# PEDIATRIC SOLID ORGAN TRANSPLANTATION

This book is dedicated to the memory of Amir H. Tejani, MD (1933–2002) whose inspiration and foresight led to the first edition of this textbook, *Pediatric Solid Organ Transplantation*, and to the founding of The International Pediatric Transplant Association (IPTA).

# Pediatric Solid Organ Transplantation

SECOND EDITION

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# Preface to the Second Edition

Over six years has passed since the first edition of *Pediatric Solid Organ Transplantation* was published. This book remains the only textbook devoted to the field of pediatric transplantation. The concept for the first edition was that of the late Amir Tejani, an individual who relentlessly promoted the field of pediatric transplantation.

Transplantation of the major organs has now become routine in many countries across the globe, and some of the best results of transplantation are now being achieved in the youngest of recipients, including neonates and infants. In parallel with the growth and success of transplantation in children, the International Pediatric Transplant Association (IPTA), and its journal *Pediatric Transplantation*, have both flourished since the first edition of this book. Like the journal, this book is an official publication of the IPTA, and many of IPTA's members have contributed to this second edition.

In the second edition, the strengths of the first edition have been maintained, while new features have been added. The addition of three new co-editors has ensured that all facets of pediatric transplantation are represented in the editorial team. The organ specific sections have all been revised by experts (new and old) to keep the text up-to-date in this rapidly evolving field. Consistency has been applied to the content of all the organ specific sections to enhance the overall structure of the text. Section Two on Immunosuppression and its Complications has been expanded by four chapters to include sections on mechanisms of action, therapies for the sensitized patient, post-transplant lymphoproliferative disorders and organ toxicities of immunosuppressive therapy. A new section has been added (Section Eight) to emphasize the many special issues that effect survivors of pediatric transplantation. This new section focuses on topics related to quality of life; an issue of profound importance as the number of long-term survivors of pediatric solid organ transplantation continues to grow. A wide variety of topics ranging from growth and cognitive development to adherence and transition to adult care are covered in this important new section.

The editors wish to express their sincere appreciation to all the authors who contributed their time and expertise to this project. The end result reflects the sum of these excellent individual contributions. In addition, we would like to thank Kerrie Roberts who coordinated all the early stages of manuscript submission with endless forbearance and efficiency. We also would like to thank Rebecca Huxley and Maria Khan of Blackwell Publishing without whom this project would not have been possible. The final stages of production were carried out by Alice Nelson who assisted the editors in resolving the remaining issues and whose contribution to the final success of this publication is inestimable. The patience, collegiality and professionalism of these individuals has helped create a work that we hope will serve as the definitive reference for all those interested in improving the care and quality of life of children undergoing solid organ transplantation.

We believe Dr Tejani would be very pleased with this new edition of *Pediatric Solid Organ Transplantation*.

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January 2007

# Preface to the First Edition

Pediatric solid organ transplantation has experienced exciting and substantial advances in the past decade consequent to continued improvement in clinical outcomes.

The data base of the North American Pediatric Renal Transplant Cooperative Study has demonstrated the value of a registry in changing clinical practice, and has stimulated the development of similar registries in other pediatric solid organ transplants such as liver and heart. The evolution of pediatric transplantation to its own independent status has been marked by several international meetings followed by the creation of the Journal of Pediatric Transplantation and, most recently, by the establishment of the International Pediatric Transplant Association (IPTA). Guided by the Pediatric Committee of the American Society of Transplantation, certification of programs in renal, hepatic and heart and lung transplants is now underway.

To provide a more cohesive approach to the teaching of pediatric transplantation in various subspecialities, the council of IPTA has initiated the compilation of this book. In organizing the structure of the text, the Editors have recognized that there is a central core of information that is common to all clinical transplantation. Grouped together in the first two sections of the book, this core is intended to provide information about the immune system, rejection and tolerance, immunosuppressives and infections: issues common to all organs. Organ specific sections are structured in a common format designed to cover transplantation while maintaining the unique needs of the organ speciality.

The Editors wish to express their sincere appreciation to all the authors who contributed their time and expertise to this project. We are particularly grateful to the Associate Editors who contributed to the conception and realization of the book. It is our hope that this comprehensive text will strengthen intra-speciality cooperation, familiarize pediatric physicians and surgeons with the salient aspects of each other's respective areas and help improve the quality of life of children undergoing transplants.

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SECTION ONE

# Immunology and Genetics

# 1 The Immune Response to Organ Allografts

*Manikkam Suthanthiran*

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

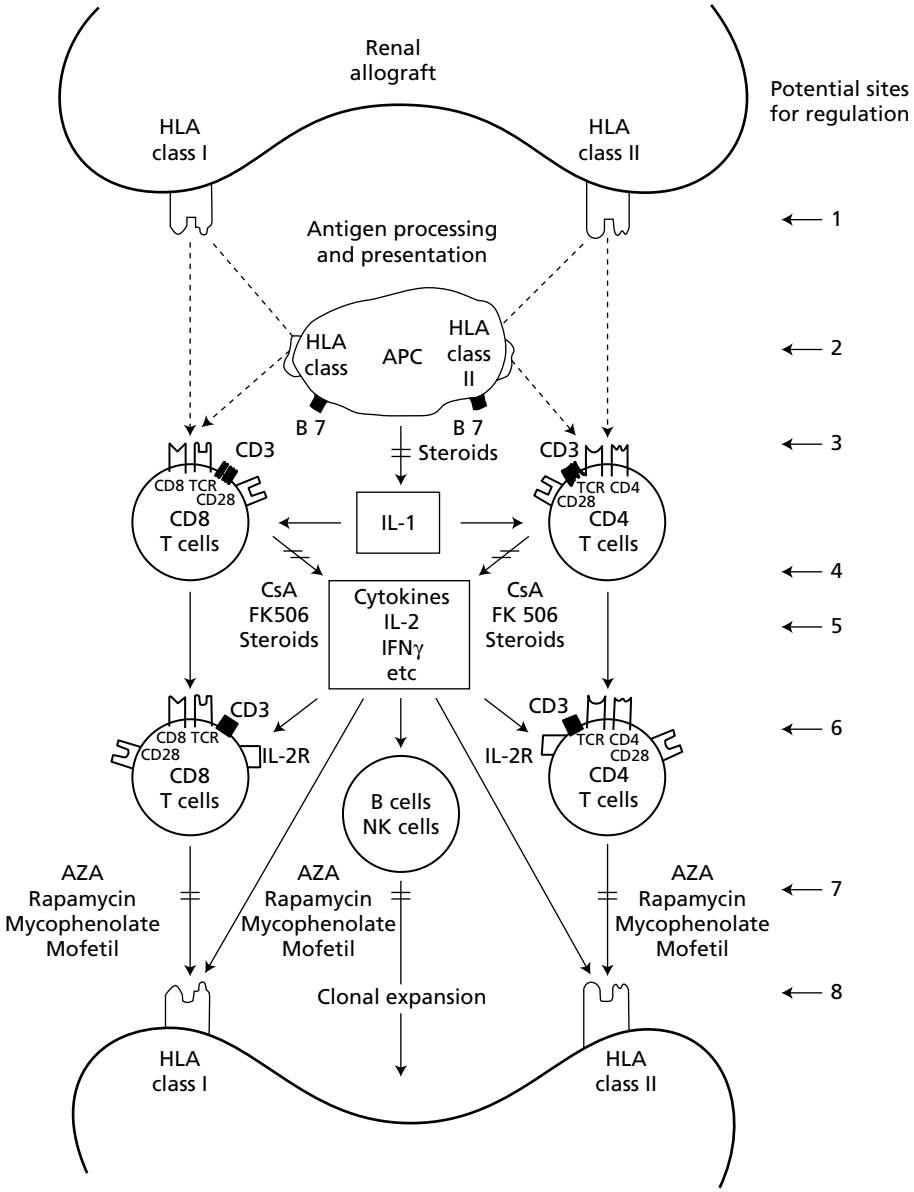


**Fig. 1.1** The antiallograft response. Schematic representation of human leukocyte antigens (HLA), the primary stimuli for the initiation of the antiallograft response; cell surface proteins participating in antigenic recognition and signal transduction; contribution of the cytokines and multiple cell 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].

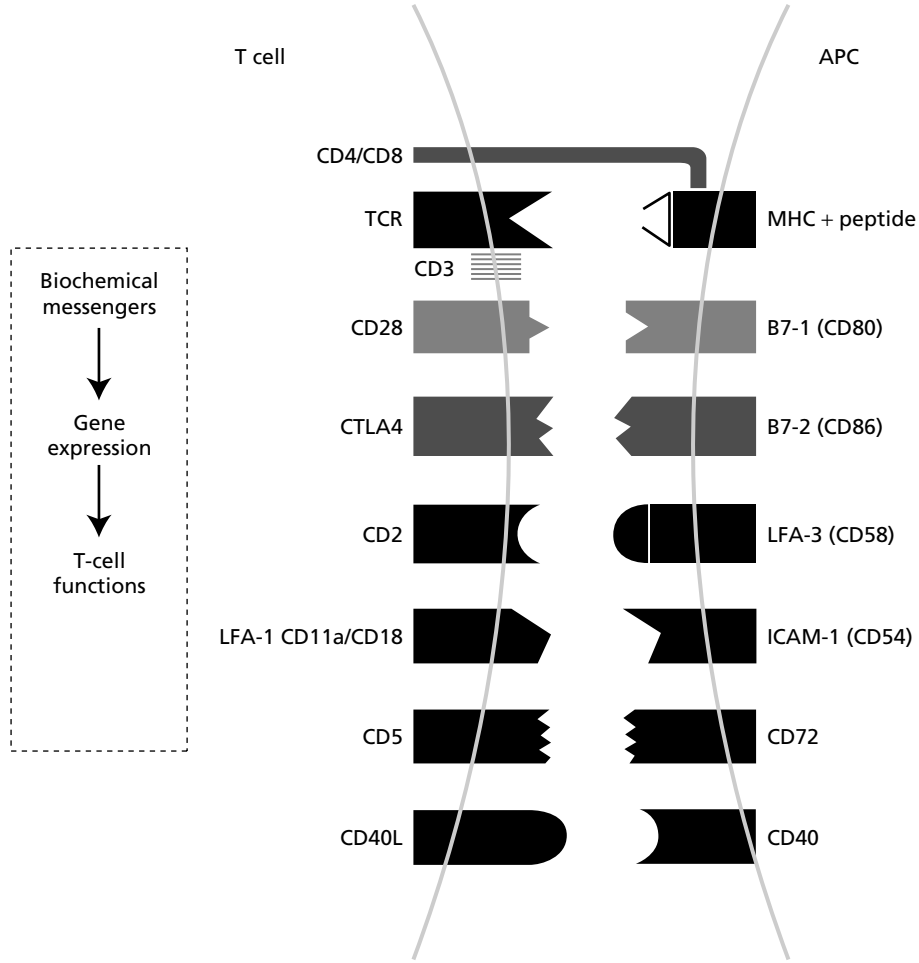


**Fig. 1.2** T-cell/antigen-presenting cell contact sites. In this schema of T-cell activation, the antigenic signal is initiated by the physical interaction between the clonally variant T-cell antigen receptor (TCR) α, β-heterodimer and the antigenic peptide displayed by MHC on antigen-presenting cells (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 ($\alpha$, $\beta$, $\gamma$) [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 $\beta$ and $\gamma$ chains that are expressed in low abundance upon resting T cells; expression of these genes is amplified in activated T cells. The $\alpha$-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 $\beta$ and $\gamma$ 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 $\beta$ 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 $\gamma$ 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, IFN$\gamma$ and tumor necrosis factor-$\alpha$ (TNF$\alpha$) 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

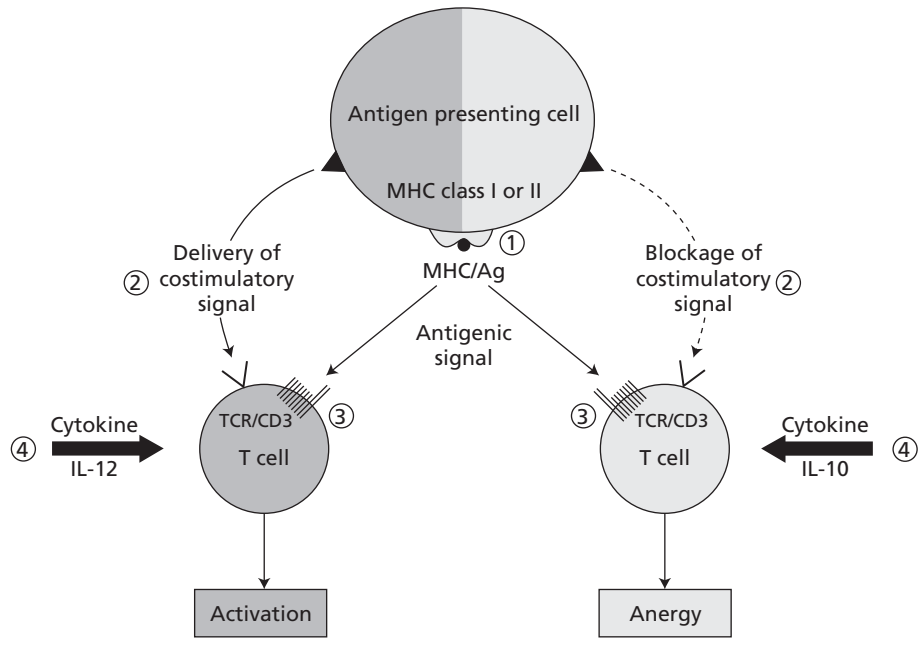


**Fig. 1.3** T-cell activation/anergy decision points. Several potential sites for the regulation of T-cell signaling are shown. The antigenic peptide displayed by major histocompatibility complex (MHC) (site 1), costimulatory signals (site 2), T-cell antigen receptor (TCR) (site 3), and cytokine signaling (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

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# 2 Allorecognition Pathways

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Allorecognition is the ability of T cells to respond to foreign histocompatibility antigens of other members of the same species. The major histocompatibility complex (MHC) molecules were originally discovered by their ability to induce serologic responses and rejection of tumor and skin grafts in mice [1,2]. It is now clear that the MHC antigens are the primary antigens responsible for causing graft rejection and are generally associated with a brisk time course of rejection. Allograft rejection is a T-cell-dependent process [3]; animals that lack T cells do not reject an allograft. T lymphocytes initiate the immune response, which ultimately results in graft rejection. Allorecognition is the essential first step for triggering the cascade of events that results in rejection of the graft. Once activated, they secrete cytokines and chemokines to activate and attract various effector cells, such as $CD8^+$ T cells and macrophages into the allograft. They are also able to interact with B cells which will secrete highly specific alloreactive antibodies. These cells in turn mediate the effector mechanisms of allograft destruction. Experimental and clinical data have confirmed that there are two distinct, nonmutually exclusive pathways of T-cell allorecognition: the "direct" and "indirect" pathways (Fig. 2.1) [4–6]. The "direct" pathway describes the ability of T cells through the T-cell receptor to engage and respond to *intact* allogeneic MHC molecules on the surface of antigen-presenting cells (APCs). This pathway is responsible for the vigorous *in vitro* response demonstrated in the primary allogeneic mixed lymphocyte reaction (MLR). *In vivo*, the direct pathway appears to be the principal route of T-cell sensitization leading to acute allograft rejection. The "indirect" pathway refers to the recognition of donor antigens presented as peptides in association with self-MHC by recipient APCs. This corresponds to the normal pathway of T-cell recognition of foreign or autoantigens in the context of self-MHC molecules such as the case in infections and autoimmune diseases. The following includes recent data about the role of allorecognition pathways in allograft rejection, both at the stage of priming of T cells and of effector phase of rejection. The importance of these pathways in developing tolerance-inducing strategies in organ transplantation is also reviewed.

## DIRECT PATHWAY OF ALLORECOGNITION

*Direct* refers to cell recognition of a whole intact foreign MHC molecule on the surface of donor cells. Although the specific peptide (typically derived from endogenous proteins, including MHC antigens, see below) bound in the groove of the MHC molecule may be important in this recognition process, it does not restrict this response. The graft, which includes donor bone marrow-derived APCs, usually expresses several class I and II MHC molecules that differ from the recipient's MHC molecules, and which can directly stimulate recipients T cells. Donor APCs prime $CD4^+$ and $CD8^+$ T cells through the direct pathway. However, as these donor APCs are destroyed during the priming process, direct T-cell priming is likely to be time-limited. Thus, direct allorecognition may account for early acute cellular rejection. Consistent with this concept, direct alloreactivity was not detectable in the peripheral blood of a cohort of renal and lung allograft recipients with chronic allograft dysfunction several years after transplantation [7–9].

Two features of the direct pathway serve to define the strength of the allogeneic response [10]. First of all, the precursor frequency of T cells that directly recognize allo-MHC is unusually high, 100–1000 times higher than the response to nominal antigens [11]. Second, unlike the response to nominal antigens, the direct response to allo-MHC requires no previous exposure or priming (i.e. can be initiated by naïve T cells). Over the years, several theories have emerged to explain the molecular basis of the strength of the direct pathway of allorecognition.

Matzinger and Bevan [12] hypothesized that the high precursor frequency of alloreactive T cells is secondary to the high frequency of different allogeneic determinants presented on allogeneic APCs (determinant frequency theory). Formulated prior to our understanding of MHC-restricted peptide presentation, this model suggested that the each allo-MHC molecule was an "interaction antigen" and could form "binary complexes" with all other cell surface proteins on the membrane surface. The resulting complex of allo-MHC + X (where X is a cell surface protein) could interact with the T-cell receptor. Each allo-MHC could form a large

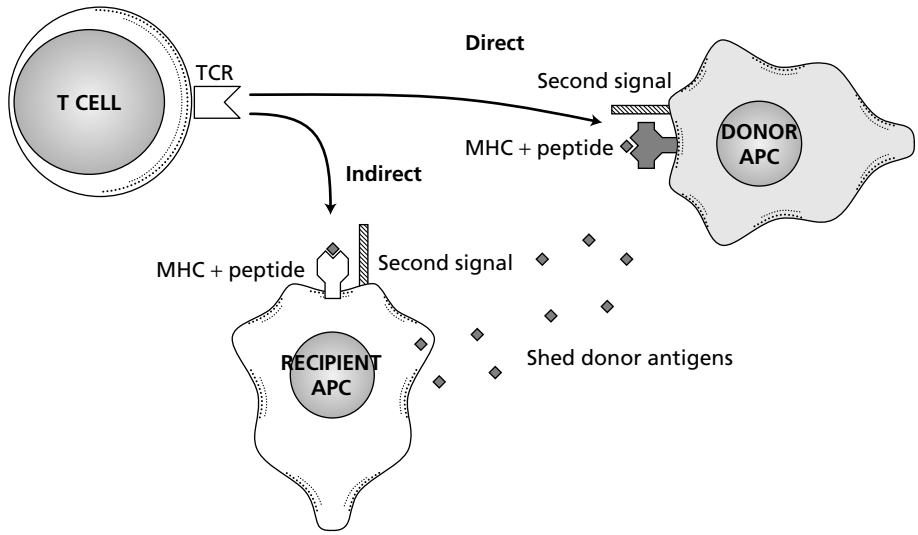


**Fig. 2.1** Pathways of T-cell allorecognition. In the "direct" pathway, recipient T cells recognize intact allo-MHC + peptide complex on the surface of donor antigen-pesenting cells (APCs). In the "indirect" pathway, recipient T cells recognize processed allopeptides (derived primarily from MHC antigens) presented by recipient APCs.

number of different antigenic complexes with different cell surface receptors and create a high frequency of antigenic determinants. This theory predicts that the high precursor frequency of alloreactive T cells results from the ability of a single allogeneic APC to stimulate many T-cell clones because of the large number of different determinants created by allo-MHC and a cell surface receptor.

Bevan [13] later proposed an alternative theory to explain the phenomenon of alloreactivity. In this theory, alloreactive T cells recognize a determinant on the foreign MHC molecule itself, and the peptides being presented by the MHC is not of central importance. All the foreign MHC molecules (approximately 100 000) can serve to stimulate T cells with low-affinity receptors by creating a high density of foreign determinants (high determinant density theory). In contrast, the density of foreign determinants created by the presentation of a foreign peptide in the context of self-MHC would be quite low, as most of the self-MHC molecules would be presenting self-peptides. When the determinant density is high, as in the case of allogeneic APCs, even low-affinity T cells can be activated.

The determinant frequency and determinant density theories need not be mutually exclusive. In fact, experimental evidence now supports the notion that both the conformation of the MHC molecule itself and the peptide–MHC complex can determine alloreactivity [14–17].

Lechler *et al.* [18] used results from studies of specific HLA-DR primed T-cell clones to postulate that allorecognition is structurally heterogeneous and varies according to the responder and stimulator MHC types. In closely related MHC combinations, allorecognition would be caused by T cells recognizing novel endogenous peptides that have never been encountered by responder T cells. In more disparate MHC combinations, the alloresponse would be directed primarily against the residues on the allo-MHC itself, and the bound peptide would have a minimal role. T-cell recognition of allo-MHC would thus be secondary to "molecular mimicry" by the allo-MHC of the three-dimensional complex of self-MHC + peptide. The "molecular mimicry" hypothesis may explain how a T cell positively selected to recognize foreign antigens in the context of self-MHC can now recognize and react to an intact allo-MHC molecule.

The concept of positive and negative selection in the thymus also helps to explain the strength of the alloimmune response [19]. During development, T cells with receptors of too high affinity are deleted (negative selection), whereas those with too low affinity are not selected. The end result of this selection is that TCRs of intermediate affinity exit the thymus and enter the periphery. Within an individual, clonal deletion occurs early in development. Potentially, autoreactive clones (with too high affinity for self) are deleted; failure of deletion of some clones may lead to autoimmunity. In the case of transplantation across an allelic difference, however, the recipient's T cells do not contact allo-MHC molecules during development in the thymus and thus escape the deletion (negative selection) imposed by interaction with self-MHC. Thus, the end result is the large number of donor MHC–peptide complexes on the graft to which a potential recipient has not been tolerized during ontogeny. Moreover, the relatively low affinity of any given TCR for its ligand suggest that each T cell could potentially recognize more than one MHC–peptide complex [20]. The high density of alloantigens on the surface of an allograft additionally contributes to the strong T-cell response.

## STRUCTURE OF THE MHC MOLECULE AND ALLORECOGNITION

A key to our understanding of the molecular basis of allorecognition was the resolution of the X-ray crystal structure of the human class I MHC HLA-A2 molecule by Wiley and

Strominger [21]. The polymorphic sequences of the MHC molecule were found to be primarily within the peptide-binding groove, which contained not a single peptide but rather a heterogeneous population of peptides [22,23]. The implication of this important discovery was that a single MHC gene actually presented an array of thousands of different peptides within the peptide-binding groove [24–26]. MHC polymorphism thus serves to diversify the identity of different peptides that could bind within the peptide-binding site.

The concept that a number of different peptides could fit into the groove of a single MHC molecule is consistent with the high determinant frequency theory of Matzinger and Bevan. The high frequency of foreign determinants could reflect the diversity of "interaction antigens" created by a single allo-MHC molecule presenting a multitude of different cellular peptides.

## ROLE OF ENDOGENOUS PEPTIDES IN ALLORECOGNITION

Emerging studies with T-cell clones have demonstrated that MHC-bound endogenous peptides are integral to T-cell allorecognition. For example, alloreactivity of T cells to class II MHC molecules is diminished after incubation of class II-bearing APCs with an exogenous influenza peptide, suggesting that the exogenous peptide blocked presentation of a particular endogenous peptide required for allorecognition [27]. Furthermore, several studies using $CD4^+$ T-cell clones showed that reactivity to cells expressing the appropriate class II molecule depended on the type of cell expressing the class II antigens [28,29]. Alloreactivity required not only the appropriate allogeneic class II molecule but also the proper endogenous peptide expressed by the particular cell type.

The identification of mutant cell lines, such as T2 and RMA-S [30–32], that are defective in processing and transporting endogenous peptides further confirmed the necessity of MHC-bound peptides for allorecognition. When the human T2 cell line was transfected with the murine class I $K^b$ gene, $K^b$ was expressed on the cell surface in normal levels. However, all the $K^b$ molecules lacked peptide. Most $K^b$-specific cytotoxic T lymphocyte (CTL) clones could not recognize the T2-$K^b$ transfectants unless the cells were loaded with cytoplasmic peptides [33]. One clone was able to recognize empty $K^b$ molecules, but the level of lysis was 10- to 100-fold lower than the level of lysis observed after peptide loading. Thus, it appears that the MHC-bound peptides create or stabilize the conformational determinants necessary for the interaction between the T-cell receptor (TCR) and MHC receptor. The resulting structural unit of recognition is a trimolecular complex formed by the TCR, the MHC molecule, and the MHC-bound peptide.

While numerous studies suggest that the majority of T cells recognize allo-MHC + peptide complex, there is evidence that some alloreactive T cells can recognize empty MHC molecules. Human T-cell clones specific for the human class I HLA-A2 antigen were able to respond to empty HLA-A2 molecules [34]. Further studies using the RMA-S cell line, which lacks the peptide transporter gene, have demonstrated that CTLs can lyse targets expressing empty class I molecules [35,36].

In addition to the importance of the presence of peptides bound to the peptide-binding groove, conformational changes of the MHC–peptide complex may also be important. Bluestone *et al*. [14,37] demonstrated that conformational changes induced by the peptide bound to the peptide-binding groove in class I MHC can alter T-cell alloreactivity. Thus, alloreactivity of some T-cell clones may be peptide dependent but not peptide specific. The conformational determinants created by MHC binding to peptide may determine whether or not the TCR can interact with the MHC molecule. Two different peptides may produce similar conformational determinants within the peptide-binding groove of the same MHC. This concept would predict the observation of cross-reactive allorecognition to unrelated peptides, which is supported by the literature.

## INDIRECT PATHWAY OF ALLORECOGNITION

*Indirect* refers to T-cell recognition of nonself-MHC-derived peptides (allopeptides) in the context of self-MHC molecules expressed on recipient APCs. In this case, similar to the physiologic pathway of antigen recognition, the peptide sequence determines the response. Indirect presentation could occur through a number of mechanisms: soluble donor MHC molecules are shed from the graft, drain through the bloodstream or lymphatics to the recipient secondary lymphoid organs where they would be processed and/or presented by recipient APCs to recipient T cells. Alternatively, donor graft cells that migrate to recipient secondary secondary lymphoid organs could be endocytosed by recipient APCs. Third, recipient monocyte–macrophages entering the donor graft could endocytose donor antigens and present the peptides to recipient T cells. Interestingly, recent emerging data demonstrate that not only $CD4^+$ T cells, as traditionally thought, but also $CD8^+$ T cells can be primed through the indirect pathway of allorecognition and contribute to graft destruction [38].

Unlike the direct pathway, the indirect pathway requires previous priming to antigen. Moreover, the precursor frequency of T cells that can recognize a specific antigen through the indirect pathway is relatively low; 100–1000 times lower than that for directly alloreactive T cells. Until recently, most investigations in transplantation only addressed the direct pathway of allorecognition and very little was known about the contribution of the indirect pathway in graft rejection. The existence of the indirect pathway of allorecognition was originally suggested by Lechler and Batchelor [39,40] in the early 1980s based on rodent studies with passenger cell