innate immune cells that bind to the Fc region of the antibody to cause allograft injury. Indeed, the presence of macrophages in the allograft, defined by positive CD68 staining, has been associated with AMR [117, 118]. Furthermore, a study in experimental transplant models has recently shown that alloantibodies may facilitate cellular rejection by functioning as opsonins to activate alloreactive T-cells [119].

Although B-cells are mainly implicated in allograft rejection as being producers of antibodies, these cells may also contribute to graft destruction via antibody-independent mechanisms. B-cell graft infiltrates have been found in acutely rejected renal allografts [120, 121], where they may function as APCs. Alloantigen presentation by B-cells has been shown to contribute to the progression of acute vascularized allograft rejection in an experimental transplant model of cardiac rejection [122]. Additionally, B-cells secrete a variety of cytokines and chemokines that can affect the migration and function of other immune cells. Furthermore, B-cells may be involved in the formation of lymphoid-like structures in the allograft [123].

Innate Immunity in Transplantation

Though research in transplant rejection and tolerance induction has primarily focused on the adaptive immune system, the contribution of innate immune factors such as complement, TLRs, and innate immune cells in these processes is increasingly being appreciated. The innate immune system can be triggered by microbial products or by endogenous components from damaged or stressed cells. Innate immune cells express a variety of pattern recognition receptors (PRRs) such as TLRs that recognize PAMPs derived from pathogens as well as DAMPs derived from cells. Examples of DAMPs include heat shock proteins, peptidoglycan, heparin sulfate, and high-mobility group box protein 1 (HMGB1). During transplantation, surgical trauma, ischemia, preservation, and reperfusion inevitably result in tissue injury leading to the release of DAMPs. These structures will rapidly attract and activate innate immune cells. Although this activation is generally not sufficient to induce graft rejection without the presence of adaptive immunity, it has become clear that these innate immune cells can initiate an inflammatory cascade that can activate and regulate alloantigen immune responses. The production of chemokines and pro-inflammatory cytokines, release of radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), activation of APCs, attraction of adaptive immune cells, and induction of stress markers on graft cells can further induce graft damage and may result in delayed graft function or graft loss. Furthermore, innate immune cells can augment graft rejection by binding alloantibodies or immune complexes through the expression of Fc receptors, thereby triggering antibody-dependent cellular cytotoxicity (ADCC), and acting as APCs after internalization of damaged graft cells.

Complement System

The complement system consists of soluble proteins that assist the immune system by marking cells for elimination, agglutinating pathogens, disrupting cell membranes through pore forma-

tion, attracting innate immune cells, and modulating adaptive immune cells. Complement proteins are mainly produced by the liver, but some, such as C3, can also be produced by macrophages, monocytes, and epithelial cells. These proteins circulate through the periphery as inactive precursors. When activated, complement proteins are converted into an active form via an enzyme-triggered cascade, resulting in amplification of the response. Activation of the complement system can occur via three pathways: the classical pathway, the alternative pathway, and

the lectin pathway (Fig. 8). **The classical pathway** is triggered by activation of the C1 complex, where C1q binds to antibody-antigen immune complexes or non-Ig molecules such as C-reactive protein and serum amyloid P [124–126]. **The lectin pathway** is activated through binding of mannose-binding lectin (MBL) or ficolin to carbohydrate structures on pathogens [124]. Both MBL and ficolin circulate in the serum as complexes with MBL-associated proteins. **The alternative pathway** is continuously triggered at a low level through spontaneous hydrolysis of C3

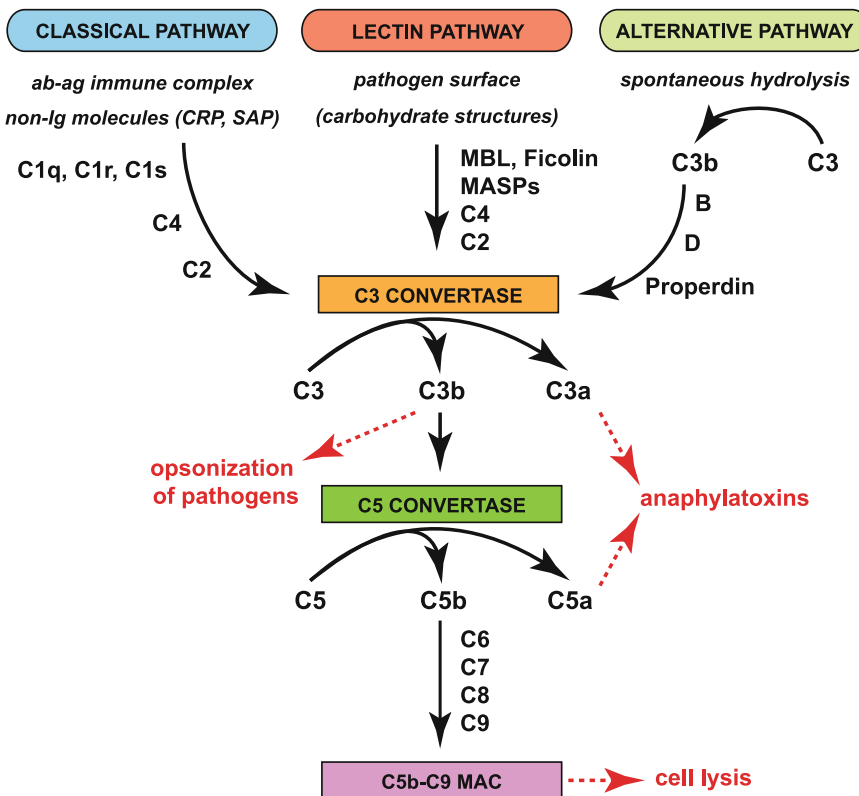


Fig. 8 *The complement system.* The complement system can be activated via three pathways: the classical pathway, the alternative pathway, and the lectin pathway. Early events of each of these pathways involve a series of enzyme reactions in which inactive complement enzyme precursors, so-called zymogens, are cleaved to yield active serine proteases. The three pathways converge at the production of C3-convertase, which cleaves C3 into C3a and C3b. Binding of C3b to the pathogen surface promotes the opsonization of pathogens by phagocytic cells. Moreover, binding of C3b to C3-convertase forms a

C5-convertase, which cleaves C5 into C5a and C5b. C5b initiates a series of reactions in which the terminal complement components C5b, C6, C7, C8, and C9 form a membrane attack complex. This complex forms pores in the target cell, resulting in cell lysis. C3a and C5a act as anaphylatoxins, triggering degranulation of mast cells and neutrophils, attracting phagocytes to the site of inflammation, and modulating T-cell responses; *ab* antibody, *ag* antigen, *CRP* C-reactive protein, *MAC* membrane attack complex, *MASPs* MBL-associated proteins, *MBL* mannose-binding lectin, *SAP* serum amyloid P

into C3b [124]. Each pathway follows a series of enzyme reactions leading to generation of C3-convertase that cleaves C3 into C3a and C3b. C3b binds to the surface of pathogens, thereby promoting the opsonization by phagocytic cells. In addition, C3b binds to C3-convertase to form a C5-convertase that cleaves C5 into C5a and C5b. Both C3a and C5a act as anaphylatoxins, triggering degranulation of mast cells and neutrophils, attracting phagocytes to the site of inflammation, and modulating T-cell responses [124]. C5b initiates the generation of the membrane attack complex (MAC), comprised of C5b, C6, C7, C8, and C9, that creates pores in the target cell, resulting in cell lysis.

Complement activation in transplantation can occur during I/R injury and rejection of the donor graft. Complement plays a pivotal role in the pathogenesis of reperfusion damage; studies in experimental transplant models have shown that complement-depleted or complement-deficient mice are protected from I/R injury [127]. Mechanisms of injury include pore formation by the membrane attack complex causing cell lysis and death, the attraction and degranulation of neutrophils, and direct parenchymal action of C5a. In kidney transplantation, complement-mediated reperfusion damage may be dependent on local production of C3 rather than circulating C3 [128]. In the clinical setting, local expression of C3 and other complement products in donor kidneys before transplant surgery is related to donor death and correlated with early and late graft function [129]. Local production of C3 also contributes to cell-mediated graft rejection; mice receiving kidneys from C3-deficient donors rejected their grafts in a less vigorous manner than mice receiving wild-type grafts, presumably due to a defective T-cell immune response [130]. Similarly, in the absence of the complement regulator decay-accelerating factor (DAF, CD55), uncontrolled local C3 production accelerated T-cell-mediated graft rejection in a heart transplant model [131]. In humans, detection of products of complement activation in the serum, urine, or graft is associated with acute cell-mediated rejection in kidney, heart, and liver

transplants [132–135]. Complement can facilitate T-cell alloreactivity by promoting T-cell priming and survival as well as enhancing APC function [127, 136]. Furthermore, production of alloantibodies is dependent on complement; mice with deficient classical pathway function showed impaired allospecific IgG response [137]. Mechanisms by which complement contributes to alloantibody production include C3 opsonization of donor antigens, thereby promoting antigen retention in the B-cell areas of secondary lymphoid organs, reducing the threshold for B-cell stimulation through cross-linking the BCR with CD21, and enabling class switching from IgM to IgG antibodies [127, 138]. DSAs in turn can activate the complement cascade. Binding of C1q to antibodies bound to the graft endothelium activates the classical pathway leading to the production of C5a and the membrane attack complex. These factors activate endothelial cells, macrophages, neutrophils, and platelets and stimulate the coagulation cascade, inducing endothelial cell damage and vascular thrombosis. The role of complement in both early and late AMR is well recognized. C4d deposition is detected in many of the most aggressive cases of AMR and is recognized as part of the criteria by both the Banff and ISHLT classifications for the diagnosis of AMR [139–141]. Complement inhibition with a humanized anti-C5 antibody (eculizumab) has been shown to decrease the incidence of early AMR in sensitized patients [142].

Interestingly, cases in ABOi kidney transplantation have been described in which C4d deposition is observed in the graft in the absence of damage or loss of graft function [143, 144]. This occurrence is referred to as **accommodation**, a condition in which a graft seemingly acquires resistance to immune-mediated injury, despite the presence of pathogenic antibody and complement [145]. Several mechanisms of accommodation have been proposed, including regulation of complement by complement regulatory proteins such as CD55 and CD59 and the production of anti-apoptotic proteins such as heme oxygenase 1 (HO1) by endothelial cells.

Toll-Like Receptors

TLRs, transmembrane proteins that function as PRRs, are expressed on various immune cells, including macrophages, DCs, mast cells, and B-cells, as well as on nonimmune cells, such as endothelial and epithelial cells. Eleven TLRs have been described in humans and 13 in mice, all recognizing distinct PAMPs derived from pathogens and/or DAMPs derived from endogenous cell components (Table 1) [146, 147]. Binding of TLRs to their ligand triggers signal transduction via the myeloid differentiation factor 88 (MyD88) pathway, except for TLR3 which signals through the Toll/IL-1R domain-containing adaptor protein inducing IFN- β

(TRIF) pathway and TLR4 which uses both pathways. Downstream effects of TLR signaling lead to activation of innate and adaptive immune responses via the production of cytokines and chemokines, upregulation of co-stimulatory molecules, and augmentation of effector functions. During I/R injury, endogenous ligands derived from damaged cells can activate TLR signaling, leading to an inflammatory immune response and thereby promoting further damage and cell death. Animal model studies have shown that TLR4 and TLR2 signaling is involved in hepatic, renal, and myocardial I/R injury [148]. In humans, signaling through TLR4 expressed on the donor kidney and liver was associated with inflammation and injury following cold preservation and transplantation; expression of a TLR4 variant with diminished binding to DAMPs was linked to less inflammation and initial good graft function [149, 150]. TLR stimulation at time of transplantation can also contribute to allospecific immune responses and prevents tolerance induction by co-stimulatory blockade [151, 152]. Absence of MyD88 signaling promotes allograft tolerance by impairing DC function and T-cell activation and increasing T-cell susceptibility to Treg suppression [153]. Absence of MyD88 in a minor histocompatibility (H-Y)-mismatched mouse model resulted in prolonged skin graft survival due to reduced DC numbers and subsequently impaired Th1 immunity [154]. DC function and Th1 responses were also impaired in a MHC-mismatched mouse model in the absence of MyD88, though acute rejection was not prevented, indicating that signaling via the MyD88 pathway can participate to, but is not necessary for, allograft rejection [155]. In clinical transplantation, TLR4 signaling was associated with acute allograft rejection in liver and lung transplant recipients [156, 157]. Thus, early TLR activation by DAMPs released following I/R injury may contribute to induction of alloimmunity, resulting in allograft rejection. In addition, TLR signaling may also explain why organs that are constantly exposed to PAMPs, such as the lung and intestine, are more prone to rejection than the liver, kidney, and heart.

Table 1 Toll-like receptors and some of their potential ligands^a

Receptor	DAMPs	PAMPs
TLR1		Triacyl lipoproteins
TLR2	HSPs	Lipoproteins
	HMGB1	Lipoteichoic acid
	Hyaluronan	Peptidoglycan Zymosan
TLR3	Self-dsRNA	Viral dsRNA
TLR4	HSPs	LPS
	Fibronectin	Fusion protein (RSV)
	Fibrinogen	
	Heparin sulfate	
	Hyaluronan	
	HMGB1	
TLR5		Flagellin
TLR6		Diacyl lipoproteins
		Lipoteichoic acid
		Zymosan
TLR7	Self-ssRNA	Viral ssRNA
TLR8	Self-ssRNA	Viral ssRNA
TLR9	HMGB1	CpG-containing DNA
	Self-DNA	
TLR10		N.D.
TLR11		Uropathogenic bacteria

^aBased on [146, 147]

dsRNA double-stranded RNA, *HMGB1* high-mobility group box protein 1, *HSPs* heat shock proteins, *LPS* lipopolysaccharides, *N.D.* not determined, *RSV* respiratory syncytial virus, *ssRNA* single-stranded RNA

Innate Immune Cells

Innate immune cells can be divided into granulocytes (neutrophils, basophils, mast cells, eosinophils), phagocytes (monocytes, macrophages, DCs), and lymphocytes (NK-cells). In contrast to adaptive immune cells, innate immune cells are not antigen specific, but respond to broadly expressed PAMPs and DAMPs derived from pathogens and damaged cells, respectively. Most of these cells have the stand-alone capability to recognize and kill possible threats. In addition, they strongly interact with each other and with adaptive immune cells for most effective protection against harmful conditions.

Granulocytes

Granulocytes, also known as polymorphonuclear leukocytes because of their segmented lobular nucleus, are characterized by granules in the cytosol that contain various proteins, including proteolytic enzymes, toxins, and cytokines. Historically, granulocytes were mainly considered as the first line of cytotoxic effector cells, but more recent evidence has shown that these cells also act as important modulators of adaptive immunity through production of cytokines and chemokines and presentation of antigens [158–163].

Neutrophils are the most abundant granulocytes and circulate in the blood in a resting state. When primed by components such as bacterial products, cytokines, or chemokines, neutrophils migrate to the site of inflammation, where they encounter activation signals to trigger killing of pathogens by phagocytosis, secretion of antimicrobial proteins and proteases, production of ROS, and generation of neutrophil extracellular traps (NETs) [164]. Neutrophils are rapidly recruited into the transplanted graft following I/R injury [165, 166]. Through the expression of Fc and complement receptors, neutrophils can interact with antibodies and complement and contribute to AMR. In lung transplant patients, neutrophilic infiltration was related to the presence of DSAs and associated with graft dysfunction and AMR [167]. In experimental lung transplant models, it was shown that graft-infiltrating neutrophils can also promote cell-

mediated rejection by interacting with DCs, thereby skewing T-cell differentiation toward Th1 [168], and by directly enhancing T-cell activation through increased co-stimulation following bacterial infection [169].

Eosinophils reside in the thymus and gastrointestinal tract and eliminate parasites, bacteria, and viruses through the release of toxic granule proteins and lipid mediators [159].

Basophils, the least common of the granulocytes, secrete immune mediators, such as histamine, vasoactive amines, and endothelial interacting substances [162]. In addition, these cells express high-affinity receptors for IgE.

Mast cells are similar to basophils in appearance and function but differ in development, life cycle, and localization: whereas basophils are present in the blood, mast cells reside in tissues [170]. Besides their role in the elimination of parasitic, bacterial, and viral infections, eosinophils, basophils, and mast cells are essential components in allergic inflammation and asthma [170]. Their potential role in alloimmune responses, however, remains to be elucidated. Experimental studies have suggested that eosinophils participate in allograft rejection in the context of Th2 alloimmune responses [42]. Although studies from two decades ago have demonstrated that graft-infiltrating eosinophils can be detected in rejecting human allografts, suggesting that these cells may be involved in allograft rejection [171–173], more recent literature on the role of these cells in alloimmune responses is sparse. Mast cells have been shown to be essential for the induction of peripheral tolerance by Tregs in a transplant model [174]. IL-9, produced by activated Tregs, recruited mast cells into the graft; neutralization of this cytokine accelerated allograft rejection. A possible tolerance-inducing mechanism of mast cells may be production of mast cell protease (MCP)6, which can degrade pro-inflammatory cytokines, such as IL-6 [175]. Of note, mast cell degranulation impaired Treg function, thereby breaking peripheral tolerance and inducing cell-mediated allograft rejection [176], which suggests that allergic inflammation may alter the balance between immune regulation and activation.

Phagocytes

Monocytes are mononuclear phagocytes that circulate in the peripheral blood. These cells are typically one of the first immune cells that enter the inflamed site to contribute to the elimination of pathogens through phagocytosis, production of ROS, and secretion of inflammatory cytokines [177, 178]. Upon inflammatory signals, monocytes can differentiate into either macrophages or DCs [179]. Recent studies in humans have shown that monocytes can be subdivided in three subsets with distinct features based on the expression of CD14 and CD16 (FC γ RIII): CD14⁺⁺CD16⁻ (classical), CD14⁺⁺CD16⁺ (intermediate), and CD14⁺CD16⁺⁺ (nonclassical) monocytes [180, 181]. Classical monocytes are highly efficient at phagocytosis, whereas intermediate monocytes express high levels of MHC class II molecules and are potent T-cell stimulators. Nonclassical monocytes exhibit a highly motile behavior, constantly patrolling the vessel walls for signs of infection or damage. In transplantation, infiltration of monocytes into the graft is associated with acute rejection and dysfunction in renal transplant recipients [182, 183]. Monocytes can participate in the initiation of cell-mediated rejection by interacting with graft endothelial cells, which induces the production of chemokines that attract T-cells into the graft [184]. In addition, differentiation of intra-graft monocytes into mature IL-12-expressing DCs can stimulate T-cell proliferation and IFN- γ production [185]. The finding that binding of MHC class I antibodies to graft endothelial cells promotes monocyte recruitment into the graft indicates a role for monocytes in AMR, likely by differentiating into macrophages [186].

Macrophages are highly versatile immune cells that are key players not only in activation and modulation of immune responses but also in various physiological processes, including tissue development, homeostasis and repair, as well as pathologic processes, including tumor development and fibrosis. Most tissues in the body contain tissue-resident macrophages, where they are strategically located to form the first line of defense against invading pathogens, and maintain tissue homeostasis by clearing dead cells and repairing tissue damage. Tissue-resident

macrophages are named differently dependent on the tissue where they are located, such as Kupffer cells (liver), alveolar macrophages (lung), osteoclasts (bone), and microglia (central nervous system). During inflammation, macrophages accumulate at the inflamed site following in situ proliferation as well as via recruitment of monocytes that differentiate into distinct activated macrophage subsets depending on the environmental signals [187]. Stimulation with TLR ligands and IFN- γ and TNF- α triggers phagocytosis, production of pro-inflammatory cytokines, chemokines, ROS and RNS, and expression of inducible nitric oxide synthase (iNOS), which allows for effective pathogen uptake and digestion. This macrophage subset is generally classified as the classically activated macrophage, or M1, and plays an important role in immune responses against pathogens and antitumor reactivity. Stimulation with IL-4 and IL-13 drives the differentiation of monocytes into so-called alternatively activated macrophages, or M2, which have anti-inflammatory properties and promote wound healing and fibrosis. Stimulation with IL-10, prostaglandins, or immune complexes can give rise to macrophages with immunoregulatory properties, so-called regulatory macrophages (Mregs). Although macrophages are often categorized as M1-polarized or M2-polarized, it is now appreciated that macrophages are a highly heterogeneous population and represent a spectrum of activated phenotypes with diverse functions [187, 188].

Macrophages play a pivotal role in I/R injury as well as allograft rejection [189, 190]. Studies in experiment transplant models have shown that macrophages accumulate in the transplanted graft early during I/R injury in kidney, liver, heart, and lung transplant recipients. Macrophages contribute to I/R damage through production of pro-inflammatory mediators, release of extracellular matrix-degrading enzymes, and activation of adaptive immunity. TLR signaling has been suggested to be important for macrophage-induced I/R injury [190, 191]. Macrophage infiltration is also associated with cell-mediated rejection as well as AMR [192–194]. During rejection, macrophages can cause damage to the

allograft presumably via production of ROS, RNS, and pro-inflammatory cytokines and induction of T-cell activation via antigen presentation and cytokine production [192]. Chronically activated macrophages are involved in the development of chronic allograft vasculopathy by promoting fibrosis [195]. Mechanisms include the activation of myofibroblasts and smooth muscle cells through production of pro-fibrotic mediators such as platelet-derived growth factor (PDGF) and TGF- β , the secretion of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs), and the induction of Th2 and Th17 immune responses [188].

Although macrophages clearly can play a graft-destructive role in transplantation, these cells can also have graft-protective functions. Macrophages are important for tissue repair following I/R injury [189]. In addition, these cells are involved in the inhibition and reversal of fibrosis through phagocytosis of dead cells and cellular debris and by the production of anti-fibrotic mediators such as IL-10 [188]. Furthermore, Mregs have been described that can suppress immune responses directed at the allograft [196]; these cells are further discussed in section "Immune Regulation." Whether intra-graft macrophages have a beneficial or detrimental effect during I/R injury and rejection most likely depends on environmental factors and the status of macrophage activation and differentiation.

Dendritic cells (DCs), named for their tree-like structure (dendron is the Greek word for tree), are a rare (~1 % of total lymphoid cells) cell population derived from monocytes and bone marrow progenitor cells. Unlike other innate immune cells, the primary function of DCs is not the frontline elimination of pathogens, but the activation and regulation of adaptive immunity through antigen presentation. Immature DCs have a high phagocytic capacity, expressing a large array of PRRs including TLRs, and circulate through the peripheral blood, tissues, and lymphoid organs in search of PAMPs or DAMPs. These cells express low levels of MHC and co-stimulatory molecules and are poor APCs. Following binding and internalization of patho-

gens or components of damaged tissue, immature DCs undergo maturation and transform into potent APCs, migrating to lymphoid tissues to present processed protein and lipid antigens to T-cells via upregulated MHC molecules and other nonclassical antigen-presenting molecules (CD1 family) [197]. DCs can also route captured antigens in non-degradative intracellular compartments back to the surface to present unprocessed antigens to B-cells [75]. In addition, DCs upregulate co-stimulatory molecules on their surface and produce various cytokines that activate and drive differentiation of T-cells and B-cells. In humans, DCs are most commonly categorized into myeloid DCs (mDCs) and plasmacytoid DCs (pDCs), but can be further divided into various subsets based on phenotype, localization, and function in vivo [198, 199]. These various subsets provide a highly heterogeneous APC population that shapes adaptive immune responses according to environmental cues. For example, mDCs can produce large quantities of IL-12 upon activation, which drives T-cell differentiation into a Th1 phenotype and B-cell differentiation into IgM-producing ASCs. In contrast, pDCs have a primary role in antiviral immune responses by secretion of type 1 IFN (IFN- α /IFN- β) after activation by viral components through TLR7 and TLR9 [198]. DCs can also activate innate immune cells, such as NK-cells and NKT-cells [197]. Besides their key role in immune activation, DCs are also essential for induction and maintenance of central and peripheral tolerance [200].

It is well accepted that DCs play a central role in the activation of alloantigen-specific T-cells after transplantation [201]. Donor "passenger" DCs that are transplanted with the allograft can migrate to the secondary lymphoid organs to present alloantigens to T-cells via the direct pathway. Recipient DCs can internalize and process cellular components from damaged or dead graft cells and present alloantigens via the indirect pathway. Furthermore, recipient DCs may acquire intact MHC-peptide complexes from donor cells and present them to T-cells via the semi-direct pathway [20]. Conversely, evidence is rising that DCs are also key components for the downregulation of immune responses directed at

the allograft. Experimental animal studies have demonstrated that both donor DCs and recipient DCs can contribute to the development of allograft tolerance [202–205]. DCs that promote tolerance, so-called tolerogenic DCs, are discussed in greater depth in section “Immune Regulation.”

FDCs, which are important for B-cell selection during GC reactions, differ from mDCs and pDCs in ontogeny, localization, and function [206]. Unlike mDCs and pDCs, FDCs are not derived from bone marrow progenitor cells, but are of mesenchymal origin. These cells are non-migratory cells that reside in the follicles of the B-cell areas of the secondary lymphoid organs. FDCs do not internalize and process antigens for presentation in the context of MHC molecules, but rather present antigens bound in immune complexes to B-cells via complement receptors CD21 and CD35. The role of FDCs in allograft rejection remains to be elucidated, but it seems plausible that these cells contribute to the selection of high-affinity alloantigen-specific ASCs and memory cells and promote the generation of DSAs.

NK-Cells

NK-cells are effector cells of the innate immune system that can kill target cells via the granzyme/perforin pathway as well as the Fas/FasL pathway (Fig. 5) without the need of prior activation, allowing for a fast immune response [207]. Human NK-cells can be subdivided into different subsets based on their expression of CD56 and CD16 (FCγRIII): the CD56^{dim}CD16^{high} subset is the more cytotoxic subset, whereas the CD56^{bright}CD16^{-/dim} subset is less cytotoxic, but produces large quantities of cytokines, such as IFN-γ, TNF-β, IL-13, and IL-10 [208]. The activation of NK-cells involves both inhibitory and activating receptors that include human killer cell immunoglobulin-like (KIR) receptors (homologue in rodents: Ly49 receptor family) and the conserved CD94/NKG2 receptor family [209]. The recognition of MHC class I molecules by inhibitory receptors prevents activation of NK-cells. Cells that are infected with pathogens, such as viruses, or are transformed (tumor cells)

downregulate expression of these molecules and can become targets for NK-cells. The absence of MHC class I molecules is generally not sufficient for NK-cell activation, and interaction of activating receptors with ligands that are expressed on potential target cells following infection, stress, or transformation is required. NKG2D is one of the best-characterized activating receptors and binds to stress-induced MICA and MICB in humans. When both MHC class I molecules and ligands for activating receptors are expressed on a cell, the NK-cell may be activated or inhibited depending on the strength of signals. NK-cell activation can also occur by engagement of CD16 to the Fc portion of antibodies bound to cells, resulting in ADCC, or by cytokines, including IL-12, IL-15, and IL-18 [207].

Graft-infiltrating NK-cells have been associated with acute and chronic rejection in both mice and humans [210–213]. NK-cells activated by IL-15 are themselves capable of mediating acute rejection in the absence of adaptive immune cells, presumably by inducing cell lysis through the granzyme/perforin pathway [214]. In humans, granzyme-expressing NK-cells have been detected in kidney allografts during acute cellular rejection [215]. Another mechanism by which NK-cells may mediate rejection is the secretion of pro-inflammatory cytokines and chemokines that promote lymphocyte recruitment to the graft [216, 217]. In addition, it was demonstrated that NK-cells contribute to acute rejection by providing help to T-cells in the absence of CD28 co-stimulation, promoting T-cell proliferation and cytokine production [218]. NK-cell-mediated T-cell help has also been proposed as a mechanism of cardiac allograft vasculopathy [212]. Furthermore, the development of antibody-mediated cardiac allograft vasculopathy was shown to be NK-cell dependent in mice [219]. In humans, graft infiltration of NK-cells was found to be associated with AMR in kidney transplants [220]. Whereas these data indicate a role for NK-cells in allograft rejection, several experimental transplant models have shown that these cells can also contribute to immune regulation and the induction of transplantation tolerance. Possible regulatory mechanisms include

perforin-mediated killing of donor APCs, thereby inhibiting the activation of alloreactive T-cells [221, 222], inhibition of homeostatic proliferation of CD8⁺ T-cells, likely through competition for IL-15 [223], and the production of the immunoregulatory cytokine IL-10 [224].

Immunologic Memory

After clearance of a foreign antigen, the vast majority of effector cells die through apoptosis (contraction phase of an immune response) and only a small number of these cells differentiate into memory cells that can survive great lengths of time (memory phase). This so-called immunologic memory allows the immune system to respond more rapidly and effectively to antigens it has encountered previously [225]. Immunologic memory has long been considered the hallmark of adaptive immunity, but recent studies have shown that innate immune cells, such as NK-cells and monocytes/macrophages, also exhibit memory-like characteristics [226]. Compared to naïve cells, memory cells have distinct phenotypic and functional characteristics. Typically, memory cells have a lower activation threshold, are more sensitive to a low dose of antigens, are less dependent on co-stimulation, and respond more vigorously than naïve cells. In humans, memory T-cells can be distinguished from naïve T-cells by the surface expression of different CD45 isoforms: naïve T-cells express CD45RA, whereas memory T-cells express CD45RO. Both memory CD4⁺ T-cells and CD8⁺ T-cells can be further divided into two subsets: central memory T (T_{cm})-cells and effector memory T (T_{em})-cells [227]. T_{cm}-cells express the homing receptors CCR7 and CD62L and primarily reside in secondary lymphoid organs. In contrast, T_{em}-cells do not express CCR7, but rather chemokine receptors and adhesion molecules that promote the migration to sites of inflammation. Whereas T_{em}-cells exhibit immediate effector function upon antigen reencounter, restimulated T_{cm}-cells show little effector function, but rapidly proliferate and differentiate into T_{em}-cells. The memory T-cell pool is maintained through slow

cell divisions, which are not dependent on antigen stimulation but on homeostatic cytokines, such as IL-7 and IL-15 [228].

B-cell memory consists of long-lived plasma cells and memory B-cells. Long-lived plasma cells continuously secrete antibodies to maintain the antibody levels in the periphery. Memory B-cells are quiescent cells that rapidly and strongly respond to BCR stimulation. Upon reactivation, these cells acquire the same functional properties as naïve B-cells, including clonal expansion, differentiation into ASCs or GC B-cells, and affinity maturation, but with faster kinetics [229]. In comparison to the naïve B-cell response, the memory B-cell response results in an increased production of antibodies with higher affinity and switched isotypes. In humans, memory B-cells have been defined by their expression of CD27, a member of the TNF receptor superfamily, yet CD27⁻ memory B-cells have also been described [230]. Like memory T-cells, the persistence of memory B-cells over long periods of time is independent of antigens [231]; however, the factors that maintain the memory B-cell pool are not yet elucidated.

Although immunologic memory plays an intrinsic part in the long-term protection against pathogens, the adverse role of immunologic memory in transplantation has been well recognized. Memory directed against alloantigens can be generated via antigen exposure at the time of blood transfusion, pregnancy, or a previous transplant. In addition, alloantigen-specific memory cells may also be induced by homeostatic proliferation under lymphopenic conditions or by heterologous immunity, a process in which a response to one antigen generates effector/memory cells with cross-reactive specificities to other unrelated antigens [232]. Several studies have shown that memory T-cells contribute to acute and chronic rejection [233], and the presence of these cells has been associated with poor transplant outcomes [234]. Notably, memory T-cells alone are sufficient to induce allograft rejection and do not require secondary lymphoid organs [235]. The presence of DSAs in sensitized patients is likely the product of long-lived plasma cells. The increase of DSA levels in sensitized

patients undergoing acute rejection shortly after transplantation suggests that alloantigen-specific memory B-cells exist and rapidly differentiate into ASCs [236]. Furthermore, continuous activation and differentiation of memory B-cells into plasma cells may result in gradual accumulation of DSAs, resulting in antibody-mediated chronic allograft injury [237]. Due to their unique characteristics, memory cells have distinct sensitivities to therapeutic agents compared to naïve cells and it is generally believed that current immunosuppressive regimens are less effective in containing memory responses [238, 239]. Novel strategies are being explored that specifically target memory cells; however, a major challenge remains in the selective inhibition of alloantigen-specific memory cells while maintaining protective ones.

Hematopoietic Stem Cell Transplantation

During HSCT, multipotent hematopoietic stem cells, generally derived from the bone marrow, peripheral blood, or umbilical cord blood, are transferred to a patient with a malignant or non-malignant blood or bone marrow disorder in order to restore marrow and immune function. If the source of the hematopoietic stem cells is allogeneic, complications may occur due to mismatches between transplantation antigens of the donor and recipient. Just like in solid organ transplantation, the patient's immune system may recognize the transplanted cells as foreign and reject the graft. In addition, the transplanted donor immunocompetent cells themselves may recognize antigens of the recipient as foreign and attack the host's body, a process termed GvHD. GvHD can be subdivided into **acute GvHD** (aGvHD) and **chronic GvHD** (cGvHD), and is discussed in detail in Chapter "Hematopoietic Stem Cell Transplantation".

The development of aGvHD consists of different phases that provide positive feedback loops to maintain the process [240, 241]. Myelosuppressive and immunosuppressive conditioning treatments to remove or destroy the host's cells and make space for the graft can cause tissue damage and

induce inflammation in the host. Studies have shown that damage to the gastrointestinal tract plays a major role in the amplification of aGvHD [242]. The release of PAMPs, such as bacterial lipopolysaccharide (LPS) or bacterial CpG DNA repeats, activates innate immune cells that contribute to tissue damage and the production of large quantities of pro-inflammatory cytokines, thereby activating other innate and adaptive immune cells. Patients treated with non-myeloablative condition regimens were found to have reduced incidences of aGvHD [243]. Studies in experimental transplant models have shown that both donor and recipient hematopoietic APCs as well as recipient non-hematopoietic APCs, such as parenchymal tissue cells, are pivotal for the development of aGvHD [244–246]. APCs activate T-cells that can cause tissue damage via cytotoxic pathways [247, 248]. In addition, activated T-cells also produce pro-inflammatory cytokines, further amplifying the pro-inflammatory cytokine environment. The overproduction of pro-inflammatory cytokines, also known as cytokine storm, provides a positive feedback loop to the innate and adaptive immune cells. If this process is not controlled, it can result in organ damage. The primary organs affected in the acute process are the skin, liver, and gastrointestinal tract, although other sites may be affected [240].

The immunologic processes of cGvHD differ from those of aGvHD; whereas acute GvHD resembles a toxic, sepsis-like syndrome, cGvHD appears more as an autoimmune-like systemic syndrome [241, 249]. Th2 cell responses seem to predominate in cGvHD, although Th1 responses may also contribute [250, 251]. Th2 cytokines recruit macrophages and other effector cells that produce fibrogenic cytokines such as TGF- β and PDGF to the site of inflammation where they can promote the proliferation and activation of tissue fibroblasts. Eosinophils, which are activated by the Th2 cytokine IL-5, may also contribute to fibrotic processes in cGvHD; increased numbers of eosinophils were detected in children with cGvHD [252]. Furthermore, B-cell dysregulation appears to be a factor for the development of cGvHD. The presence of autoantibodies was found to be elevated in patients with cGvHD

[253], suggesting an emergence of self-reactive B-cells. B-cell activation may be due to the high levels of BAFF that are present during cGvHD [254]. Other factors that can promote the development of cGvHD are damage to the thymus caused by conditioning regimens and/or aGvHD which can lead to dysregulation of central tolerance mechanisms during the reconstitution of the immune system and decreased numbers of Tregs [249]. cGvHD can occur in almost any organ, but mainly affects oral and ocular mucosal surfaces and the skin, lungs, kidneys, liver, and gut [241, 255].

A beneficial graft-versus-host response in HSCT is the graft-versus-leukemia (GvL) response, in which the donor cells recognize and eliminate tumor cells. Studies in experimental transplant models have shown that both CD4+ and CD8+ T-cells play an important role in this process [256, 257]. In clinical transplantation, patients receiving bone marrow grafts depleted of T-cells were found to have a higher incidence of relapse of leukemia [258]. T-cell mechanisms that mediate GvL reactivity include elimination of tumor cells via cytotoxic pathways and cytokine production [259]. Other cells that contribute to the GvL response are NK-cells [260]. Donor-derived NK-cells can become activated when they express inhibitory receptors specific for a donor KIR epitope that is not expressed by the host cells. NK-cell reactivity can eliminate leukemia relapse and protect patients against GvHD in clinical transplantation [261].

Immune Regulation and Tolerance in Transplantation

Immune regulation is an imperative part of the immune system for the control of inflammatory responses, maintenance of immune homeostasis, and prevention of autoimmune reactivity. Several central and peripheral mechanisms are in place to ascertain that the immune system properly distinguishes between self and foreign and to avoid over-reactivity to pathogens. Under certain conditions, these mechanisms induce **immune tolerance**, a state of unresponsiveness to substances

that have the capacity to elicit an immune response. Of note, immune tolerance is not a deficiency of the immune system to respond to a specific antigen, but rather an active process to prevent reactivity to that particular antigen. Immune tolerance can be divided into **central tolerance**, which occurs during lymphocyte development in primary lymphoid organs, and **peripheral tolerance**, which is established in the periphery to control mature lymphocytes. Since the generation of TCRs and BCRs occurs through a random rearrangement of the receptor genes, central and peripheral tolerance mechanisms are crucial for the elimination of self-reactive T-cells and B-cells that inevitably develop during this process.

Central tolerance involves deletion of self-reactive clones during lymphocyte development. Maturing T-cells are exposed to self-antigens in the thymic medulla through presentation by thymic DCs and medullary thymic epithelial cells (mTECs) [262]. Self-antigens are imported into the thymus via circulation or are presented via endogenous expression. mTECs can synthesize and express tissue-specific antigens, which is regulated by the autoimmune regulator (AIRE) protein [262]. T-cell clones with high affinity for self-antigens are eliminated by induction of apoptosis. High-affinity self-reactive immature B-cells are eliminated during development in the bone marrow by receptor editing or clonal deletion [263]. Recognition of self-antigens results in a halt of B-cell maturation, internalization of the BCR, and reactivation of the Ig gene rearrangement program, leading to the expression of a new BCR. Failure to generate a non-self-reactive functional BCR will induce cell death through the upregulation of pro-apoptotic factors and inhibition of B-cell survival factors of the BCL-2 family. These central tolerance mechanisms, however, do not remove all self-reactive cells. Cells that bear receptors with high avidity for self-antigens that are not expressed in the thymus or bone marrow as well as those with receptors that are of sufficient low avidity for self-antigens may escape from the primary lymphoid organs. For these cells, various **peripheral tolerance** mechanisms are in place to ensure immune tolerance [264]. T-cells that

recognize self-antigens in the periphery in the absence of co-stimulatory molecules may be deleted through apoptosis or enter a state of functional inactivity (anergy). Immature B-cells that have high-avidity interactions with self-antigens may be deleted, whereas those with low-avidity interactions may become anergic. Ignorance (unawareness) is a state of non-reactivity to self-antigens due to physical separation of self-reactive cells and their target antigens (i.e., immunoprivileged sites such as the testes or anatomical barriers such as the blood-brain barrier). Furthermore, peripheral cells with regulatory properties may actively suppress self-reactive cells.

In 1945, Owen [265] was one of the first to show that immune tolerance can be induced not only to self-antigens but also to non-self-antigens; he observed the coexistence of two types of erythrocytes in the blood of nonidentical (dizygotic) twin cattle that shared a placenta. These observations were confirmed by Billingham, Brent, and Medawar [266], who showed that tolerance to non-self-antigens can be induced by injections of foreign cells into fetal mice and chick embryos. It is the work by Medawar (who is considered the “father of transplant immunology”) together with the theories of immune tolerance formulated by Burnet and Fenner that form the fundamental basis of induced immune tolerance [267]. Inducing immune tolerance to transplants, a state of unresponsiveness to the allograft in a competent immune system without immunosuppression, is an ultimate goal in transplantation. This objective has been proven to be a major challenge. The allograft contains a vast array of different antigens to which the immune system can respond; tolerance may develop to some antigens, but not others, possibly through different mechanisms. Furthermore, tolerance may occur despite immunosuppression; recognition of immunosuppressed patients who have established tolerance to their allograft is complicated due to the lack of suitable tools to identify them. Although rare, operational tolerance, generally defined as stable graft function without clinical features of chronic rejection and in the absence of any immunosuppressive drugs [268], has been observed in transplant recipients after successful

intentional weaning of immunosuppression (more frequently in liver transplant recipients than other organ recipients) and in non-adherent patients who have stopped their medication [269, 270]. Furthermore, certain strategies may induce tolerance, such as combined kidney/bone marrow transplantation with non-myeloablative conditioning or total lymphoid irradiation [271, 272]. Whereas these findings indicate that transplant tolerance is clinically possible, reliable induction of long-term tolerance to allografts is still elusive. Knowledge of immunoregulatory mechanisms that can induce immune tolerance to transplants is key to influence the balance between rejection and acceptance.

Deletion

Deletion of alloreactive cells would be a very effective mechanism to protect the graft from rejection. In experimental transplant models, the phenomenon of central tolerance has been exploited to induce tolerance to allografts. Direct delivery of alloantigens in the thymus has been shown to delete thymocytes expressing alloantigen-specific TCRs and to induce donor-specific unresponsiveness [273, 274]. In addition, tolerance to donor antigens has been achieved through intrathymic deletion of donor-reactive T-cells in mixed chimeras prepared with a non-myeloablative conditioning regimen and allogeneic bone marrow transplantation [275, 276]. Other studies have provided evidence that mechanisms of peripheral deletion of donor-reactive T-cells are crucial for the induction of transplant tolerance [277]. B-cell tolerance can be induced by mixed chimerism strategies, presumably via either deletion or anergy or by both mechanisms [278]. In the clinical setting, donor-specific B-cell tolerance has been demonstrated in infant recipients of ABOi hearts: these recipients remained selectively deficient in antibody production to the donor blood group over time after transplantation, while non-self, non-donor ABO antibodies developed normally [279]. Tolerance in this setting may occur through deletion of donor-reactive B-cells.

Regulatory Immune Cells

Regulatory immune cells are important components for the maintenance of immune homeostasis and the downregulation of immune reactivity. Besides the control of autoimmunity, these cells can also regulate immune responses directed against alloantigens and could therefore be key players in the induction of transplant tolerance. Regulatory cell subsets have been found within both adaptive and innate immune cells.

Regulatory T-Cells

T-cell subsets with regulatory properties have been described within various T-cell populations, including CD4⁺ T-cells, CD8⁺ T-cells, CD4-CD8- double-negative (DN) T-cells, and $\gamma\delta$ T-cells [280]. Although all these regulatory subsets may potentially contribute to the control of alloimmune responses, attention in transplantation has particularly focused on CD4⁺ T-cells that constitutively express CD25, the IL-2 receptor α -chain, and FOXP3, a transcription factor important for their development, maintenance, and function [281, 282]. These CD4⁺CD25⁺FOXP3⁺ Tregs can be divided into two subsets: Tregs that develop in the thymus during a multistep progression involving TCR recognition, APCs, co-stimulatory molecules, and cytokines [283] and, as described above, those that develop in the periphery from CD4⁺ T-cells in the presence of Treg-inducing factors, such as TGF- β [58]. The phenotypic characteristics of thymus-derived and peripheral-derived Tregs are very similar and it is difficult to distinguish between these subsets in the periphery. The mechanisms by which CD4⁺CD25⁺FOXP3⁺ Tregs mediate their suppressive function can be subdivided into four different “modes of action,” including suppression by inhibitory cytokines, cytotoxicity, metabolic disruption, and modulation of DC maturation or function [284]. Numerous studies have shown that the balance of graft-destructive T-cells and Tregs is critical for the control of alloimmune responses and the induction of tolerance [285]. Adoptive transfer of Tregs can prevent both acute and chronic rejection [286] and GvHD [287]. In the clinical setting,

lower frequencies and inadequate regulatory function of Tregs have been observed in the peripheral blood of both acute and chronic rejecting organ transplant patients compared with clinically stable ones [288]. Although these findings may imply that a stable clinical condition is possibly due to a maintained phenomenon of immune regulation that is lacking in patients with rejection, it is also possible that Tregs in rejecting transplant patients migrate to other compartments, such as the transplanted organ itself, to exert their suppressive function locally. Various studies have found higher FOXP3 expression levels in rejecting grafts compared to clinically stable grafts, suggesting that FOXP3⁺ cells may not prevent rejection but are rather involved in “damage control” [289]. High FOXP3 expression levels during acute rejection were associated with successful reversal of the rejection episode [290]. The fact that Tregs may not be able to prevent graft rejection in the clinical setting is likely due to the relatively small number of these cells compared to the high frequency of effector cells. Furthermore, it has been shown in an experimental transplant model that memory T-cells are resistant to regulation by Tregs [291]. Thus, the presence of preexisting alloantigen-specific memory cells at time of transplant may tip the balance away from regulation by Tregs toward graft destruction. Using strategies that increase the frequency or function of Tregs, either in vivo or via expansion in vitro followed by adoptive transfer, may shift the balance in favor of regulation of alloimmune responses and acceptance of the graft [280].

NKT-Cells

NKT-cells are a conserved subpopulation of $\alpha\beta$ T-cells expressing NK lineage receptors that are restricted to the recognition of glycolipid antigens presented by the non-polymorphic MHC class I-like molecule CD1d [292]. Although various NKT-cell subsets exist, the most studied and best-characterized subset is the invariant NKT (iNKT)-cell subset, which is defined by the expression of a semi-invariant TCR composed of a V α 24-J α 18 chain associated with a V β 11 chain in humans and V α 14-J α 18 with V β 8, V β 7, and

V β 2 in mice. In humans, iNKT-cells can be CD4+, CD8+, or DN, while these cells are exclusively CD4+ or DN in mice. Upon activation, NKT-cells can produce large quantities of pro- and anti-inflammatory cytokines, including IFN- γ , IL-4, IL-13, and IL-10, that can affect both innate and adaptive immune responses. Although there are some data showing that NKT-cells may contribute to graft damage [293, 294], more evidence suggests that NKT-cells play a protective role in transplantation. Several experimental transplant model studies have shown that NKT-cells are essential for the induction of tolerance [295–297]. The beneficial role of NKT-cells in prolonging graft survival has been attributed to their IL-10 production [298].

Regulatory B-Cells

Although it was long considered that B-cells mainly have a graft-destructive role in transplantation by differentiating into ASCs and producing allospecific antibodies, recent studies have demonstrated that B-cells can also contribute to the regulation of alloimmune responses and the induction of tolerance [299]. Experimental transplant models have shown that adoptive transfer of B-cells can induce long-term acceptance of allografts [300]. In addition, B-cells appear to be critical for certain protocols that induce tolerance, such as anti-CD45RB and/or anti-TIM-1 treatment [301, 302]. Ligation of TIM-1 induced TIM-1+ Bregs that could transfer long-term acceptance of islet allografts in an IL-10-dependent manner [303]. In the clinical setting, B-cells may be involved in tolerance mechanisms in operational tolerant patients. Kidney transplant patients with stable graft function in the absence of immunosuppressive medication had increased numbers of B-cells in their peripheral blood as well as enhanced expression of B-cell differentiation and activation genes compared with subjects receiving immunosuppression [304–306]. Moreover, B-cells from operational tolerant patients produced more IL-10 than those from immunosuppressed patients with stable graft function, suggesting potential regulatory properties of B-cells in tolerant patients [307]. Whether B-cells are responsible for the induction

of tolerance in these operational tolerant patients or whether they contribute to the maintenance of tolerance in the absence of immunosuppression remains to be elucidated.

Regulatory Macrophages

Mregs are a distinct subset of macrophages with immunoregulatory properties. In culture, these cells can be induced by a wide range of different stimuli, including IL-10, IFN- γ , prostaglandins, immune complexes, and macrophage colony-stimulating factor (M-CSF) [187, 196]. In addition, interaction with Tregs or B-1 cells can induce macrophages to acquire Mreg-like characteristics [308, 309]. Mregs produce large quantities of the inhibitory cytokine IL-10, which can suppress Th1 and Th2 immune responses and promote the differentiation of IL-10-producing Tregs. Mregs are currently being explored as a cell-based therapy in solid organ transplantation. In a pilot clinical study, two living-donor kidney transplant recipients were reduced to low-dose tacrolimus monotherapy with excellent graft function after treatment with *in vitro* cultured Mregs, indicating therapeutic potential of these cells [196, 310].

Tolerogenic Dendritic Cells

Besides their key role in immune activation, it has become clear that DCs are also crucial players in the downregulation of immune responses and the induction of tolerance. Initially it was thought that the state of maturity determined whether a DC can stimulate or regulate an alloimmune response, with an immature state being tolerogenic. Indeed, studies have shown that immature mDC either derived from the donor or the recipient can prolong graft survival and promote transplant tolerance in experimental transplant models [311–313]. Immature, maturation-resistant tolerogenic DCs can be generated with low doses of granulocyte-macrophage colony-stimulating factor (GM-CSF) in the absence of IL-4 or by pharmacological manipulation with rapamycin, dexamethasone, or vitamin D3 [314–317]. However, it has been shown that mature donor-derived mDCs also have the ability to regulate alloimmune reactivity, suggesting that DC

maturation does not distinguish between immunostimulatory and immunoregulatory DCs [318]. The suppressive mechanisms of mDCs include clonal deletion, induction of Tregs, and inhibition of effector and memory T-cell responses as well as B-cell responses [319]. pDCs can also promote tolerance in transplantation; preoperative infusion of pDCs has been shown to induce allogeneic T-cell hyporesponsiveness and prolong graft survival [320, 321]. pDCs can acquire alloantigens in the graft and home to lymph nodes, where they mediate alloantigen-specific Treg development [322]. In the clinical setting, higher ratios of peripheral pDCs to mDCs were found in pediatric liver transplant recipients who had been successfully withdrawn from immunosuppression and those undergoing prospective drug weaning compared with patients on maintenance immunosuppression [323]. Furthermore, high PD-L1/CD86 ratios on pDC were found to correlate with elevated Treg frequency in operationally tolerant pediatric liver transplant recipients [324]. Clinical trials involving tolerogenic DCs for patients with cancer have been ongoing for over a decade, but the use of DCs as a tolerogenic therapy is now also being investigated in transplantation [325].

Myeloid-Derived Suppressor Cells

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population composed of progenitor cells of macrophages, granulocytes, DCs, and immature myeloid cells. Despite their heterogeneity, most MDSCs express common phenotypic markers, such as CD33 and low levels of MHC class II molecules in humans and Gr-1 and CD11b in mice [326]. The expansion and activation of MDSCs are regulated by factors produced by activated T-cells, stromal cells, and tumor cells. Activated MDSCs can suppress proliferation and cytokine production of B-cells, T-cells, and NK-cells. Mechanisms of suppression include activation of iNOS and arginase-1 and via the production of ROS. Although it has been demonstrated that MDSCs can mediate the development of Tregs [327], it has also been shown that MDSCs can suppress the generation of peripheral-derived Tregs [328]. The regulatory

role of MDSCs has been well-studied in cancer immunobiology, but evidence is now also emerging in transplantation from experimental transplant models demonstrating their contribution to the regulation of immune responses to alloantigens and the induction of tolerance [329, 330].

Summary

The immune system has evolved into an extraordinary system with intricate and dynamic mechanisms to protect the host from substances that are foreign or malignant. Transplantation of allogeneic or xenogeneic cells, tissues, or organs typically results in rejection of the transplanted graft by the recipient's immune system. In HSCT, the transplanted immunocompetent cells themselves can also attack the antigens of the host's body, a process termed GvHD. In addition, the physical process of cell or organ procurement and transplantation can induce thermal and metabolic stress responses in the graft, which result in cell damage and death. The subsequent release of cellular components can also activate the immune system.

Antigens that are involved in graft rejection include MHC molecules (HLA), ABO blood group antigens, minor histocompatibility antigens, and autoantigens.

The immune system is divided into the adaptive immune system and the innate immune system. The adaptive immune system is composed of T-cells and B-cells, specialized lymphocytes that express receptors specific to antigens. Both cell types contribute to transplant rejection and graft dysfunction, but can also regulate allo-immune responses.

T-cells express TCRs that recognize foreign antigens presented as peptide fragments in the context of self-MHC molecules. Allorecognition by TCRs can occur via the direct pathway, the indirect pathway, and the semi-direct pathway. Binding of the TCR to its specific MHC-peptide complex (signal 1) together with interaction of co-stimulatory receptors on T-cells with their ligands

expressed on APCs (signal 2) induce T-cell activation. The co-receptors CD4 and CD8, specific for MHC class II and class I, respectively, increase the binding affinity. Binding of cytokines to their receptors on T-cells delivers signal 3, triggering T-cell proliferation. T-cell activation is followed by differentiation into effector T-cells, which include cytotoxic T-cells, Th1 cells, Th2 cells, Th17 cells, Tfh cells, Th9 cells, and Tregs. Cytotoxic CD4+ and CD8+ T-cells can eliminate donor cells via the Fas/FasL pathway and the granzyme/perforin pathway.

B-cells express BCRs that recognize antigens in their native or unprocessed form. B-cells can be activated by TI pathways via TLR stimulation (TI type 1 activation) or cross-linking of BCRs (TI type 2 activation) or by a TD pathway via T-cell help. Upon TD activation, B-cells either differentiate into short-lived ASCs or undergo clonal expansion, SHM, and isotype switching in GCs to develop into long-lived ASCs or memory B-cells. Other B-cell subsets include MZ B-cells, B-1 cells, and Bregs. Antibodies fix complement and activate innate cells. Besides production of DSAs, B-cells may contribute to graft rejection through antigen presentation and cytokine production.

The innate immune system is composed of innate factors such as complement, TLRs, and innate immune cells that form the first line of defense against pathogens and respond to damaged or stressed cells. Innate immunity plays a pivotal role in I/R injury as well as graft rejection.

The complement system consists of soluble proteins that assist the immune system by marking cells for elimination, agglutinating pathogens, disrupting cell membranes via pore formation, attracting innate immune cells, and modulating adaptive immune cells. Activation of complement can occur via the classical pathway, the lectin pathway, or the alternative pathway.

TLRs function as PRRs and are expressed on various cells, including macrophages, DCs, mast cells, B-cells, endothelial cells, and epithelial cells. TLRs recognize PAMPs derived from pathogens and DAMPs derived from endogenous cell components.

Innate immune cells are not antigen specific, but respond to PAMPs and DAMPs. These cells include granulocytes, monocytes, macrophages, DCs, and NK-cells. Innate immune cells can play a role in I/R injury as well as rejection.

Granulocytes, which include neutrophils, eosinophils, basophils, and mast cells, are characterized by granules in the cytosol that contain various proteins such as proteolytic enzymes, toxins, and cytokines. These cells are the first line of cytotoxic cells, but can also modulate adaptive immunity by production of cytokines and chemokines as well as antigen presentation.

Monocytes are mononuclear phagocytes that are typically the first cells to enter the inflamed site and contribute to the initiation of an immune response through phagocytosis, production of ROS, and secretion of cytokines. Monocytes can differentiate into either macrophages or DCs.

Macrophages are important for the activation and modulation of immune responses, various physiological processes, such as tissue development, homeostasis, and repair, and pathologic processes, such as tumor development and fibrosis.

Dendritic cells are important for the activation and regulation of adaptive immunity. Immature DCs have a high phagocytic capacity. Upon binding and internalization of pathogens or components of damaged cells, immature DCs undergo maturation and transform into professional APCs. Both donor and recipient DCs can play a

central role in the activation of alloantigen-specific T-cells after transplantation.

NK-cells can kill target cells via the granzyme/perforin pathway and the Fas/FasL pathway. Activation of NK-cells involves inhibitory receptors and activating receptors. Cells that have downregulated expression of MHC class I molecules can become targets for NK-cells.

Immunologic memory allows the immune system to respond more rapidly and effectively to antigens it has encountered previously. Although immunologic memory is important for the long-term protection against pathogens, memory against alloantigens has a detrimental role in transplantation.

Induction of immune tolerance, a state of unresponsiveness to the allograft in a competent immune system without immunosuppression, is the ultimate goal in transplantation. Immune regulatory mechanisms that may induce tolerance to transplants include deletion and regulatory immune cells, such as Tregs, NKT-cells, Bregs, Mregs, tolerogenic DCs, and MDSCs.

In conclusion, both adaptive and innate immunity contribute to allograft rejection and graft dysfunction. The dynamic cross-talk between the adaptive and innate immune system requires further understanding of both systems in order to be able to regulate alloimmune responses and to induce transplant tolerance. Using strategies that target adaptive and/or innate immunostimulatory mechanisms and promote immunoregulatory mechanisms may shift the balance from rejection to regulation of alloimmune responses and acceptance of the graft.

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