SECTION ONE

Immunology and Genetics

1 The Immune Response to Organ Allografts

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Organ transplantation has benefited significantly from advances in immunology and molecular biology. A relatively young scientific discipline, immunobiology of organ transplantation is the quientessential example of translational science that has resulted in truly life-saving remedies for those afflicted with irreparable end-organ failure. There are several commonalities in the immune response to cellular and solid organ allografts, and the essential principles are reviewed in this chapter.

T-CELL SURFACE PROTEINS, ANTIGEN RECOGNITION AND SIGNAL TRANSDUCTION

The antigen recognition complex is comprised of the clone specific T-cell antigen receptor (TCR) α and β heterodimer that is responsible for the recognition of the antigenic peptide displayed in the groove of major histocompatibility complex (MHC) encoded proteins, and the clonally invariant CD3 complex responsible for signal transduction (Table 1.1) [1–5]. Whereas the majority of peripheral blood T cells display TCR α and β heterodimer on their cell surface, a minority expresses TCR γ and δ chains.

The T-cell surface is also decorated with lineage specific and functional proteins that contribute to the immune synapse between the T cells and the antigen presenting cells (APCs). Peripheral blood T cells express either the CD4 protein or the CD8 protein on their cell surface and the CD4 and CD8 proteins bind nonpolymorphic domains of human leukocyte antigen (HLA) class II (DR, DP, DQ) and class I (A, B, C) molecules, respectively, and contribute to the associative recognition process termed MHC restriction. Kinetic models of the immune synapse suggest that a critical threshold of

Table 1.1 Cell-surface proteins important for T-cell activation.* (Reproduced from Suthanthiran *et al.* [52] with permission.)

T-cell surface	APC surface	Functional response	Consequence of blockade
LFA-1 (CD11a, CD18)	ICAM (CD54)	Adhesion	Immunosuppression
ICAM1 (CD54)	LFA-1 (CD11a, CD18)		
CD8, TCR, CD3	MHCI	Antigen recognition	Immunosuppression
CD4, TCR, CD3	MHCII		
CD2	LFA3 (CD58)	Costimulation	Immunosuppression
CD40L (CD154)	CD40		
CD5	CD72		
CD28	B7-1 (CD80)	Costimulation	Anergy
CD28	B7-2 (CD86)		
CTLA4 (CD152)	B7-1 (CD80)	Inhibition	Immunostimulation
CTLA4 (CD152)	B7-2 (CD86)		

APC, antigen-presenting cell; ICAM, intercellular adhesion molecule; LFA, leukocyte function-associated antigen; MHC, major histocompatibility complex.

* Receptor/counter-receptor pairs that mediate interactions between T cells and APCs are shown in this table. Inhibition of each protein-to-protein interaction, except the CTLA4–B7-1/B7-2 interaction results in an abortive *in vitro* immune response. Initial contact between T cells and APCs requires an antigen-independent adhesive interaction. Next, the T-cell antigen receptor complex engages processed antigen presented within the antigen-presenting groove of MHC molecules. Finally, costimulatory signals are required for full T-cell activation. An especially important signal is generated by B7-mediated activation of CD28 on T cells. Activation of CD28 by B7-2 may provide a more potent signal than activation by B7-1. CTLA4, present on activated but not resting T cells, imparts a negative signal.

TCR to MHC-peptide engagements is obligatory to stabilize the TCR/peptide physical contacts and the redistribution of cell surface proteins. An important consequence is the co-clustering of the TCR/CD3 complex with the T-cell surface proteins that include integrins such as leukocyte function-associated antigen 1 (LFA-1) and nonintegrins such as CD2 [6–8].

The immunologic synapse consists of a multiplicity of T-cell surface protein forms and clusters, thereby creating a platform for antigen recognition and generation of various crucial T-cell activation-related signals. The synapse begins to form when the initial adhesions between T-cell surface proteins and APC surface proteins are formed. These adhesions create intimate contact between T cells and APCs and thereby provide an opportunity for T cells to recognize antigen. Antigen-driven T-cell activation, a tightly regulated, preprogrammed process, begins when T cells recognize intracellularly processed fragments of foreign proteins (approximately 8–16 amino acids) embedded within the groove of the MHC proteins expressed on the surface of APCs. Some recipient T cells directly recognize the allograft (i.e. donor antigen(s) presented on the surface of donor APCs), while other T cells recognize the donor antigen after it is processed and presented by self-APCs [9].

Following activation by antigen, the TCR/CD3 complex and co-clustered CD4 and CD8 proteins are physically associated with intracellular protein–tyrosine kinases (PTKs) of two different families, the src (including $p59^{fyn}$ and $p56^{lck}$) and

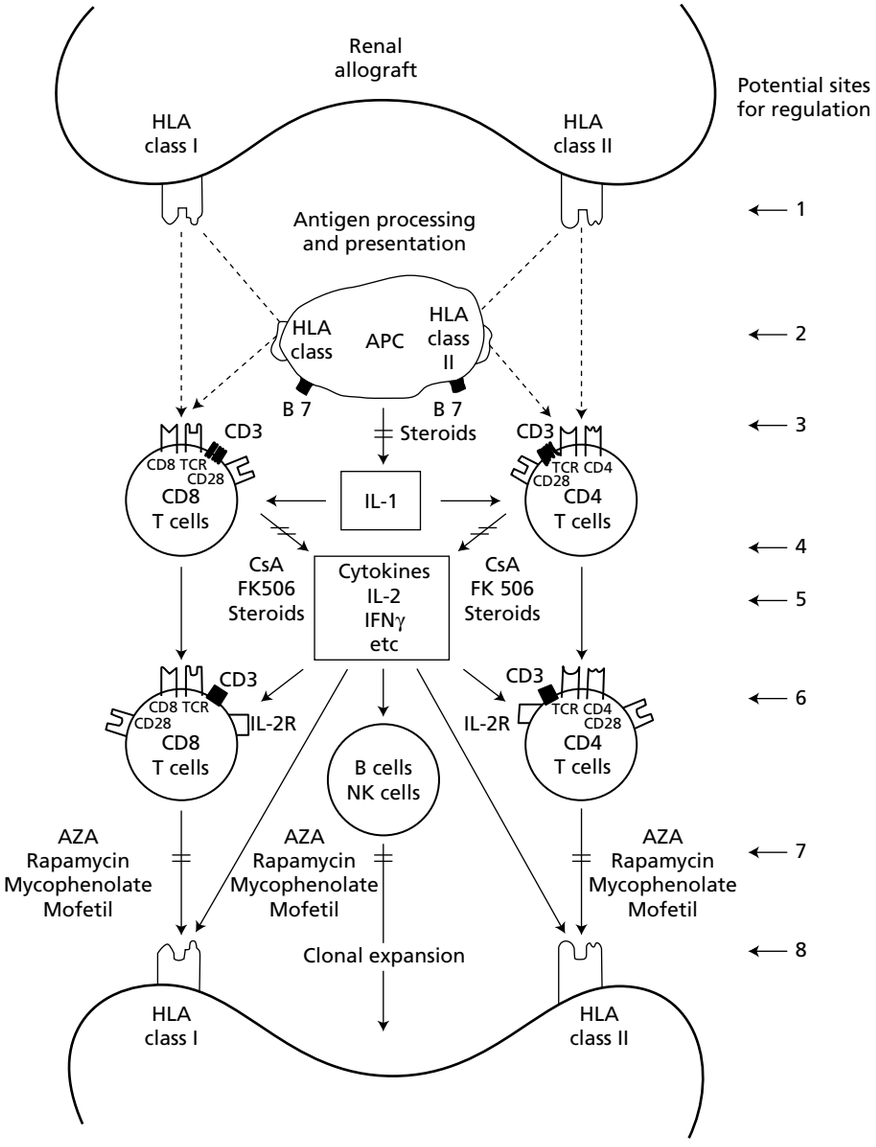

Fig. 1.1 The antiallograft response. Schematic representation of human leukocyte antigens (HLA), the primary stimuli for the initiation of the antiallograft response; cell surface proteins participating in antigenic recognition and signal transduction; contribution of the cytokines and multiple cell types to the immune response; and the potential sites for the regulation of the antiallograft response. Site 1: Minimizing histoincompatibility between the recipients and the donor (e.g. HLA matching). Site 2: Prevention of monokine production by antigen-presenting cells (e.g. corticosteroids). Site 3: Blockade of antigen recognition (e.g. OKT3 mAbs). Site 4: Inhibition of T-cell cytokine production (e.g. cyclosporin A [CsA]). Site 5: Inhibition of cytokine activity (e.g. anti-interleukin-2 [IL-2] antibody). Site 6: Inhibition of cell cycle progression (e.g. anti-IL-2 receptor antibody). Site 7: Inhibition of clonal expansion (e.g. azathioprine [AZA]). Site 8: Prevention of allograft damage by masking target antigen molecules (e.g. antibodies directed at adhesion molecules). HLA class I: HLA-A, B and C antigens; HLA class II: HLA-DR, DP and DQ antigens. IFNγ, γ-interferon; NK cells, natural killer cells. (Reproduced from Suthanthiran *et al.* [51] with permission.)

Table 1.2 Cellular elements contributing to the antiallograft response. (Reproduced from Suthanthiran *et al*. [52] with permission.)

Cell type	Functional attributes
T cells	The $CD4^+$ T cells and the $CD8^+$ T cells participate in the antiallograft response. $CD4^+$ T cells recognize antigens presented by HLA class II proteins, and $CD8^+$ T cells recognize antigens presented by HLA class I proteins. The CD3/TCR complex is responsible for recognition of antigen and generates and transduces the antigenic signal
$CD4^+$ T cells	$CD4^+$ T cells function mostly as helper T cells and secrete cytokines such as IL-2, a T-cell growth/death factor, and IFNγ, a proinflammatory polypeptide that can upregulate the expression of HLA proteins as well as augment cytotoxic activity of T cells and NK cells. Recently, two main types of $CD4^+$ T cells have been recognized: $CD4^+$ Th1 and $CD4^+$ Th2. IL-2 and IFNγ are produced by $CD4^+$ Th1 type cells, and IL-4 and IL-5 are secreted by $CD4^+$ Th2 type cells. Each cell type regulates the secretion of the other, and the regulated secretion is important in the expression of host immunity
$CD8^+$ T cells	$CD8^+$ T cells function mainly as cytotoxic T cells. A subset of $CD8^+$ T cells expresses suppressor cell function. $CD8^+$ T cells can secrete cytokines such as IL-2, IFNγ, and can express molecules such as perforin, granzymes that function as effectors of cytotoxicity
APCs	Monocytes/macrophages and dendritic cells function as potent APCs. Donor's APCs can process and present donor antigens to recipient's T cells (direct recognition) or recipient's APCs can process and present donor antigens to recipient's T cells (indirect recognition). The relative contribution of direct recognition and indirect recognition to the antiallograft response has not been resolved. Direct recognition and indirect recognition might also have differential susceptibility to inhibition by immunosuppressive drugs
B cells	B cells require T-cell help for the differentiation and production of antibodies directed at donor antigens. The alloantibodies can damage the graft by binding and activating complement components (complement-dependent cytotoxicity) and/or binding the Fc receptor of cells capable of mediating cytotoxicity (antibody-dependent, cell-mediated cytotoxicity)
NK cells	The precise role of NK cells in the antiallograft response is not known. Increased NK cell activity has been correlated with rejection. NK cell function might also be important in immune surveillance mechanisms pertinent to the prevention of infection and malignancy

APCs, antigen presenting cells; IFN, interferon; IL, interleukin; NK, natural killer; TCR, T-cell antigen receptor.

ZAP-70 families. The CD45 protein, a tyrosine phosphatase, contributes to the activation process by dephosphorylating an autoinhibitory site on the $p56^{lck}$ PTK. Intracellular domains of several TCR/CD3 proteins contain activation motifs that are crucial for antigen-stimulated signaling. Certain tyrosine residues within these motifs serve as targets for the catalytic activity of src family PTKs. Subsequently, these phosphorylated tyrosines serve as docking stations for the SH2 domains (recognition structures for select phosphotyrosine-containing motifs) of the ZAP-70 PTK. Following antigenic engagement of the TCR/CD3 complex, select serine residues of the TCR and CD3 chains are also phosphorylated.

The wave of tyrosine phosphorylation triggered by antigen recognition encompasses other intracellular proteins and is a cardinal event in initiating T-cell activation. Tyrosine phosphorylation of the phospholipase $C\gamma_1$ activates this coenzyme and triggers a cascade of events that lead to full expression of T-cell programs: hydrolysis of phosphatidylinositol 4,5-biphosphate (PIP_2) and generation of two intracellular messengers, inositol 1,4,5-triphosphate (IP_3) and diacylglycerol [10]. IP_3, in turn, mobilizes ionized calcium from intracellular stores, while diacylglycerol, in the presence of increased cytosolic free Ca^{2+}, binds to and translocates protein kinase C (PKC) – a phospholipid/Ca^{2+}-sensitive protein serine/threonine kinase – to the membrane in its enzymatically active form. Sustained activation of PKC is dependent on diacylglycerol generation from hydrolysis of additional lipids such as phosphatidylcholine.

The increase in intracellular free Ca^{2+} and sustained PKC activation promote the expression of several nuclear regulatory proteins (e.g. nuclear factor of activated T cells [NF-AT], nuclear factor kappa B [NF-κB], activator protein 1 [AP-1]) and the transcriptional activation and expression of genes central to T-cell growth (e.g. interleukin-2 [IL-2] and receptors for IL-2 and IL-15).

Calcineurin, a Ca^{2+}- and calmodulin-dependent serine/threonine phosphatase, is crucial to Ca^{2+}-dependent, TCR-initiated signal transduction [11]. Inhibition by cyclosporine and tacrolimus (FK506) of the phosphatase activity of calcineurin is considered central to their immunosuppressive activity [12,13].

Allograft rejection is contingent on the coordinated activation of alloreactive T cells and APCs (Fig. 1.1 and Table 1.2). Through the intermediacy of cytokines and cell-to-cell interactions, a heterogeneous contingent of lymphocytes, including $CD4^+$ helper T cells, $CD8^+$ cytotoxic T cells, antibody-forming B cells, and other proinflammatory leukocytes are recruited into the antiallograft response [14].

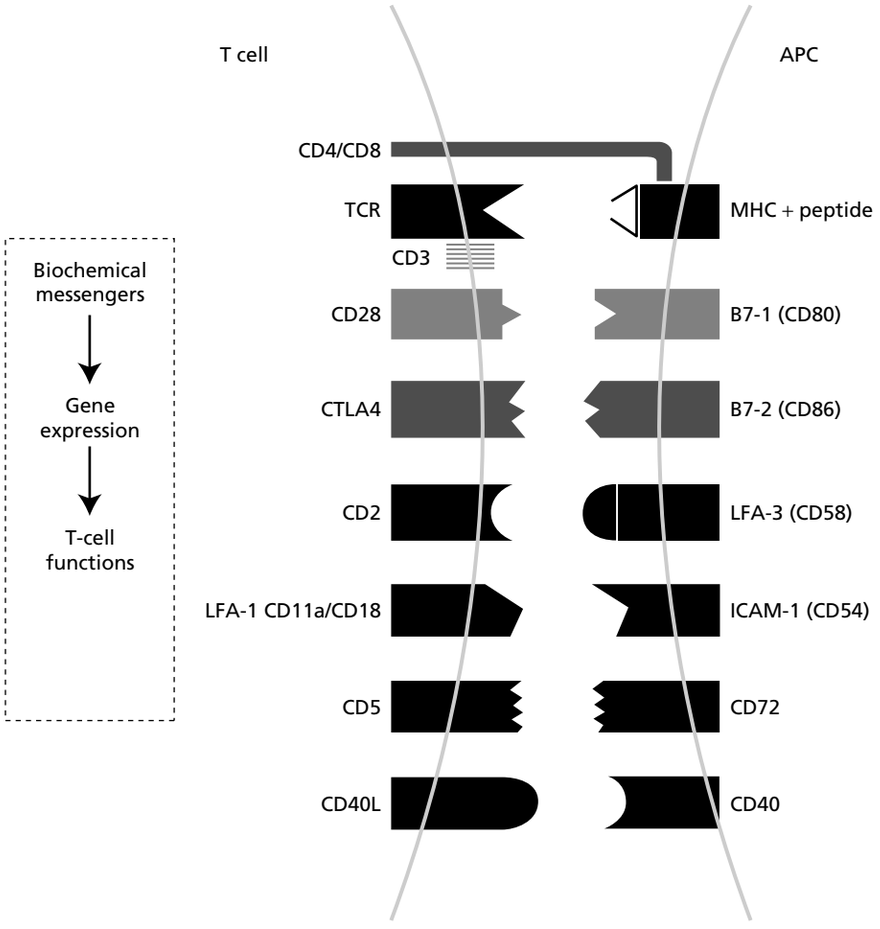

Fig. 1.2 T-cell/antigen-presenting cell contact sites. In this schema of T-cell activation, the antigenic signal is initiated by the physical interaction between the clonally variant T-cell antigen receptor (TCR) α, β-heterodimer and the antigenic peptide displayed by MHC on antigen-presenting cells (APCs). The antigenic signal is transduced into the cell by the CD3 proteins. The CD4 and the CD8 antigens function as associative recognition structures, and restrict TCR recognition to class II and class I antigens of MHC, respectively. Additional T-cell surface receptors generate the obligatory costimulatory signals by interacting with their counter-receptors expressed on the surface of the APCs. The simultaneous delivery to the T cells of the antigenic signal and the costimulatory signal results in the optimum generation of second messengers (such as calcium), expression of transcription factors (such as nuclear factor of activated T cells), and T-cell growth promoting genes (such as interleukin [IL]-2). The CD28 antigen as well as the CTLA4 antigen can interact with both the B7-1 and B7-2 antigens. The CD28 antigen generates a stimulatory signal, and the recent studies of CTLA4-deficient mice suggest that CTLA4, unlike CD28, generates a negative signal. CD, cluster designation; ICAM-1, intercellular adhesion molecule-1; LFA-1, leukocyte function-associated antigen 1; MHC, major histocompatibility complex. (Reproduced from Suthanthiran [48] with permission.)

COSTIMULATORY SIGNALS

Signaling of T cells via the TCR/CD3 complex (antigenic signal) is necessary, albeit insufficient, to induce T-cell proliferation; full activation is dependent on both the antigenic signals and the costimulatory signals (signal two) engendered by the contactual interactions between cell surface proteins expressed on antigen-specific T cells and APCs (Fig. 1.2; see Table 1.1) [15,16]. The interaction of the CD2 protein on the T-cell surface with the CD58 (leukocyte function-associated antigen 3 [LFA-3]) protein on the surface of APCs, and that of the CD11a/CD18 (LFA-1) proteins with the CD54 (intercellular adhesion molecule 1 [ICAM-1]) proteins [17], and/or the interaction of the CD5 with the CD72 proteins [8] aids in imparting such a costimulatory signal.

Recognition of the B7-1 (CD80) and B7-2 (CD86) proteins expressed upon $CD4^+$ T cells generates a very powerful T-cell costimulus [18]. Monocytes and dendritic cells constitutively express CD86. Cytokines (e.g. granulocyte–macrophage colony-stimulating factor [GM-CSF] or γ-interferon [IFNγ])

stimulate expression of CD80 on monocytes, B cells, and dendritic cells. Many T cells express B7 binding proteins (i.e. CD28 proteins that are constitutively expressed on the surface of $CD4^+$ T cells and CTLA-4 [CD152]), a protein whose ectodomain is closely related to that of CD28, and is expressed upon activated $CD4^+$ and $CD8^+$ T cells. CD28 binding of B7 molecules stimulates a Ca^{2+}-independent activation pathway that leads to stable transcription of the IL-2, IL-2 receptors, and other activation genes resulting in vigorous T-cell proliferation. For some time, the terms CD28 and the costimulatory receptor were considered synonymous by some, but the demonstration that robust T-cell activation occurs in CD28-deficient mice indicated that other receptor ligand systems contribute to signal two [19]. In particular the interaction between CD40 expressed upon APCs and CD40 ligand (CD154) expressed by antigen-activated $CD4^+$ T cells has received great attention as a potent second signal [20].

The delivery of the antigenic signal and the costimulatory signal leads to stable transcription of the IL-2, several T-cell growth factor receptors, and other pivotal T-cell activation genes. The Ca^{2+}-independent costimulatory CD28 pathway is resistant to inhibition by cyclosporine or tacrolimus as compared to the calcium-dependent pathway of T-cell activation. In contrast, recognition of B7 proteins by CTLA-4, a protein primarily expressed on activated T cells, stimulates a negative signal to T cells and this signal is a prerequisite for peripheral T-cell tolerance [21].

The formulation that full T-cell activation is dependent on the costimulatory signal as well as the antigenic signal is significant, as T-cell molecules responsible for costimulation and their cognate receptors on the surface of APCs then represent target molecules for the regulation of the antiallograft response. Indeed, transplantation tolerance has been induced in experimental models by targeting a variety of cell-surface molecules that contribute to the generation of costimulatory signals.

INTERLEUKIN-2/INTERLEUKIN-15 STIMULATED T-CELL PROLIFERATION

T-cell proliferation occurs as a consequence of the T-cell activation-dependent production of IL-2 and the expression of multimeric high affinity IL-2 receptors on T cells formed by the noncovalent association of three IL-2 binding peptides (α, β, γ) [22–26]. IL-15 is a paracrine-type T-cell growth factor family member with very similar overall structural and identical T-cell stimulatory qualities to IL-2 [22]. The IL-2 and IL-15 receptor complexes share β and γ chains that are expressed in low abundance upon resting T cells; expression of these genes is amplified in activated T cells. The α-chain receptor components of the IL-2 and IL-15 receptor complexes are distinct and expressed upon activated, but not resting, T cells. The intracytoplasmic domains of the IL-2 receptor β and γ chains are required for intracellular signal transduction. The ligand-activated, but not resting, IL-2/IL-15 receptors are associated with intracellular PTKs [22,26–28]. Raf-1, a protein serine/threonine kinase that is prerequisite to IL-2/IL-15–triggered cell proliferation, associates with the intracellular domain of the shared β chain [29]. Translocation of IL-2 receptor-bound Raf-1 serine/threonine kinase into the cytosol requires IL-2/IL-15–stimulated PTK activity. The ligand-activated common γ chain recruits a member of the Janus kinase family, Jak 3, to the receptor complex that leads to activation of a member of the STAT family. Activation of this particular Jak-STAT pathway is prerequisite for proliferation of antigen-activated T cells. The subsequent events leading to IL-2/IL-15–dependent proliferation are not fully resolved; however, IL-2/IL-15–stimulated expression of several DNA binding proteins including bcl-2, c-jun, c-fos, and c-myc contributes to cell-cycle progression [30,31]. It is interesting and probably significant that IL-2, but not IL-15, triggers apoptosis of many antigen-activation T cells. In this way, IL-15–triggered events are more detrimental to the allograft response than IL-2. As IL-15 is not produced by T cells, IL-15 expression is not regulated by cyclosporine or tacrolimus.

IMMUNOBIOLOGY AND MOLECULAR FEATURES OF REJECTION

The net consequence of cytokine production and acquisition of cell-surface receptors for these transcellular molecules is the emergence of antigen-specific and graft-destructive T cells (see Fig. 1.1) [14]. Cytokines also facilitate the humoral arm of immunity by promoting the production of cytopathic antibodies. Moreover, IFNγ and tumor necrosis factor-α (TNFα) can amplify the ongoing immune response by upregulating the expression of HLA molecules as well as costimulatory molecules (e.g. B7) on graft parenchymal cells and APCs. We and others have demonstrated the presence of antigen-specific cytotoxic T lymphocytes (CTL) and anti-HLA antibodies during, or preceding, a clinical rejection episode [32,33]. We have detected messenger RNA (mRNA) encoding the CTL-selective serine protease (granzyme B), perforin, and Fas-ligand attack molecules and immunoregulatory cytokines, such as IL-10 and IL-15, in human renal allografts undergoing acute rejection (reviewed in reference [34]). Indeed these gene-expression events can anticipate clinically apparent rejection. More recent efforts to develop a noninvasive method for the molecular diagnosis of rejection have proved rewarding. Using either peripheral blood [35] or urinary leukocytes [36] rejection-related, gene-expression events evident in renal biopsy specimens are also detected in peripheral blood or urinary sediment specimens. We suspect that a noninvasive, molecular-diagnostic approach to rejection may prove pivotal toward detection of insidious, clinically silent rejection episodes that, although rarely detected through

standard measures, are steroid-sensitive but usually lead to chronic rejection [37].

The immune response directed at the allograft may not all be unidirectional and graft destructive; the immune repertoire appears to include both graft destructive immunity, as exemplified by the presence of granzyme B expressing cytopathic cells, and graft protective immunity, as exemplified by $FoxP3^+CD25^+CD4^+$ T-regulatory cells. Indeed, we and others have found that acute rejection of human allografts is associated not only with cytopathic cells but also with $FoxP3^+$ T-regulatory cells [38,39]. Emerging data also suggest that the outcome of an episode of acute rejection depends upon the balance between cytopathic cells and T-regulatory cells, with reversible acute rejection and renal graft salvage being associated with FoxP3 and T-regulatory cells [38].

TRANSPLANTATION TOLERANCE

There are many definitions of transplantation tolerance. We define clinical transplantation tolerance as an inability of the organ graft recipient to express a graft destructive immune response in the absence of exogenous immunosuppressive therapy. While this statement does not restrict either the mechanistic basis or the quantitative aspects of immune unresponsiveness of the host, tolerance is antigen-specific, induced as a consequence of prior exposure to the specific antigen, and is not dependent on the continuous administration of exogenous nonspecific immunosuppressants.

A classification of tolerance on the basis of the mechanisms involved, site of induction, extent of tolerance, and the cell primarily tolerized is provided in Table 1.3. Induction strategies for the creation of peripheral tolerance are listed in Table 1.4.

Table 1.3 Classification of tolerance.

A	Based on the major mechanism involved
	1. Clonal deletion
	2. Clonal energy
	3. Suppression
B	Based on the period of induction
	1. Fetal
	2. Neonatal
	3. Adult
C	Based on the cell tolerized
	1. T cell
	2. B cell
D	Based on the extent of tolerance
	1. Complete
	2. Partial, including split
E	Based on the main site of induction
	1. Central
	2. Peripheral

Table 1.4 Potential approaches for the creation of tolerance.

A	Cell depletion protocols
	1. Whole body irradiation
	2. Total lymphoid irradiation
	3. Panel of monoclonal antibodies
B	Reconstitution protocols
	1. Allogeneic bone marrow cells with or without T-cell depletion
	2. Syngeneic bone marrow cells
C	Combination of strategies A and B
D	Cell-surface molecule targeted therapy
	1. Anti-CD4 mAbs
	2. Anti-ICAM-1 + anti-LFA-1 mAbs
	3. Anti-CD3 mAbs
	4. Anti-CD2 mAbs
	5. Anti-IL-2 receptor α (CD25) mAbs
	6. CTLA4Ig fusion protein
	7. Anti-CD40L mAbs
E	Drugs
	1. Azathioprine
	2. Cyclosporine
	3. Rapamycin
F	Additional approaches
	1. Donor-specific blood transfusions with concomitant mAb or drug therapy
	2. Intrathymic inoculation of cells/antigens
	3. Oral administration of cells/antigens

Several hypotheses, not necessarily mutually exclusive and at times even complementary, have been proposed for the cellular basis of tolerance. Data from several laboratories support the following mechanistic pathways – clonal deletion, clonal anergy, and immunoregulation – for the creation of a tolerant state.

Clonal deletion

Clonal deletion is a process by which self–antigen-reactive cells, (especially those with high affinity for the self-antigens), are eliminated from the organism's immune repertoire. This process is called central tolerance. In the case of T cells, this process takes place in the thymus, and the death of immature T cells is considered to be the ultimate result of high-affinity interactions between a T cell with productively rearranged TCR and the thymic nonlymphoid cells, including dendritic cells that express the self-MHC antigen. This purging of the immune repertoire of self-reactive T cells is termed negative selection and is distinguished from the positive selection process responsible for the generation of the T-cell repertoire involved in the recognition of foreign antigens in the context of self-MHC molecules. Clonal deletion, or at least marked depletion, of mature T cells as a consequence of apoptosis can also occur in the periphery (reviewed in reference [40]). The form of graft tolerance occurring as a consequence of mixed hematopoietic chimerism entails massive deletion of alloreactive clones [41]. Tolerance to renal allografts has been

achieved in patients that have accepted a bone marrow graft from the same donor [42,43]. It is interesting that IL-2, the only T-cell growth factor that triggers T-cell proliferation as well as apoptosis, is an absolute prerequisite for the acquisition of organ graft tolerance through use of nonlymphoablative treatment regimens [44,45]. Tolerance achieved under these circumstances also involves additional mechanisms, including clonal anergy and suppressor mechanisms [46–48].

Clonal anergy

Clonal anergy refers to a process in which the antigen-reactive cells are functionally silenced. The cellular basis for the hyporesponsiveness resides in the anergic cell itself, and the current data suggest that the anergic T cells fail to express the T-cell growth factor, IL-2, and other crucial T-cell activation genes because of defects in the antigen-stimulated signaling pathway.

T-cell clonal anergy can result from suboptimal antigen-driven signaling of T cells, as mentioned earlier. The full activation of T cells requires at least two signals, one signal generated via the TCR/CD3 complex, and the second (costimulatory) signal initiated/delivered by the APCs. Stimulation of T cells via the TCR/CD3 complex alone – provision of antigenic signal without the obligatory costimulatory signal – can result in T-cell anergy/paralysis (Fig. 1.3 and Table 1.1).

B-cell activation, in a fashion analogous to T-cell activation, requires at least two signals. The first signal is initiated via the B-cell antigen receptor immunoglobulin, and a second costimulatory signal is provided by cytokines or cell surface proteins of T-cell origin. Thus, delivery of the antigenic signal alone to the B cells without the instructive cytokines or T-cell help can lead to B-cell anergy and tolerance.

Immunoregulatory (suppressor) mechanisms

Antigen-specific T or B cells are physically present and are functionally competent in tolerant states resulting from suppressor mechanisms. The cytopathic and antigen-specific cells are restrained by the suppressor cells or factors or express noncytopathic cellular programs. Each of the major subsets of T cells, the CD4 T cells and the CD8 T cells, has been implicated in mediating suppression. Indeed, a cascade involving MHC antigen-restricted T cells, MHC antigen-unrestricted T cells, and their secretory products have been reported to collaborate to mediate suppression. Recently, a subset of $CD4^+$ T cells, the $CD4^+$ $CD25^+$ cells that express FoxP3, has been identified to mediate potent suppressive activity [49,50].

At least four distinct mechanisms have been advanced to explain the cellular basis for suppression: (i) An antiidiotypic regulatory mechanism in which the idiotype of the TCR of the original antigen-responsive T cells functions as an immunogen and elicits an antiidiotypic response. The elicited antiidiotypic regulatory cells, in turn, prevent the further responses of the idiotype-bearing cells to the original sensitizing stimulus; (ii) The veto process by which recognition by alloreactive T cells of alloantigen-expressing veto cells results in the targeted killing (veto process) of the original alloreactive T cells by the veto cells; (iii) Immune deviation, a shift in $CD4^+$ T-cell programs away from Th1-type (IL-2, IFNγ expressing) toward the Th2-type (IL-4, IL-10 expressing) program; and (iv) The production of suppressor factors or cytokines. (e.g. the production of TGF-β by myelin basic protein-specific CD8 T cells or other cytokines with antiproliferative properties.) The process leading to full tolerance is infectious. Tolerant T cells recruit nontolerant T cells into the tolerant state [47]. The

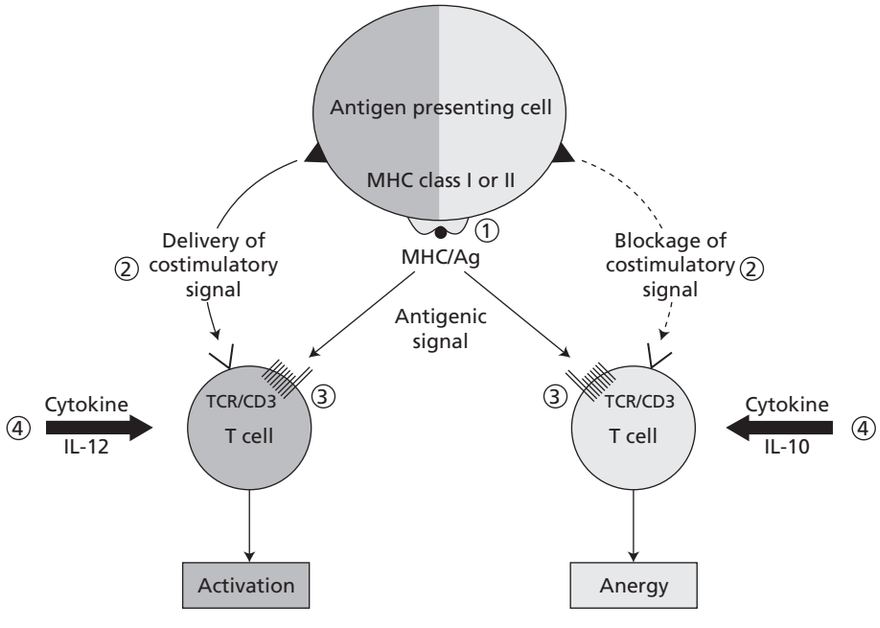

Fig. 1.3 T-cell activation/anergy decision points. Several potential sites for the regulation of T-cell signaling are shown. The antigenic peptide displayed by major histocompatibility complex (MHC) (site 1), costimulatory signals (site 2), T-cell antigen receptor (TCR) (site 3), and cytokine signaling (site 4) can influence the eventual outcome. Altered peptide ligands, blockade of costimulatory signals, downregulation of TCR, and interleukin (IL)-10 favor anergy induction, whereas fully immunogenic peptides, delivery of costimulatory signals, appropriate number of TCRs, and IL-12 prevent anergy induction and facilitate full activation of T cells. (Reproduced from Suthanthiran [48] with permission.)

tolerant state also establishes a condition in which foreign tissues housed in the same microenvironment as the specific antigen to which the host has been tolerized are protected from rejection [47]. Tolerance is a multistep process [46–48].

Clearly more than one mechanism is operative in the induction of tolerance (see Fig. 1.3). The tolerant state is not an all-or-nothing phenomenon but is one that has several gradations. Of the mechanisms proposed for tolerance, clonal deletion might be of greater importance in the creation of self-tolerance, and clonal anergy and immunoregulatory mechanisms might be more applicable to transplantation tolerance. More recent data suggest both clonal depletion and immunoregulatory mechanisms are needed to create and sustain central or peripheral tolerance. From a practical viewpoint, a nonimmunogenic allograft (e.g. located in an immunologically privileged site or physically isolated from the immune system) might also be "tolerated" by an immunocompetent organ-graft recipient.

Authentic tolerance has been difficult to identify in human renal allograft recipients. Nevertheless, the clinical examples, albeit infrequent, of grafts functioning without any exogenous immunosuppressive drugs (either due to noncompliance of the patient or due to discontinuation of drugs for other medical reasons) does suggest that some long-term recipients of allografts develop tolerance to the transplanted organ and accept the allografts. The recent progress in our understanding of the immunobiology of graft rejection and tolerance and the potential to apply molecular approaches to the bedside hold significant promise for the creation of a clinically relevant tolerant state and transplantation without exogenous immunosuppressants – the ultimate goal of the transplant physician.

ACKNOWLEDGMENTS

This chapter is adapted from an earlier chapter entitled "Immunobiology and immunopharmacology of renal allograft rejection" by M. Suthanthiran, C. Hartono and T.B. Strom. In: Schrier RW, ed. *Diseases of the Kidney and Urinary Tract*, 8th edn. Philadelphia, PA: Lippincott, Williams & Wilkins, 2006: 2540–52. The authors are grateful to Ms. Linda Stackhouse for her meticulous help in the preparation of this chapter.

REFERENCES

1 Unanue ER, Cerottini JC. Antigen presentation. *FASEB J* 1989;**3**:2496–502.
2 Jorgensen JL, Reay PA, Ehrich EW, *et al*. Molecular components of T cell recognition. *Annu Rev Immunol* 1992;**10**:835–73.
3 Germain RN. MHC-dependent antigen processing and peptide presentation: providing ligands for T lymphocyte activation. *Cell* 1994;**76**:287–99.
4 Acuto O, Cantrell D. T cell activation and the cytoskeleton. *Annu Rev Immunol* 2000;**18**:165–84.
5 Dustin ML, Cooper JA. The immunological synapse and the actin cytoskeleton: molecular hardware for T cell signaling. *Nature Immunol* 2000;**1**:23–9.
6 Brown MH, Cantrell DA, Brattsand G, *et al*. The CD2 antigen associates with the T-cell antigen receptor CD3 antigen complex on the surface of human T lymphocytes. *Nature* 1989;**339**:551–3.
7 Suthanthiran M. A novel model for the antigen-dependent activation of normal human T cells: transmembrane signaling by crosslinkage of the CD3/T cell receptor-alpha/beta complex with the cluster determinant 2 antigen. *J Exp Med* 1990;**171**:1965–79.
8 Beyers AD, Spruyt LL, Williams AF. Molecular associations between the T-lymphocyte antigen receptor complex and the surface antigens CD2, CD4, or CD8 and CD5. *Proc Natl Acad Sci USA* 1992;**89**:2945–9.
9 Shoskes DA, Wood KJ. Indirect presentation of MHC antigens in transplantation. *Immunol Today* 1994;**15**:32–8.
10 Nishizuka Y. Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. *Science* 1992;**258**:607–14.
11 Clipstone NA, Crabtree GR. Identification of calcineurin as a key signalling enzyme in T-lymphocyte activation. *Nature* 1992;**357**:695–7.
12 O'Keefe SJ, Tamura J, Kincaid RL, *et al*. FK506- and CsA-sensitive activation of the IL-2 promoter by calcineurin. *Nature* 1992;**357**:692–4.
13 Liu J, Farmer JD Jr, Lane WS, *et al*. Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. *Cell* 1991;**66**:807–15.
14 Suthanthiran M, Strom TB. Renal transplantation. *N Engl J Med* 1994;**334**:365–76.
15 Schwartz RH. T cell anergy. *Sci Am* 1993;**269**:62–3; 66–71.
16 Suthanthiran M. Signaling features of T cells: implication for the regulation of the anti-allograft response. *Kidney Int Suppl* 1993;**43**:S3–11.
17 Dustin ML, Springer TA. T-cell receptor cross-linking transiently stimulates adhesiveness through LFA-1. *Nature* 1989;**341**:619–24.
18 Lenschow DJ, Walunas TL, Bluestone JA. CD28/B7 system of T cell costimulation. *Annu Rev Immunol* 1996;**14**:233–58.
19 Shahinian A, Pfeffer K, Lee KP, *et al*. Differential T cell costimulatory requirements in CD28-deficient mice. *Science* 1993;**261**:609–12.
20 Noelle RJ. CD40 and its ligand in host defense. *Immunity* 1996;**4**:415–9.
21 Oosterwegel MA, Greenwald RJ, Mandelbrot DA, *et al*. CTLA-4 and T cell activation. *Curr Opin Immunol* 1999;**11**:294–300.
22 Waldmann T, Tagaya Y, Bamford R. Interleukin-2, interleukin-15, and their receptors. *Int Rev Immunol* 1998;**16**:205–26.
23 Smith KA. Interleukin-2: inception, impact, and implications. *Science* 1988;**240**:1169–76.
24 Waldman TA. The interleukin-2 receptor. *J Biol Chem* 1991;**266**:2681–4.
25 Takeshita T, Asao H, Ohtani K. Cloning of the gamma chain of the human IL-2 receptor. *Science* 1992;**257**:379–82.
26 Hatakeyama M, Kono T, Kobayashi N, *et al*. Interaction of the IL-2 receptor with the src-family kinase $p56^{lck}$: identification of novel intermolecular association. *Science* 1991;**252**:1523–8.

27 Fung MR, Scearce RM, Hoffman JA, *et al*. A tyrosine kinase physically associates with the beta-subunit of the human IL-2 receptor. *J Immunol* 1991;**147**:1253–60.

28 Remillard B, Petrillo R, Maslinski W, *et al*. Interleukin-2 receptor regulates activation of phosphatidylinositol 3-kinase. *J Biol Chem* 1991;**266**:14 167–70.

29 Maslinski W, Remillard B, Tsudo M, *et al*. Interleukin-2 (IL-2) induces tyrosine kinase-dependent translocation of active Raf-1 from the IL-2 receptor into the cytosol. *J Biol Chem* 1992;**267**:15 281–4.

30 Shibuya H, Yoneyama M, Ninomiya-Tsuji J, *et al*. IL-2 and EGF receptors stimulate the hematopoietic cell cycle via different signaling pathways: demonstration of a novel role for c-myc. *Cell* 1992;**70**:57–67.

31 Taniguchi T. Cytokine signalling through non-receptor protein tyrosine kinases. *Science* 1995;**260**:251–5.

32 Strom TB, Tilney NL, Carpenter CB, *et al*. Identity and cytotoxic capacity of cells infiltrating renal allografts. *N Engl J Med* 1975;**292**:1257–63.

33 Suthanthiran M, Garovoy MR. Immunologic monitoring of the renal transplant recipient. *Urol Clin North Am* 1983;**10**:315–25.

34 Strom TB, Suthanthiran M. Prospects and applicability of molecular diagnosis of allograft rejection. *Semin Nephrol* 2000;**20**:103–7.

35 Vasconcellos LM, Schachter AD, Zheng XX, *et al*. Cytotoxic lymphocyte gene expression in peripheral blood leukocytes correlates with rejecting renal allografts. *Transplantation* 1998;**66**:562–6.

36 Li B, Hartono C, Ding R, *et al*. Noninvasive diagnosis of renal-allograft rejection by measurement of messenger RNA for perforin and granzyme B in urine. *N Engl J Med* 2001;**344**:947–54.

37 Rush D, Nickerson P, Gough J, *et al*. Beneficial effects of treatment of early subclinical rejection: a randomized study. *J Am Soc Nephrol* 1998;**9**:2129–34.

38 Muthukumar T, Dadhania D, Ding R, *et al*. Messenger RNA for FOXP3 in the urine of renal allograft recipients. *New Engl J Med* 2005;**353**:2342–51.

39 Baan CC, van der Mast BJ, Klepper M, *et al*. Differential effect of calcineurin inhibitors. Anti-CD25 antibodies and rapamycin on the induction of FOXP3 in human T cells. *Transplantation* 2005;**80**:110–7.

40 Van Parijs L, Abbas AK. Homeostasis and self-tolerance in the immune system: turning lymphocytes off. *Science* 1998;**280**:243–8.

41 Wekerle T, Sayegh MH, Hill J, *et al*. Extrathymic T cell deletion and allogeneic stem cell engraftment induced with costimulatory blockade is followed by central T cell tolerance. *J Exp Med* 1998;**187**:2037–44.

42 Sayegh MH, Fine NA, Smith JL, *et al*. Immunologic tolerance to renal allografts after bone marrow transplants from the same donors. *Ann Intern Med* 1991;**114**:954–5.

43 Spitzer TR, Delmonico F, Tolkoff-Rubin N, *et al*. Combined histocompatibility leukocyte antigen-matched donor bone marrow and renal transplantation for multiple myeloma with end stage renal disease: the induction of allograft tolerance through mixed lymphohematopoietic chimerism. *Transplantation* 1999;**68**:480–4.

44 Dai Z, Konieczny BT, Baddoura FK, *et al*. Impaired alloantigen-mediated T cell apoptosis and failure to induce long-term allograft survival in IL-2-deficient mice. *J Immunol* 1998;**161**:1659–63.

45 Li Y, Li XC, Zheng XX, *et al*. Blocking both signal 1 and signal 2 of T-cell activation prevents apoptosis of alloreactive T cells and induction of peripheral allograft tolerance. *Nature Med* 1999;**5**:1298–302.

46 Li SC, Wells AD, Strom TB, *et al*. The role of T cell apoptosis in transplantation tolerance. *Curr Opin Immunol* 2000;**12**:522–7.

47 Waldmann H. Transplantation tolerance – where do we stand? *Nature Med* 1999;**5**:1245–8.

48 Suthanthiran M. Transplantation tolerance: fooling mother nature. *Proc Natl Acad Sci USA* 1996;**93**:12 072–5.

49 Sakaguchi S, Sakaguchi N, Shimizu J, *et al*. Immunologic tolerance maintained by $CD25^{+}CD4^{+}$ regulatory T cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. *Immunol Rev* 2001;**182**:18–32.

50 Maloy KJ, Powrie F. Regulatory T cells in the control of immune pathology. *Nat Immunol* 2001;**2**:816–22.

51 Suthanthiran M, Hartono C, Strom TB. Immunobiology and immunopharmacology of renal allograft rejection. In: Schrier RW, ed. *Diseases of the Kidney and Urinary Tract*, 8th edn. Philadelphia, PA: Lippincott, Williams & Wilkin, 2006: 2540–52.

52 Suthanthiran M, Morris RE, Strom TB. Transplantation immunobiology. In: Walsh PC, Retik AB, Vaughn ED Jr, *et al*., eds. *Campbell's Urology*, 7th edn. Philadelphia, PA: W.B. Saunders, 1997:491–504.