CHAPTER 81

Immunobiology and Immunopharmacology of Renal Allograft Rejection

Choli Hartono • Terry B. Strom • Manikkam Suthanthiran

Renal transplantation is the treatment of choice for patients with irreversible renal failure and has moved from a high risk, experimental procedure to a safe, clinical procedure in the relatively short time of five decades.[1] The substantive gains in patient and graft survival owe much to an improved understanding of the antiallograft repertoire, better preservation of donor kidneys, judicious usage of immunosuppressive drugs and monoclonal/polyclonal antibodies, and the clinical application of infection prophylaxis protocols.

IMMUNOBIOLOGY OF RENAL TRANSPLANTATION

The Antiallograft Response

Allograft rejection is contingent on the coordinated activation of alloreactive T cells and antigen-presenting cells (APCs). 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 (Fig. 81.1 and Table 81.1).

T Cell Activation and the Immunologic Synapse: Signal One

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 crucial T cell activation-related signals.[2] The synapse begins to form when the initial adhesions between certain T cell (e.g., CD2, LFA-1) and APC surface proteins (e.g., CD58, ICAM-1) are formed (Table 81.2). These physical contacts between T cells and APCs provide an opportunity for the antigen reactive T cells to recognize cognate antigen. Antigen-driven T cell activation, a highly coordinated, preprogrammed process, begins when T cells recognize intracellularly processed fragments of foreign proteins (approximately 8 to 16 amino acids) embedded within the groove of the major histocompatibility complex (MHC) proteins expressed on the surface of APCs.[3–5] Some of the recipient's T cells directly recognize the allograft (i.e., donor antigen[s] presented on the surface of donor APCs) and this process is termed direct recognition whereas other T cells recognize the donor antigen after it is processed and presented by self-APCs[6] (Fig. 81.1) and this process is designated indirect recognition.

The T cell antigen receptor (TCR)–CD3 complex is composed of clonally distinct TCR α and β peptide chains that recognize the antigenic peptide in the context of MHC proteins and clonally invariant CD3 chains that propagate intracellular signals originating from antigenic recognition (Fig. 81.2).[2,7,8] The TCR variable, diversity, junction, and constant region genes (i.e., genes for regions of the clone-specific antigen receptors) are spliced together in a cassette-like fashion during T cell maturation.[7] A small population of T cells expresses TCR γ and δ chains instead of the TCR α and β chains.

CD4 and CD8 proteins, expressed on reciprocal T cell subsets, bind to nonpolymorphic domains of human leukocyte antigen (HLA) class II (DR, DP, DQ) and class I (A, B, C) molecules, respectively (Fig. 81.1 and Table 81.2).[2,7] A threshold of TCR to MHC-peptide engagements is necessary to stabilize the immunologic synapse stimulating a redistribution of cell-surface proteins and coclustering of the TCR/CD3 complex with the T cell-surface proteins.[8–10] Additional T cell surface proteins such as CD5 proteins join the synapse.[9,10] The TCR cluster already includes integrins (e.g., LFA-1) and nonintegrins (e.g., CD2)[2,8,9] that have created T cell–APC adhesions. Hence, antigen recognition stimulates a redistribution of cell-surface proteins and coclustering of the TCR/CD3 complex with the T cell-surface proteins[2,7–9] and signaling molecules. This multimeric complex functions as a unit in initiating T cell activation.

Following activation by antigen, the TCR–CD3 complex and coclustered CD4 and CD8 proteins are physically associated with intracellular protein–tyrosine kinases (PTKs)

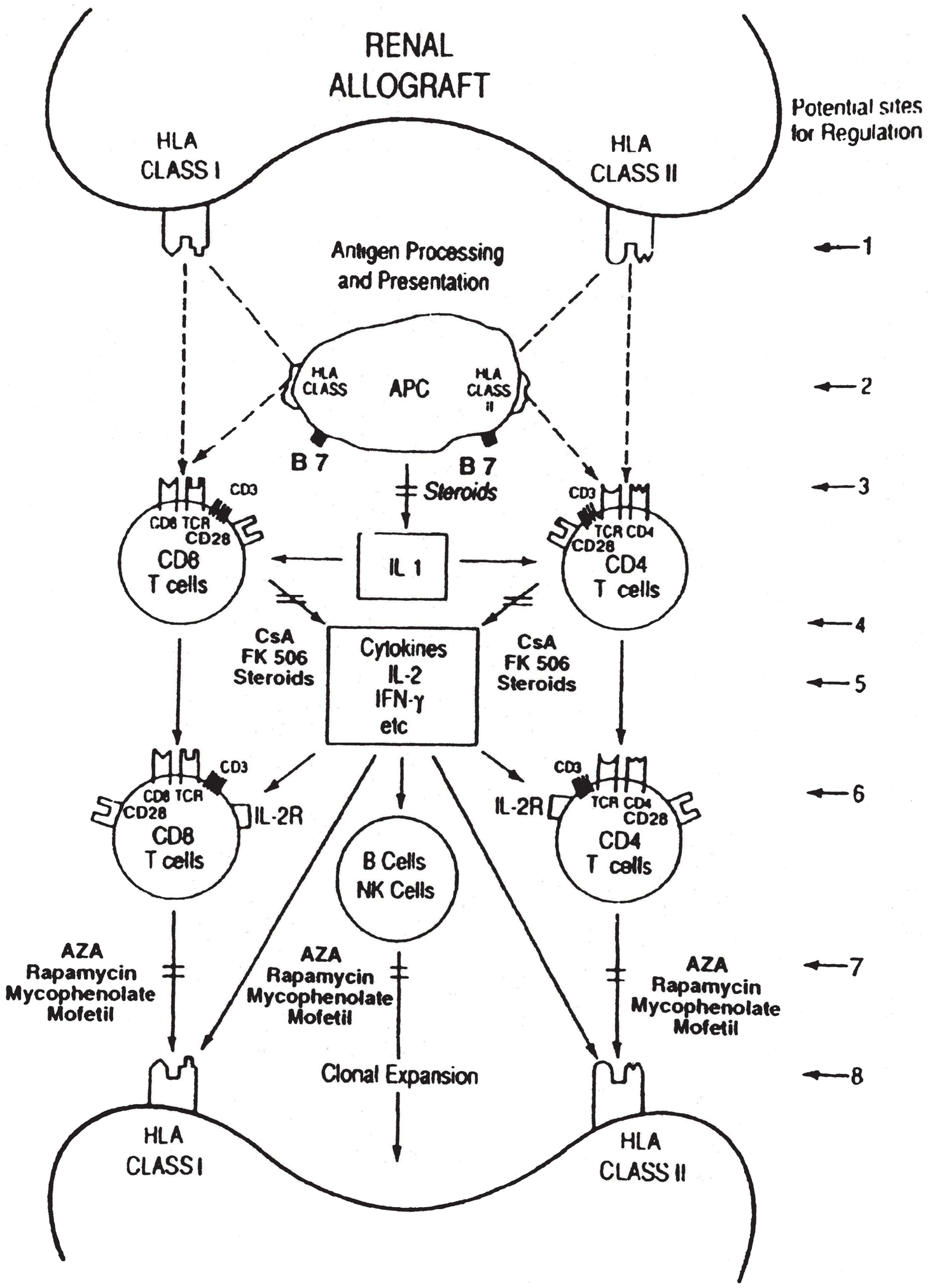

FIGURE 81.1 The antiallograft response. Schematic representation of human leukocyte antigens (HLA), the primary stimuli for the initiation of the antiallograft response, cell-surface proteins participating in antigenic recognition and signal transduction, contribution of the cytokines and multiple cell 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. *IFN-γ*, interferon-γ; *NK cells*, natural killer cells.

of two different families, the src (including $p59^{fyn}$ and $p56^{lck}$) and ZAP 70 families.[2] 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.[2,5]

The waves of tyrosine phosphorylation triggered by antigen recognition encompass other intracellular proteins and are 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 leads 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 (Fig. 81.2).[11] 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.[5,11] 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

81.1 Cellular Elements Contributing to the Antiallograft Response

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; 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 IFN-γ, a proinflammatory polypeptide that can upregulate the expression of HLA proteins as well as augment cytotoxic activity of T cells and NK cells. Recently, three main types of CD4+ T cells have been recognized: CD4+ TH1, CD4+ TH2, and CD4 TH17. IL-2 and IFN-γ are produced by CD4+ TH1 type cells, IL-4 and IL-5 are secreted by CD4+ TH2 type cells, and IL-17 family of cytokines CD4+CD17 cells. Each cell type can regulate 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 and IFN-γ 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; HLA, human leukocyte antigen; NK, natural killer; TCR, T cell antigen receptor.
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[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).[2,5,12]

Calcineurin, a Ca^{2+}- and calmodulin-dependent serine/threonine phosphatase, is crucial to Ca^{2+}-dependent, TCR-initiated signal transduction.[13,14] Inhibition by cyclosporine and tacrolimus (FK-506) of the phosphatase activity of calcineurin is considered central to their immunosuppressive activity.[15]

Costimulatory Signals: Signal Two

Signaling of T cells via the TCR/CD3 complex (signal one) is necessary, but insufficient, to induce T cell proliferation; full activation of T cells 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. 81.3 and Table 81.2).[16,17] 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,[18] and/or the interaction of the CD5 with the CD72 proteins[10] 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

81.2 Cell-Surface Proteins Important for T Cell Activation[a]

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

[a]Receptor/counterreceptor 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. Monoclonal antibodies directed at the T cell CD2 protein, used as component of a preconditioning regimen, has been associated with tolerance to histoincompatble human renal allografts.[23]

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

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powerful T cell costimulus.[19] A subset of monocytes and dendritic cells constitutively express CD80 and CD86 at low levels and cytokines (e.g., granulocyte–macrophage colony-stimulating factor [GMCSF] or interferon-γ [IFN-γ]) stimulate heightened expression of CD80 and CD86 on monocytes, B cells, and dendritic cells.[19] 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.[19] 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.[20] 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.[21]

The delivery of the antigenic first signal and the costimulatory second signal leads to stable transcription of the IL-2, several T cell growth-factor receptors, and other pivotal T cell activation genes (Table 81.2). The Ca^{2+}-independent costimulatory CD28 pathway is relatively more resistant to inhibition by cyclosporine or FK-506 as compared to the calcium-dependent pathway of T cell activation. Whereas the interactions between B7 proteins and its counter receptor CD28 result in positive costimulation, the interactions between B7 proteins by CTLA-4, a protein primarily expressed on activated T cells, result in the generation of a negative signal to T cells. This coinhibitory signal is a prerequisite for peripheral T cell tolerance.[22]

The formulation that full T cell activation is dependent on the costimulatory signal, as well as the antigenic signal, is most significant, as T cell molecules responsible for costimulation and their cognate receptors on the surface

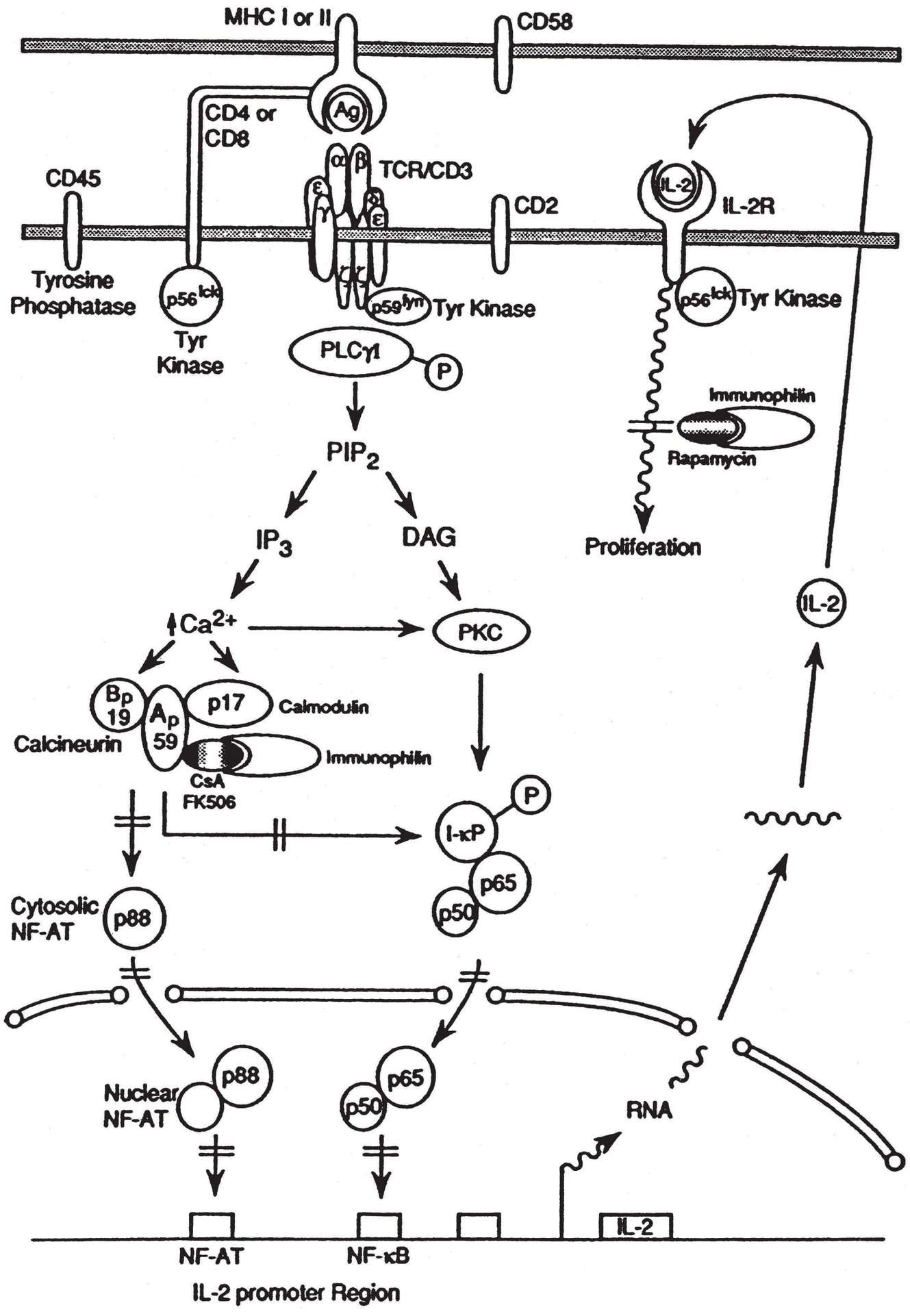

FIGURE 81.2 Signal transduction in T cells and mechanisms of action of cyclosporin A (CsA), FK-506, or rapamycin. Signaling molecules and transmembrane signaling events participating in the transduction of antigenic signals from the plasma membrane of the T cells to the nucleus are schematically shown. The sites of action of the drug (CsA/FK-506/rapamycin)–immunophilin complex are also shown. *Ag,* antigen; *Ap59* and *Bp19,* subunits of calcineurin; *DAG,* diacylglycerol; *I-κB,* inhibitory factor kappa B; *IL-2,* interleukin-2; *immunophilin,* cyclophilin or FK-binding protein; IP_3, inositol 1,4,5-triphosphate; *MHC,* major histocompatibility complex; *NF-AT,* nuclear factor of activated T cells; *NF-κB,* nuclear factor kappa B; *P,* phosphotyrosine; PIP_2, phosphatidylinositol 4,5-biphosphate; *PKC,* protein kinase C; *PLCγ1,* phospholipase C gamma-1; *Tyr kinase,* tyrosine kinase. (Adapted from Schreier MH, Baumann G, Zenke G, et al. Inhibition of T-cell signaling pathways by immunophilin drug complexes: Are side effects inherent to immunosuppressive properties? *Transplant Proc* 1993;25:502.)

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, and tolerance to histoincompatible human kidney allografts has been accomplished with a conditioning regimen that includes monoclonal antibodies directed at the CD2 protein.[23]

Interleukin-2/Interleukin-15 Stimulated T Cell Proliferation

Autocrine type of 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 (Fig. 81.2) formed by the noncovalent association of three IL-2-binding peptides (α, β, γ).[12,24–26] 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.[12] 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.[12,27–29] Raf-1, a protein serine/threonine kinase associates with the intracellular domain of the shared β chain,[30] and this association and the kinase activity are prerequisites to IL-2/IL-15-triggered cell proliferation. Translocation of IL-2 receptor-bound Raf-1 serine/threonine kinase into the cytosol requires IL-2/IL-15-stimulated

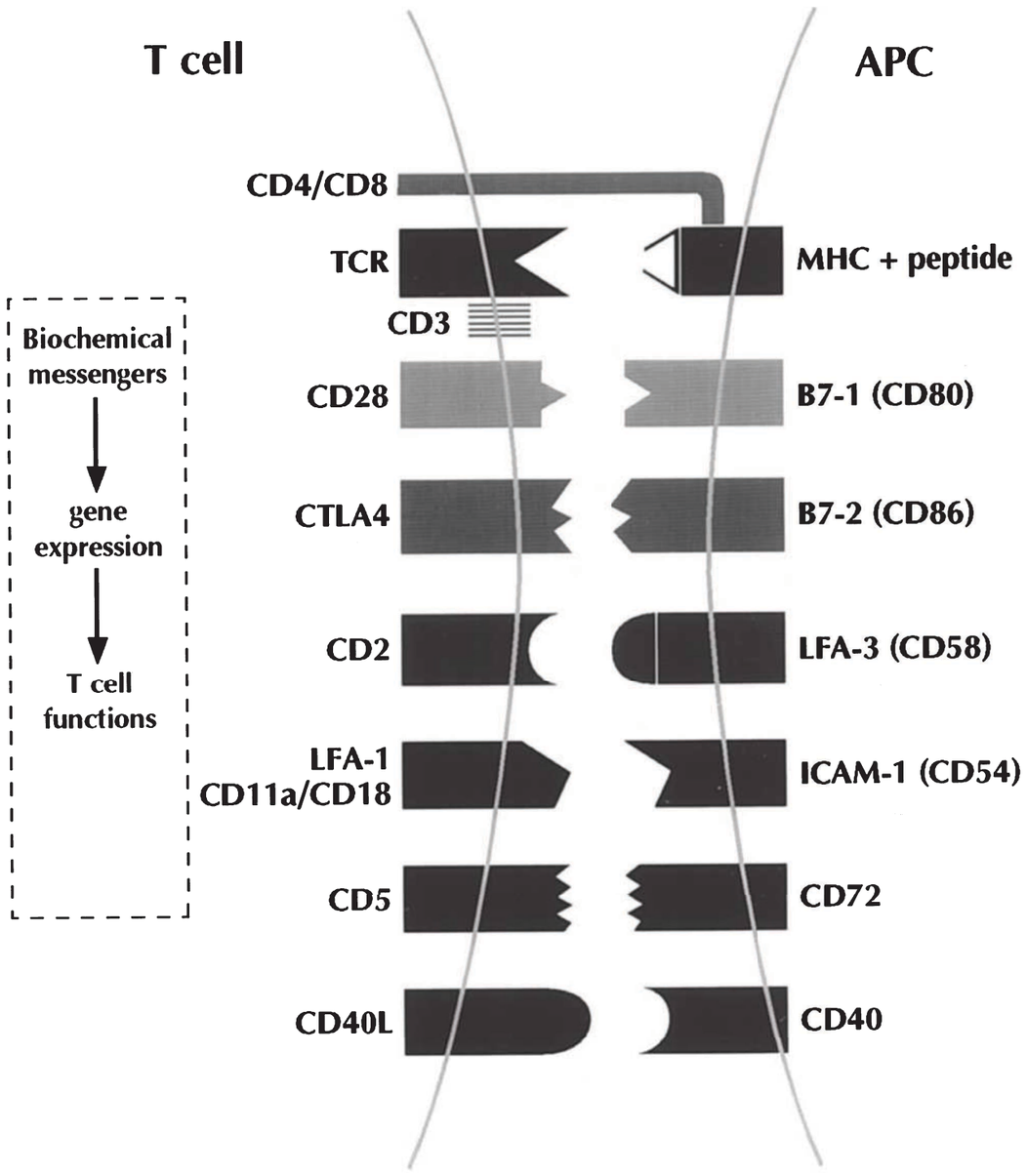

FIGURE 81.3 T cell/antigen-presenting cell contact sites. In this schema of T cell activation, the antigenic signal is initiated by the physical interaction between the clonally variant T cell antigen receptor (TCR) α-, β-heterodimer, and the antigenic peptide displayed by MHC on antigen-presenting cells (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 counterreceptors 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 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 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. (From Suthanthiran M. Transplantation tolerance: fooling mother nature. *Proc Natl Acad Sci USA.* 1996;93:12072.)

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 essential for the 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.[31,32] 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 may be more detrimental to the antiallograft response than those initiated by IL-2. As IL-15 is not produced by T cells, IL-15 expression is not regulated by cyclosporine or tacrolimus.

Humoral Rejection

Antibody-mediated rejection (AMR) is a form of humoral rejection wherein antibodies directed at the donor HLA antigens (DSAs) serve as the main effector for the immune response directed at the allograft. Antibodies directed at non-HLA antigens such as endothelial cell associated antigens and MHC class I-related chain A antigens (MICA) have also been implicated in the pathogenesis of AMR. Whereas most acute T cell mediated rejections (TMRs) are responsive to steroid therapy, AMR is typically steroid-resistant and requires additional treatment such as plasmapheresis, anti-B cell, and intravenous immunoglobulin (IVIG) therapy. The incidence of AMR has been estimated at less than 10% but appears to be on the rise due to multiple reasons including acute TMR being effectively prevented by current immunosuppressive regimens, better definition of AMR, and transplantation of individuals with humoral presenitization and repeat transplants. Patients with AMR invariably harbor anti-HLA DSA although, in certain cases, histopathologic evidence of AMR may be apparent without any anti-HLA DSA. Acute AMR may occur within 1 week after engraftment even in the setting of antithymocyte globulin induction therapy. The diagnosis of AMR requires the presence of C4d complement staining in the peritubular capillaries in addition to peritubular capillary inflammation with polymorphonuclear and mononuclear leukocytes or the presence of fibrinoid changes/transumural arterial inflammation or acute tubular necrosis (ATN)-like tissue injury.[33] In the current Banff classification schema, those who present with histolgic features consistent with AMR but without concurrent intragraft C4d deposition or circulating DSA are classified as supicious for AMR—it is possible that the offending antibodies may be of the noncomplement fixing IgG subtypes and/or non-HLA antibodies (because most screening assays for DSA utilize HLA as target antigens).

A novel form of humoral rejection has also been documented. Antibodies directed against two epitopes of the angiotensin II type I (AT_1) receptor have been associated with refractory vascular allograft rejection in a series of 16 patients and these patients did not have anti-HLA antibodies at the time of incident humoral rejection.[34]

Immunobiology and Molecular Diagnosis 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

and antibody producing B cells/plasma cells (Fig. 81.1). Cytokines facilitate not only the T cell effector arm and TCR but also the B cell/plasma cell arm by promoting the production of cytopathic antibodies. Moreover, cytokines such as IFN-γ and tumor necrosis factor-α (TNF-α) 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 (Fig. 81.1). 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.[35,36] We have detected messenger RNA (mRNA) encoding the CTL-selective serine protease (granzyme B), perforin, Fas-ligand attack molecules, and immunoregulatory cytokines, such as IL-10 and IL-15, in human renal allografts undergoing acute rejection.[37] Indeed, these gene expression events may 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[38] or urinary leukocytes[39] rejection-related, gene expression events evident in renal biopsy specimens are robustly detected in peripheral blood or urinary sediment specimens. Initial results from large-scale multicenter trials (e.g., Clinical Trials in Organ Transplantation, CTOT-04) support the hypothesis that noninvasive diagnosis of acute TMR is feasible by measurement of genes encoding cytotoxic attack molecules in urine, and the urinary cell mRNA profiles may anticipate the future development of acute TMR.[40] We speculate as well that a noninvasive, molecular diagnostic approach to rejection would be of value toward the detection of insidious, clinically silent rejection episodes that, although rarely detected through standard measures, are steroid-sensitive but usually lead to chronic rejection.[41]

Immunopharmacology of Allograft Rejection

Glucocorticosteroids

Glucocorticosteroids inhibit T cell proliferation, T cell-dependent immunity, and cytokine gene transcription (including IL-1, IL-2, IL-6, IFN-γ, and TNF-α gene).[42–44] Although no single cytokine can reverse the inhibitory effects of corticosteroids on mitogen-stimulated T cell proliferation, a combination of cytokines is effective.[45] The glucocorticoid and glucocorticoid–receptor bimolecular complex block IL-2 gene transcription via impairment of the cooperative effect of several DNA-binding proteins.[46] Corticosteroids also inhibit formation of free NF-κB, a DNA-binding protein required for cytokine and other T cell-activation gene expression events (Fig. 81.1 and Table 81.3).[47]

Azathioprine

Azathioprine (AZA), a thioguanine derivative of 6-mercaptopurine,[48] is a purine analog, acts as a nonspecific inhibitor of purine biosynthesis, and is an effective antiproliferative agent (Fig. 81.1 and Table 81.3).[48,49] In a randomized conversion trial from mycophenolate mofetil (MMF) to AZA in 48 stable kidney transplant recipients at 6 months following engraftment, it was observed that acute rejection rates

TABLE **81.3** **Mechanisms of Action of Small Molecule Immunosuppressants**[a]

Immunosuppressant	Subcellular Site(s) of Action
Azathioprine	Inhibits purine synthesis
Corticosteroids	Blocks cytokine gene expression
CsA/tacrolimus	Blocks Ca^{2+}-dependent T cell activation pathway via binding to calcineurin
Mycophenolate mofetil	Inhibits inosine monophosphate dehydrogenase and prevents de novo guanosine and deoxyguanosine synthesis in lymphocytes
Sirolimus/everolimus	Blocks IL-2 and other growth factor signal transduction; blocks CD28-mediated costimulatory signals
Leflunomide/FK778	Inhibits dihydroorotate dehydrogenase—a key enzyme for de novo pyramidine biosynthesis
FTY720	Phosphorylated FTY720 binds sphingolipid 1-phosphate receptor and prevents S1P signaling of cells; sequestration of lymphocytes within the lymph nodes and prevention of cell egress into the peripheral circulation

CsA, cyclosporin A; IL, interleukin.

were comparable (4.5% vs. 3.8%) after a 6-month observation period in the MMF (n = 22) or AZA (n = 26) arm. The trial participants received cyclosporine and prednisone as maintenance immunosuppressive therapy and antithymocyte globulin induction was used in 27% of the recipients maintained on MMF and 46% in the AZA conversion group. It is worth noting that high-risk patients including retransplant recipients, highly sensitized, and those with a history of steroid-resistant rejection were all excluded from the trial.[50]

The Calcineurin Inhibitors: Cyclosporine and Tacrolimus (FK-506)

Cyclosporine, a small cyclic fungal peptide, and FK-506, a macrolide antibiotic, block the Ca^{2+}-dependent antigen triggered T cell activation (signal one) (Fig. 81.2).[51] The immunosuppressive effects of cyclosporine and FK-506 are dependent on the formation of a heterodimeric complex that consists of the drug cyclosporine or FK-506 and its respective cytoplasmic receptor "immunophilin" proteins, cyclophilin and FK-binding protein (FKBP), respectively. The heterodimeric cyclosporine–cyclophilin complex and the FK-506–FKBP complex target and bind calcineurin and inhibit its phosphatase activity (Table 81.3). The inhibition of the enzymatic activity of calcineurin is considered central to the immunosuppressive effects of cyclosporine and FK-506.

One of the well-documented consequences of calcium/calmodulin dependent activation of calcineurin is dephosphorylation of cytoplasmic NF-AT in T cells, import of NF-AT into the nucleus, binding of NF-AT with its nuclear partmer, and transcription of the IL-2 gene. The cyclosporine–FK-506 mediated inhibition of phosphatase activity of calcineurin results in the lack of dephosphorylation of cytoplasmic NF-AT and retention of the phosphorylated NF-AT in the cytoplasm. In addition to inhibiting the expression of NF-AT, cyclosporine also inhibits other DNA-binding proteins, such as NF-κB and AP-1.[52]

The phosphorylation status of transcription factors can also affect their DNA binding ability and interaction with the rest of the transcriptional machinery. For example, the DNA binding activities of c-jun increase upon dephosphorylation.

Blockade of cytokine gene activation does not totally account for the antiproliferative effect of cyclosporine and FK-506. It is significant that cyclosporine as well as FK-506, in striking contrast to their inhibitory activity on the induced expression of IL-2, enhance the expression of transforming growth factor-β (TGF-β).[53,54] Because TGF-β is a potent inhibitor of T cell proliferation and generation of antigen-specific CTL,[55] heightened expression of TGF-β must contribute to the antiproliferative/immunosuppressive activity of cyclosporine/tacrolimus. This TGF-β inducing effect of cyclosporine/tacrolimus also suggests a mechanism for some of the complications (e.g., renal fibrosis and tumor metastasis) of therapy with calcineurin inhibitors, because TGF-β is a fibrogenic and proangiogenic cytokine.

Mycophenolate Mofetil and Enteric-Coated Mycophenolate Sodium

MMF is a semisynthetic derivative of mycophenolic acid (MPA). MMF inhibits allograft rejection in rodents, diminishes proliferation of T and B cells, decreases generation of cytotoxic T cells, and suppresses antibody formation.[56–58] MMF inhibits inosine monophosphate dehydrogenase (IMPDH), an enzyme in the de novo pathway of purine synthesis. Lymphocytes are dependent on this biosynthetic pathway to satisfy their guanosine requirements (Table 81.3).[58] Early clinical trials have utilized MMF to replace azathioprine in the cyclosporine- and steroid-based immunosuppressive regimen. These controlled, prospective trials have shown a diminished incidence of early acute rejection episodes.[58–60] Although follow-up studies over a 3-year period have indicated an advantage for MMF over azathioprine,[60] a recent randomized trial comparing MMF with azathioprine in recipients of a first kidney transplant from a deceased donor found similar levels of acute rejection in the first 6 months of transplantation.[61]

Enteric-coated mycophenolate sodium (EC-MPS) was developed to improve the gastrointestinal tolerability of MPA. An international phase III, randomized, double-blinded, parallel group trial demonstrated the therapeutic equivalence of MMF and EC-MPS.[62] The two parallel groups received equivalent concomitant antibody induction, corticosteroids, and calcineurin inhibitor (CNI) therapy. At 12 months, the incidence of acute rejection, graft loss, and death was comparable for both treatment groups. Interestingly, in the phase III pivotal trial gastrointestinal complications were not significantly different between MMF and EC-MPS. Within 12 months of enrollment, dose changes were required for gastrointestinal adverse events in 19.5% versus 15% of subjects (P = not significant [NS]) in the MMF and EC-MPS groups, respectively.[62]

Sirolimus (Rapamycin)

Rapamycin[63–65] is a macrocyclic lactone isolated from Streptomyces hygroscopicus that, like FK-506, binds to FKBP. However, rapamycin and FK-506 affect different and distinctive sites in the signal transduction pathway (Fig. 81.2 and Table 81.3). Whereas rapamycin blocks IL-2 and other growth factor-mediated signal transduction, FK-506 (or cyclosporine) has no such capacity. Also, the rapamycin–FKBP complex, unlike the FK-506–FKBP complex, does not bind calcineurin. The antiproliferative activity of the rapamycin–FKBP complex is linked to blockade of the activation of the 70-kDa S6 protein kinases and blockade of expression of the bcl-2 proto-oncogene. Rapamycin also blocks the Ca^{2+}-independent CD28-induced costimulatory pathway. Substitution of rapamycin for azathioprine in a triple-therapy regimen

reduced the frequency and severity of acute rejection.[66] The CONVERT trial, an international randomized, prospective, open-label study tested the efficacy and safety of converting CNI-based maintenance therapy for renal transplant recipients to a sirolimus-based CNI-free immunosuppressive regimen.[67] The mean Nankivell glomerular filtration rates (GFRs) at 12 months were 63.6 mL per min vs. 61.1 mL per min ($P = .006$) and at 24 months were 62.6 mL per min vs. 59.9 mL per min ($P = .009$) in the converted CNI-free group. The rejection, graft survival, and patient survival rates were similar in both CNI and sirolimus groups. The malignancy rates were significantly lower after conversion to sirolimus (3.8% vs. 11.0%, $P < .001$). The mean urinary protein/creatinine ratios or calculated daily proteinuria at 24 months was higher after sirolimus conversion when compared to baseline (mean ± standard deviation [SD], 0.72 ± 1.50 vs. 0.04 ± 0.04, $P < .001$). In the Spare-the-Nephron trial, 299 patients were randomized to MMF/CNI or MMF/sirolimus.[68] Iothalamate estimation of GFR at 1 year showed the mean percentage improvement from baseline of GFR was higher in the MMF/sirolimus group when compared to the MMF/CNI group (24.4% vs. 5.2%, $P = .012$). The percentage change in GFR from baseline at 2 years remained higher in the CNI-free group but was not statistically significant (8.6% vs. 3.4%, $P = .54$).

Everolimus (RAD)

Everolimus is a derivative of rapamycin. The use of everolimus in phase II clinical trials involving cyclosporine, steroids, and basiliximab induction resulted in excellent graft survival at 36 months.[69] In a short-term phase III trial, everolimus was comparable to MMF with cyclosporine and steroids in preventing acute rejection.[70] The U.S. Food and Drug Administration (FDA) approved everolimus in 2010 for the prevention of kidney transplant rejection following its approval in 2009 for the treatment of advanced renal cell carcinoma in patients who have failed sunitinib or sofrafenib therapy.

Leflunomide

Leflunomide is a synthetic isoxazole derivative that inhibits dihydroorotate dehydrogenase—a key enzyme for de novo pyrimidine synthesis. It belongs to the family of drugs known as malonitrilamides and is currently approved for the treatment of rheumatoid arthritis. Leflunomide has antiviral effects against cytomegalovirus, herpes simplex virus type 1, and polyomavirus (BK virus).[71–73] A short-term, open-label, prospective crossover trial of leflunomide comprised of 22 patients with chronic renal allograft dysfunction found 100% patient survival and 91% graft survival at 6 months posttransplantation, and was well tolerated, with anemia being the most common adverse effect.[74]

FK778

FK778 is an analog of the active metabolite (A771726) of leflunomide. A phase II European multicenter randomized, double-blind, and FK778 dose-controlled trial was performed in 149 renal transplant recipients divided into three groups: group 1, high-level FK778/tacrolimus/steroids; group 2, low- level FK778/tacrolimus/steroids; and group 3, placebo/tacrolimus/steroids.[75] The incidence of acute rejection in groups 1, 2, and 3 were 28.6%, 25.9%, and 34.8%, respectively, and patients who reached target levels had a lower incidence of acute rejection. Anemia was a commonly reported complication and was observed in 43% in group 1, 31% in group 2, and 20% in group 3. A phase II randomized, open-label, two-arm, parallel-group, multicenter trial tested the efficacy of FK778 against BK nephropathy in comparison to standard of care (reduction of immunosuppression). The treatment group had a statistically significant reduction in BK viremia but without significant improvement in renal function or histology based on the Drachenberg criteria. When compared to the standard of care, the FK778 treated group also experienced multiple rejection epidoses and had a higher incidence of biopsy-proven acute rejection.[76]

Muromonab–CD3 (OKT3)

OKT3 is a murine monoclonal antibody directed against the CD3 component of the T cell receptor complex. It was initially tested for its efficacy as an antirejection agent and was found to be superior to corticosteroids in the treatment of acute rejection of renal allografts. Later, OKT3 was utilized as an induction agent in renal transplantation and for the treatment of steroid-resistant acute rejection. The OKT3 associated first dose reaction as a result of cytokine release may be severe and include fever, chills, respiratory symptoms, and headaches. Currently, OKT3 has lost favor with the transplant community primarily because of the first dose reaction and because of the availability of other induction agents. A humanized preparation (HuM291) that potentially reduces cytokine release reaction is being investigated[77] and may restore CD3–directed therapy in organ transplanttion.

Antithymocyte Globulin

Immunizing either rabbits or equines with human thymocytes produces antithymocyte globulin preparations. The antibodies generated are polyclonal in nature and are directed against several cell-surface antigens including: CD2, CD3, CD4, CD8, CD11a, CD18, CD25, CD44, CD45, HLA-DR, and HLA class I heavy chain.[78] Antithymocyte globulin (ATG) preparations are used both as an induction agent, especially in high-risk renal transplant recipients, and for the treatment of acute rejection. In a steroid rapid-withdrawal protocol using calcineurin inhibitor and MMF or sirolimus, rabbit ATG was selected as the induction agent for low-risk, mostly Caucasian, renal transplant recipients and the actuarial acute rejection-free graft survival was 92% at 3 years.[79] The surviving peripheral T cell subsets were analyzed in five patients following antibody-mediated T cell depletion therapy with ATG.[80] The study found a significant reduction in the absolute lymphocyte population but heterogeneity in the degree of depletion of T cell subsets. Whereas both CD8+ naïve T cells

and CD4+ naïve T cells were depleted by over 98%, both CD4+CD25+ T cell subset and CD4+CD45RA-CD62L- T cell subset were only depleted by 90% (P = .001). The CD4+CD45RA-CD62L-effector memory T cells represented 88 ± 3% of the postdepletion resistant T cell subset in contrast to their usual prevalence of 10% to 20% in normal volunteers. These memory T cells may potentially serve as the progenitor cells for mounting an immune response against the allograft even in the setting of lymphopenia. The CD4+CD25+ may include regulatory T cells and their relative sparing may be of significance for counter-regulating the anti-allograft response. Clearly more data with a larger cohort are needed but the initial implication of the study is that T cell depletion therapy with ATG has differential effects on T cell subsets with some beneficial (a sparing of regulatory T cells) and some detrimental (lack of full depletion of memory T cells).

Interleukin-2 Receptor Antagonists: Basiliximab and Daclizumab

IL-2 receptor antagonists (IL-2Ra) inhibit allograft rejection by competitively binding CD25 antigen (IL-2 receptor α chain or Tac subunit) on activated T lymphocytes. Both basiliximab (chimeric human/murine monoclonal $IgG_{1\kappa}$) and daclizumab (humanized monoclonal IgG1) are commonly utilized as induction agents for renal transplantation. A meta-analysis of clinical trials involving monoclonal antibodies directed at CD25 showed that, when combined with standard double or triple immunosuppressive regimens, the use of these antibodies reduced the incidence of acute rejection by 34% and by 49% the incidence of steroid-resistant rejection.[81] The meta-analysis also showed that the efficacy of anti-CD25 monoclonal antibodies in preventing acute rejection was similar to that of OKT3 and that of polyclonal antibody preparations and, importantly, with fewer side effects. A large prospective randomized international trial tested the efficacy of a 5-day course of ATG versus two doses of basiliximab induction therapy in 278 deceased-donor renal transplants at risk for acute rejection or delayed graft function.[82] Participants in the ATG group (n = 141) had lower incidence of acute rejection at 12 months when compared to the basiliximab group (n = 137) (15.6% vs. 25.5%). However, the incidence of delayed graft function (40.4% vs. 44.5%), death (4.3% vs. 4.4%), as well as graft loss (9.2% vs. 10.2%), were similar in the two groups.

Alemtuzumab

Alemtuzumab is a humanized monoclonal $IgG_{1\kappa}$ directed against CD52, which is a glycoprotein expressed on B and T cells, natural killer (NK) cells, monocytes, and macrophages. It is approved by the FDA for the treatment of B cell chronic lymphocytic leukemia. In a pilot study of 29 primary renal transplant recipients treated with alemtuzumab and sirolimus monotherapy, profound and sustained depletion of lymphocytes was observed; however, 8 of 29 patients developed acute rejection with 7 requiring conversion to standard triple therapy.[83] In an investigation of 44 renal allograft recipients treated with alemtuzumab, tacrolimus (trough levels of 5 to 7 ng per mL), and MMF (500 mg twice a day), and with a median follow-up of 9 months, four patients developed acute rejection and four developed infection and the patient and graft survival rates were 100%.[84] The use of alemtuzumab induction was associated with an elevated serum B-cell activating factor (BAFF) level in kidney transplantation and may increase the risk of humoral rejection in the absence of concomitant CNI maintenance therapy.[85]

The INTAC study group tested alemtuzumab against conventional induction agents in a randomized prospective multicenter trial.[86] High risk participants received rabbit ATG whereas low risk participants in the trial received basiliximab conventional induction therapy. All participants received tacrolimus and MMF maintenance immunosuppression with rapid steroid discontinuation after 5 days of therapy. When compared to basiliximab, alemtuzumab induction in the low risk group resulted in a significantly lower rate of biopsy-proven acute rejection at 6 months (2% vs. 18%, P < .001), 12 months (3% vs. 20%, P < .001), and 36 months (10% vs. 22%, P ≤ .003). When compared to rabbit ATG, alemtuzumab induction in the high-risk group resulted in equivalent rates of biopsy-proven acute rejection at 6 months (6% vs. 9%; P = .49), 12 months (10% vs. 13%; P = .53), and 36 months (18% vs. 15%; P = .63). Patient survival at 3 years was similar between alemtuzumab and basiliximab or ATG in the low risk (95% vs. 98%; P = .19) or high risk (99% vs. 91%; P = .07) groups. After censoring for deaths, graft survival at 3 years was similar between alemtuzumab and basiliximab or ATG in the low-risk (97% vs. 94%; P = .17) or high-risk (91% vs. 84%; P = .32) groups. However, the rates of late biopsy-proven acute rejection (between 12 and 36 months) were higher in the alemtuzumab cohort when compared to participants in the conventional induction cohorts (8% vs. 3%, P = .03) thus suggesting that surveillance for late rejection is important when using alemtuzumab as an induction agent.

Rituximab

Rituximab is a chimeric murine/human monoclonal $IgG_{1\kappa}$ directed against the CD20 antigen expressed on the surface of B cells. It was FDA approved for the treatment of CD20-positive, B cell non-Hodgkin lymphoma. Initial experience with rituximab has shown promising results in the treatment of steroid-resistant acute renal allograft rejection.[87] Rituximab has also been used as a component of a preconditioning regimen to prepare patients for renal transplantation from ABO incompatibile donors.[88] In an open-label, controlled trial randomizing rituximab against daclizumab induction, the study was closed after excessive acute rejection episodes in the rituximab arm (first five of six patients) in the initial 3 months after transplant (83% vs. 14%). Both arms received steroid-free maintenance with tacrolimus and MMF. All episodes of rejection responded to intravenous methylprednisolone and the GFR was similar at 1 year in the 2 arms (44.4 ± 8.1 vs.

48.9 ± 10.6 mL/min/1.73 m^2). The authors hypothesized that rituximab therapy, by disrupting regulatory B cells and transiently increasing the release of inflammatory cytokines, contributed to an anti-allograft immune response.[89]

Intravenous Immune Globulins

IVIG is used to treat a variety of autoimmune diseases based on its immodulatory effects.[90] In the renal transplantation arena, IVIG is being utilized to reduce humoral immunity in two distinct settings: (1) to reduce the level of preexisting anti-HLA antibodies and convert a positive crossmatch recipient to a negative crossmatch recipient,[91] and (2) to treat humoral rejection.[92] It has been reported that a combination desensitizing regimen of IVIG and rituximab facilitated, in a safe manner, rapid transplantation of 16 of 20 highly sensitized patients.[93]

FTY720

FTY720 is a synthetic analog derived from the ascomycete Isaria sinclairii. The phosphorylated metabolite, FTY720-phosphate, is the biologically active compound. FTY720 affects the normal trafficking of lymphocytes and prevents their transmigration from lymph nodes to the allograft by binding lymphocytic sphingolipid 1-phosphate $(S1P)_1$ receptors. This process prevents the signaling of lymphocytes by serum S1P and the egress into the periphery in response to systemic inflammation.[94] In the first human trial of FTY720 in stable renal allograft recipients, transient but asymptomatic bradycardia was noted following 10 of 24 doses examined.[95] In a randomized, multicenter, double-blind, and placebo-controlled, phase I study of stable renal allograft recipients, a dose-dependent decrease in peripheral blood lymphocytes was observed.[96] A phase IIA trial comparing FTY720 to MMF in combination with cyclosporine and steroids in de novo renal transplant recipients showed equivalent efficacy and safety with regard to prevention of acute rejection.[97] A 12-month phase III international randomized trial compared three groups of patients: group 1, reduced dose cyclosporine and FTY720 (5 mg); group 2, full dose cyclosporine and FTY720 (2.5 mg); and group 3, MMF and full dose cyclosporine.[98] All study patients received corticosteroids as part of the maintenance regimen. Participants in group 1 had a prohibitively higher risk of acute rejection and the group was discontinued from the trial on the recommendation by the study Data Safety Monitoring Board (DSMB). Participants in groups 2 and 3 had similar rates of acute rejection at 22%. Analysis of a composite efficacy endpoint showed that group 2 did not achieve statistical noninferiority compared to group 3. The rate of discontinuation of study drugs was also higher in group 2 versus group 3 with patients receiving FTY720 having an increased incidence of macular edema. A second phase III trial failed to demonstrate any benefit of combining FTY720 and reduced dose cyclosporine when compared to the MMF-based standard of care regimen for de novo kidney transplant recipients.[99]

Belatacept

Belatacept (BMS-224818) is a fusion protein of cytotoxic T lymphocyte-associated antigen 4 and Fc piece of immunoglobulin (CTLA4Ig) and was designed to block the B7/CD28 costimulatory pathway. A phase II trial comparing belatacept to cyclosporine (in a regimen consisting of basiliximab, MMF, and corticosteroids) yielded promising results in preventing acute rejection of renal allografts.[100] Phase III trials, BENEFIT and BENEFIT-EXT, were conducted to test the effectiveness of belatacept as part of a CNI-free regimen.[101,102] The BENEFIT study was a 3-year randomized, parallel group designed trial conducted at 100 transplant sites worldwide. Following basiliximab induction, participants were randomized to one of three groups consisting of a more intensive belatacept regimen, a less intensive belatacept regimen, or cyclosporine with the addition of maintenance MMF and corticosteroids. The incidence of acute rejection at 12 months was higher in the belatacept groups (22% and 17%) compared to the cyclosporine group (7%). More participants also developed type IIa and IIb rejections in the belatacept cohorts but without an increase in donor-specific antibody production when compared to the cyclosporine-treated group. The mean GFR was superior at 12 months for the belatacept groups compared to the cyclosporine group. The BENEFIT-EXT trial was a 3-year randomized, multicenter trial performed at 79 transplant sites worldwide to test the benefit of a CNI-free regimen containing belatacept in patients undergoing high risk transplant from expanded criteria donors. Following basiliximab induction, participants were randomized to one of three groups consisting of a more intensive belatacept regimen, a less intensive belatacept regimen, or cyclosporine with the addition of maintenance MMF and corticosteroids. The incidence of acute rejection was not different among the three groups but CNI-free belatacept regimens resulted in more type IIb rejections. The mean GFR was significantly higher at 12 months for the more intensive belatacept group (52.1 mL/min/1.73 m^2) but not significant for the less intensive belatacept group (49.5 mL/min/1.73 m^2) compared to the cyclosporine group (45.2 mL/min/1.73 m^2). Both the BENEFIT and BENEFIT-EXT trials showed that neither the more intensive nor less intensive belatacept regimens were noninferior to cyclosporine on patient and graft survival. In the 2-year follow-up report, the salutary effects on GFR remained apparent for both the BENEFIT and BENEFIT-EXT trials with 16 to 17 mL per min and 8 to 10 mL per min higher GFR observed in the belatacept cohort of both trials when compared to the CNI group.[103] Belatacept was approved by the FDA in 2011 for the prophylaxis of organ rejection in kidney transplant recipients.

Alefacept

Alefacept, LFA3-Ig, is a dimeric fusion protein made by linking the CD2 binding portion of the human lymphocyte function-associated antigen-3 (LFA-3) to the Fc portion of human IgG1. In addition to pretransplant whole blood

donor-specific transfusion (DST), costimulatory blockade using CTLA4-Ig for 8 weeks and sirolimus for 90 days, alefacept given weekly for 8 weeks was able to significantly prolong renal allograft survival in rhesus monkeys when compared to control animals.[104] LFA3-Ig and CTLA4-Ig combination therapy given to the animals prevented the development of alloantibodies and five of eight treated monkeys had greater than 90 days of rejection-free period.[104] A phase II randomized, open-label, parallel group, multicenter trial has recently been completed to test alefacept in de novo kidney transplant using tacrolimus, MMF, and steroids.[105] The primary endpoint was incidence of biopsy proven acute rejection (BPAR) at 6 months. The trial enrolled 309 subjects randomized to the following four arms: control (basilixmab induction with full dose tacrolimus, MMF, and steroids), alefacept/low dose tacrolimus/MMF/steroids (A), alefacept/full dose tacrolimus/steroids (B), and every other week alefacept/low dose tacrolimus/MMF/steroids (C). The authors found that the incidence of BPAR was significantly higher in group A when compared to the control arm (26.3% vs. 12.7%; $P < .05$) whereas the MMF replacement arm (B) and group C had similar rates of BPAR when compared to the control arm (18.8% and 16.7%, respectively). At six months, patient and graft survival as well as renal function were similar in all groups.[105]

Janus Kinase (JAK)3 inhibitor

JAK3 inhibitor (CP-690,550) inhibits the tyrosine kinase required for signal transduction downstream of cytokine receptors and is important for the activation and function of T-cells as well as NK cells. A phase IIA randomized, open-label, multicenter trial was conducted in de novo kidney transplant recipients.[106] Following induction with monoclonal antibodies directed at the IL-2Ra, participants were randomized to lower dose JAK3 inhibitor (CP15), higher dose JAK3 inhibitor (CP30), and tacrolimus along with maintenance MMF and corticosteroids. The trial was converted into an exploratory study without sufficient power to address its primary and secondary objectives due to four cases of BK nepropathy (BKN) that occurred in the CP30 group. The CP15 group had a similar rejection rate when compared to the tacrolimus control group. Paradoxically, the CP30 group had a higher rejection rate than the tacrolimus control group. In this trial, combination JAK3 inhibition with MMF therapy yielded excessive viral opportunistic infections such as BKN and CMV disease.

Efalizumab

Efalizumab is a humanized IgG1 anti-CD11a monoclonal antibody approved by the FDA for treatment of psoriasis. LFA-1, a member of the heterodimeric B2 integrin family and adhesion molecule on T cell, interacts with ICAM-1 (CD54) on APC to facilitate T-cell activation. CD11a and CD18 constitute the alpha and beta chains of LFA-1. Although an early phase II study in kidney transplants was promising, a voluntary phased withdrawal of the product in the U.S. market was instituted in 2009 due to increased risk of progressive multifocal leukoencephalopathy.[107]

Eculizumab

Eculizumab is a humanized monoclonal antibody against complement 5a molecule and is approved by the FDA for the treatment of paroxysmal nocturnal hematuria. Several case reports described the use of eculizumab in renal transplant recipients with atypical hemolytic-uremic syndrome, as salvage therapy for antibody-mediated rejection, and renal transplant patients with catastrophic antiphospholipid antibody syndrome.[108-110]

Bortezomib

Bortezomib is a proteasome inhibitor and is approved by the FDA for the treatment of multiple myeloma and mantle cell lymphoma. Proteasomes are large cytosolic protease complexes and with ubiquitin they perform basic housekeeping protein degradation in all eukaryotic cells. The ubiquitin-proteasome pathway is essential for numerous important physiologic functions such as oncogenesis, inflammation, apoptosis, cell cycle progression, and immune activation. Plasma cells are professional antibody secreting cells and in the process of producing antibodies they are subjected to tremendous intracellular stress leading to proteasomal insufficiency and cell death if accumulation of polyubiquitinated proteins are left unchecked.[111] Even nonmalignant plasma cells are susceptible to proteasome inhibition. Preliminary studies have been completed testing bortezomib in antibody-mediated kidney rejection and appear promising.[112] Clearly further studies with larger cohorts are needed to fully define the usefulness of bortezomib.

Immunosuppressive Regimens

Immunologic considerations, including antirejection therapy, are organized around a few general principles. The first consideration is careful patient preparation and, in the circumstance of living donor renal transplantation, selection of the best available ABO-compatible HLA match in the event that several potential living related donors are available for organ donation. Second is a multitiered approach to immunosuppressive therapy similar, in principle, to that used in chemotherapy; several agents are used simultaneously, each of which is directed at a different molecular target within the allograft response (Fig. 81.1 and Table 81.3). Additive/synergistic effects are achieved through application of each agent at a relatively low dose, thereby limiting the toxicity of each individual agent while increasing the total immunosuppressive effect. Third is the principle that higher immunosuppressive drug doses and/or more individual immunosuppressive drugs are required to gain early engraftment and to treat established rejection than are needed to maintain immunosuppression in the long term. Hence, intensive induction and lower dose maintenance drug protocols are used. Fourth is careful investigation of each

episode of posttransplant graft dysfunction, with the realization that most of the common causes of graft dysfunction, including rejection, can (and often do) coexist. Successful therapy, therefore, often involves several simultaneous therapeutic maneuvers. Fifth is the appropriate reduction or withdrawal of an immunosuppressive drug when that drug's toxicity exceeds its therapeutic benefit.

The basic immunosuppressive protocol used in most transplant centers involves the use of at least two and often three drugs, each directed at a discrete site in the T cell activation cascade (Fig. 81.1) and each with distinct side effects. Although a calcineurin inhibitor plus MMF plus glucocorticoids is the most widely used regimen, there are concerns regarding nephrotoxicity associated with long-term use of calcineurin inhibitors[113] and several popular variations of the "triple" drug protocol are being explored in the clinic. A calcineurin-free regimen consisting of sirolimus, MMF, and glucocorticosteroids has been reported to result in better renal function.[114] In a randomized controlled trial of cyclosporine withdrawal in recipients of first deceased donor renal grafts, a 3-month course of cyclosporine followed by azathioprine and steroid maintenance therapy was superior to continuous cyclosporine-alone protocol.[115] Early cyclosporine withdrawal from a regimen of cyclosporine, sirolimus, and steroids has been associated with a better renal function and renal allograft histology compared to patients maintained on the three-drug regimen.[116]

Many centers employ induction therapy with antilymphocyte preparations. Monoclonal anti–CD25 antibodies or polyclonal antithymocyte antibodies are used as induction therapy in the immediate posttransplant period, thereby establishing an immunosuppressive umbrella that enables early engraftment without immediate use of calcineurin inhibitors during the early posttransplant period. During this critical period, the graft may be particularly vulnerable to CsA–tacrolimus-induced nephrotoxic effects. The incidence of early rejection episodes is reduced by the prophylactic use of anti-CD25 or antithymocyte antibodies. This protocol is particularly beneficial for patients at high risk for immunologic graft failure (e.g., broadly presensitized or retransplant patients). The efficacy of the polyclonal antilymphocyte antibody preparation (e.g., thymoglobulin) or mAbs (e.g., CAMPATH-1H) in preventing rejection is impressive, but profound lymphopenia and an increase in the incidence of opportunistic infections and lymphoma results. Because of selective targeting of IL-2R+ T cells, anti-CD25 mAb treatment appears to be safer than treatment with thymoglobulin or anti-T cell mAbs.[81] Insofar as activated but not resting T cells express the IL-2 receptor α chain, anti-CD25 mAbs are employed as humanized[117,118] or chimeric[119,120] mAbs to selectively target and destroy alloreactive T cells. These efforts are based on successful exploration and application of IL-2–receptor targeted therapy in preclinical models.[121] Low-dose tacrolimus and sirolimus protocol[122] may prove to rival the current sequential immune therapy regimens for use in patients at high risk to reject an allograft. Induction therapy protocols using either basiliximab or daclizumab have also been utilized to enable successful early steroid withdrawal in the first week of renal transplantation.[123,124]

HLA and Renal Transplantation

The genes that code for the HLA antigens are located within the short arm of chromosome 6.[125,126] The class I proteins, HLA-A, B, and C antigens, are composed of a 41-kDa polymorphic chain linked noncovalently to a 12-kDa β_2-microglobulin chain that is encoded in chromosome 15. The class I molecules are expressed by all nucleated cells and platelets. The class II molecules, HLA-DR, DP, and DQ, are composed of a chain of 34 kDa and a β chain of 29 kDa. MHC class II molecules are constitutively expressed on the surface of B cells, monocytes/macrophages, and dendritic cells. Additional lymphoid cells, such as T cells and many nonlymphoid cells, such as renal tubular epithelial cells, express class II proteins only on stimulation with cytokines.

The clinical benefits of HLA matching are readily appreciable in the recipients of renal grafts from living related donors. An analysis of the United Network for Organ Sharing (UNOS) scientific renal transplant registry data has revealed that the 1-year graft survival rate is 94% in recipients of two haplotype-matched, HLA-identical kidneys. It is 89% and 90%, respectively, when a one haplotype-matched parent or sibling is the donor (Fig. 81.4A).[127] The Collaborative Transplant Study, an international study that draws on 305 transplant centers located in 47 countries for data, has also demonstrated that the survival rate of HLA-identical transplants is superior to that of one-haplotype-matched grafts, even in the cyclosporin era.[128]

The advantage of HLA-matching is maintained beyond the first year of transplantation. UNOS registry data[127] show estimated half-lives (the time needed for 50% of the grafts functioning at 1 year posttransplantation to fail) of 26.9 years for HLA-identical grafts and 12.2 years and 10.8 years for one-haplotype-matched sibling grafts and parental grafts, respectively. Data from the Collaborative Transplant Study, comprising 22,414 living related grafts, have also revealed a substantial long-term benefit[128] of HLA matching in recipients of living related grafts.

The effect of matching for HLA in deceased donor graft recipients has been examined in a prospective U.S. study[129] in which kidneys were shared nationally on the basis of matching for HLA-A, B, and DR antigens. All transplantation centers in the United States participated in this study. The 1-year graft survival rate was 88% for HLA-matched kidneys and 79% for HLA-mismatched kidneys (Fig. 81.4B). Moreover, the benefit of HLA matching persisted beyond the first year posttransplantation; the estimated half-life of the HLA-matched renal graft was 17.3 years and that of HLA-mismatched renal allografts was 7.8 years.

Since the inception of the U.S. national kidney-sharing program in 1987, more than 7,500 deceased donor kidneys have been distributed to transplantation centers located in 48 states, and a recent analysis confirmed and extended the

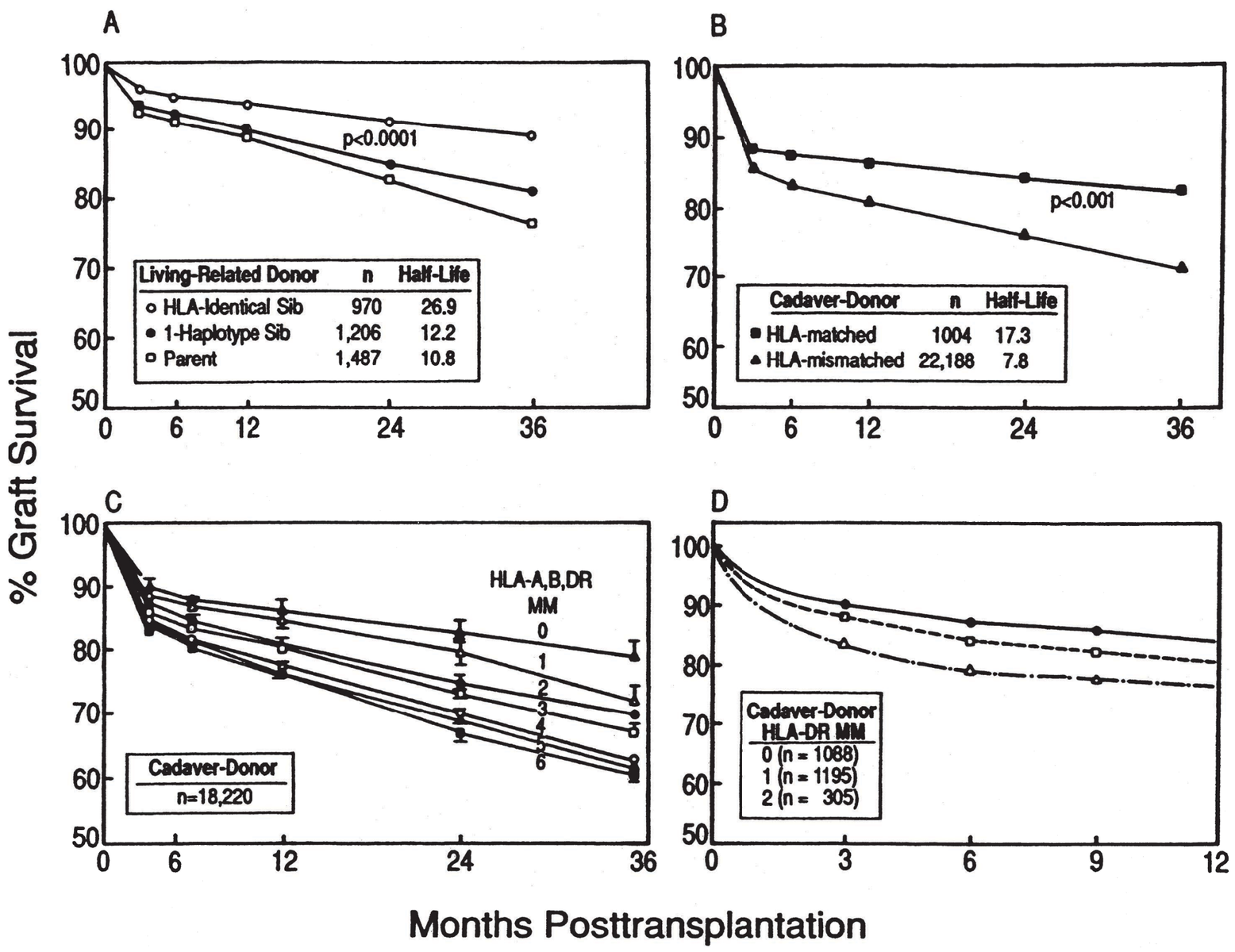

FIGURE 81.4 Impact of human leukocyte antigen (HLA) matching on renal allograft survival rates. **A:** The effect of haplotype matching in living related renal transplantation.[68] **B:** The superior results found with HLA-matched (A, B, and DR antigens) deceased donor renal grafts compared with HLA-mismatched deceased donor renal grafts.[70] **C:** The impact of different levels of HLA-A, B, and DR mismatching on the survival of deceased donor renal grafts.[72] **D:** The stepwise improvement in the survival of deceased donor grafts following matching for the HLA-DR antigens identified by DNA typing (number of DR mismatches: •—•, 0;—, 1; •—Δ—•, 2 .[75]

observation that HLA-matched transplants have a superior outcome compared to HLA-mismatched transplants.[130] The estimated 10-year rate of deceased donor graft survival was 52% for HLA-matched transplants and was 37% for HLA-mismatched transplants. Furthermore, the incidence of rejection was lower in HLA-matched transplants compared to mismatched ones. Interestingly, the mean duration of cold-ischemia time of nationally shared kidneys was not that different from locally transplanted kidneys; it was 23 hours compared to 22 hours for nonshared kidneys.[130]

A stepwise increase in the survival rate of deceased donor renal allografts has also been documented with increasing levels of HLA-A, B, and DR antigen matching (Fig. 81.4C). The improvement in the graft survival rate following HLA matching is more apparent when matching is based on better resolved HLA antigens (HLA split antigens) than when based on broad HLA antigens; the improvement in the graft survival rate between the best-matched and the worst-matched grafts increases with time.[131] In the UNOS registry data, the difference in the graft survival rate between the best-matched and worst-matched recipient was 10% at 1-year posttransplantation and this difference increased to 18% by 3 years posttransplantation. The Collaborative Transplant Study of more than 67,000 primary cadaver grafts has also demonstrated a significant correlation between the number of HLA mismatches and graft loss.[128]

A threshold level of HLA matching might exist: Allografts that are matched for four or more HLA antigens (or two or less HLA mismatches) have a superior short- as well as long-term outcome compared to less than four HLA antigen matches (or greater than two antigen mismatches).[131] It is noteworthy that the beneficial effect of different degrees of matching/mismatching for the HLA-A, B, and DR antigens,[132] with the exception of phenotypically identical HLA transplants, is more evident in white recipients as compared to black recipients of deceased donor renal allografts.[127,131]

The impact of each of the HLA loci—HLA-A locus, HLA-B locus, and HAL-DR locus—on renal allograft outcome has been investigated. Each locus impacts graft outcome. In the Collaborative Transplant Study, the influence of HLA-DR mismatches was greater than that of HLA-A or HLA-B mismatches in the first year following transplantation; with increased posttransplantation time, mismatches at any of the three loci impacted adversely on graft survival rates.[128]

Molecular techniques are currently used for the typing of HLA and for finer resolution of the HLA system.[133] The clinical advantage of molecular matching was suggested originally by the observation that the 1-year deceased donor renal graft survival rate is 87% in patients who receive kidneys that are HLA-DR identical, not only by the serologic methods but also by molecular methods (DNA-RFLP method). This figure drops to 69% for patients who receive kidneys that are not HLA-DR identical by the molecular methodology.[134] Application of molecular techniques for the identification of HLA-DR antigens has also resulted in the appreciation of a stepwise increase in the survival of deceased donor renal allografts matched for zero, one, or two HLA-DR antigens (Fig. 81.4D).[128,133] Molecular typing has also been used to detect mismatches at the HLA-A or HLA-B locus. Mismatches that were missed by conventional serologic techniques, but identified by molecular techniques, were found to adversely impact graft survival.[128]

Previous data has suggested minimal impact of matching for HLA-C locus antigens. Matching for the HLA-DP antigen, on the other hand, appears to be important in repeat but not primary grafts.[128] Emerging data support the importance of matching for HLA-C antigens. Using the polymerase chain reaction-sequence specific primer method, a cohort of 2,260 deceased-donor renal transplant recipients was typed for the HLA-C locus as well as assessed for presensitization using lymphocytotoxicity testing. Mismatching at the HLA-C locus had a significantly negative impact on graft survival in presensitized but not in non-presensitized recipients.[135]

Crossmatch

Crossmatches, testing of the recipient's serum for antibodies reacting with the donor's HLA antigens, must be performed prior to renal transplantation. The standard crossmatch test (CDC) consists of incubating the serum from the recipient with the donor's lymphocytes in the presence of rabbit serum as a source of complement.

The presence in the recipient's serum of cytotoxic antibodies directed at the donor's class I antigen (positive T cell crossmatch) is an absolute contraindication to transplantation because 80% to 90% of transplants performed in the presence of a positive crossmatch are subject to hyperacute rejection.[136] The sensitivity of the standard crossmatch test has been increased by the addition of sublytic concentrations of antihuman globulin (AHG) to the test system. The graft survival rate is about 5% lower in recipients with a positive AHG test compared to recipients with a negative AHG test.[137]

The significance of antibodies reacting with the donor's class II antigens (positive B cell crossmatch) is not fully resolved. A survival disadvantage, 7% in primary transplants and 15% in repeat transplants, however, has been noted in recipients with a positive B cell crossmatch.[137]

A number of centers are currently using flow cytometry-based methodology to detect donor-specific antibodies. Flow cytometry crossmatches permit detection of low, sublytic concentrations of complement fixing as well as noncomplement-fixing antibodies. In the UNOS kidney transplant registry data,[138] a positive flow cytometry crossmatch was associated with an increased incidence of early graft dysfunction requiring dialytic support, primary nonfunction of the allograft, prolonged hospitalization, and a greater incidence of allograft rejection. The negative impact of a positive flow cytometry crossmatch was greater in repeat transplants compared to primary transplants. Whereas a positive flow crossmatch was associated with a 5% decrease in the 3-year survival rate of primary grafts, a 19% decrease was observed in the 3-year survival of repeat grafts. In primary transplants, a T+B+ flow cytometry crossmatch and a T–B+ crossmatch had a similar outcome (76% vs. 74% at 3 years posttransplantation), and in repeat transplants a T+B+ flow cytometry crossmatch has a much inferior outcome compared to a T–B+ crossmatch (60% vs. 73% at 3 years posttransplantation).

The presence of posttransplant donor-specific anti-HLA antibodies has been shown in an international cooperative study from 36 centers to be detrimental to the survival of the kidney allograft.[139] From the study, the overall frequency of HLA antibodies found among kidney transplant recipients was 20.9%. The frequency of antibodies detected remained relatively constant in patients who were transplanted from 1 year (17.8%) to greater than 10 years (26.9%). In a separate study, data also support the negative impact of posttransplant de novo donor HLA-specific antibodies on graft outcome. In addition, they appear to predict rejection in kidney transplant recipients.[140]

Solid phase assays were introduced to enhance anti-HLA antibody detection either via enzyme-linked immunosorbent assay (ELISA)-based methods or Luminex technology (Austen, TX).[141] The ELISA technique detects anti-HLA antibodies from test serum via their binding with individual or groups of HLA molecules on the surface of the ELISA well with the appropriate specificity. A positive read is indicated by a change in color following the addition of a second enzyme-linked anti-human IgG antibody and a suitable substrate for the reaction. The Luminex platform-based method has greater sensitivity than the ELISA technique and is now widely used by transplant programs to screen for HLA-specific antibodies.

The Luminex single antigen fluorescent bead technology is based on a series of polysytrene beads with embedded fluorochromes of varying intensity that display predetermined HLA molecules on their surfaces. A test serum containing primary anti-HLA antibodies will interact with the appropriate HLA molecules, which are attached to the beads. Phycoerythrin-labelled anti-human IgG antibodies are then added to bind the primary anti-HLA antibodies. Lasers excite both the fluorochromes in the beads and the phycoerythrin bound to the primary anti-HLA antibodies. The unique combination of signals detected by the sensor determines the specificity of the anti-HLA antibody in question.

The solid-phase assays and Luminex in particular screen only for anti-HLA antibodies whereas the CDC method may detect non-HLA antibodies such as autoantibodies, antibodies directed at non-HLA molecules, and even immune complexes. The CDC assay may also pick up IgM anti-HLA antibodies whereas the Luminex method requires the addition of anti-IgM antibodies to detect anti-HLA IgM antibodies. On the other hand, the Luminex assay will detect all classes of IgG antibodies irrespective of their complement fixing abilities, which is required for detection by the CDC method. Luminex technology also permits the testing of sera following the administration of rituximab given during desensitization procedures whereas the CDC assay may yield a false positive reaction due to the interaction of the monoclonal antibodies and CD20 molecules on the B cells. A calculated panel reactive antibody (PRA) may be obtained with Luminex single antigen fluorescent bead technology by determining the frequencies of the HLA antigens in a given population. Finally, the Luminex platform assay allows a pretransplant virtual crossmatch to be performed in order to predict the success of high-risk presensitized live or deceased donor transplants.

TRANSPLANTATION TOLERANCE

Transplantation tolerance can be defined as an inability of the organ graft recipient to express a graft destructive immune response. Although this statement does not restrict either the mechanistic basis or the quantitative aspects of immune unresponsiveness of the host, true 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 81.4. Induction strategies for the creation of peripheral tolerance are listed in Table 81.5.

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 possibilities for the creation of a tolerant state: clonal deletion, clonal anergy, and immunoregulation.

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.[142] The form of graft tolerance, occurring as a consequence of mixed hematopoietic chimerism, entails massive deletion of alloreactive clones.[143] Tolerance to renal allografts has been achieved in patients who have accepted a bone marrow graft from the same donor.[144,145] 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.[146,147] Tolerance achieved under these circumstances also involves additional mechanisms, including clonal anergy and suppressor mechanisms.[148-150]

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

81.4 Classification of Tolerance

A. Based on the Major Mechanism Involved
   1. Clonal deletion
   2. Clonal anergy
   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

81.5 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

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 signal 1 without signal 2—can result in T cell anergy/paralysis (Fig. 81.5 and Table 81.2).

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 the 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.

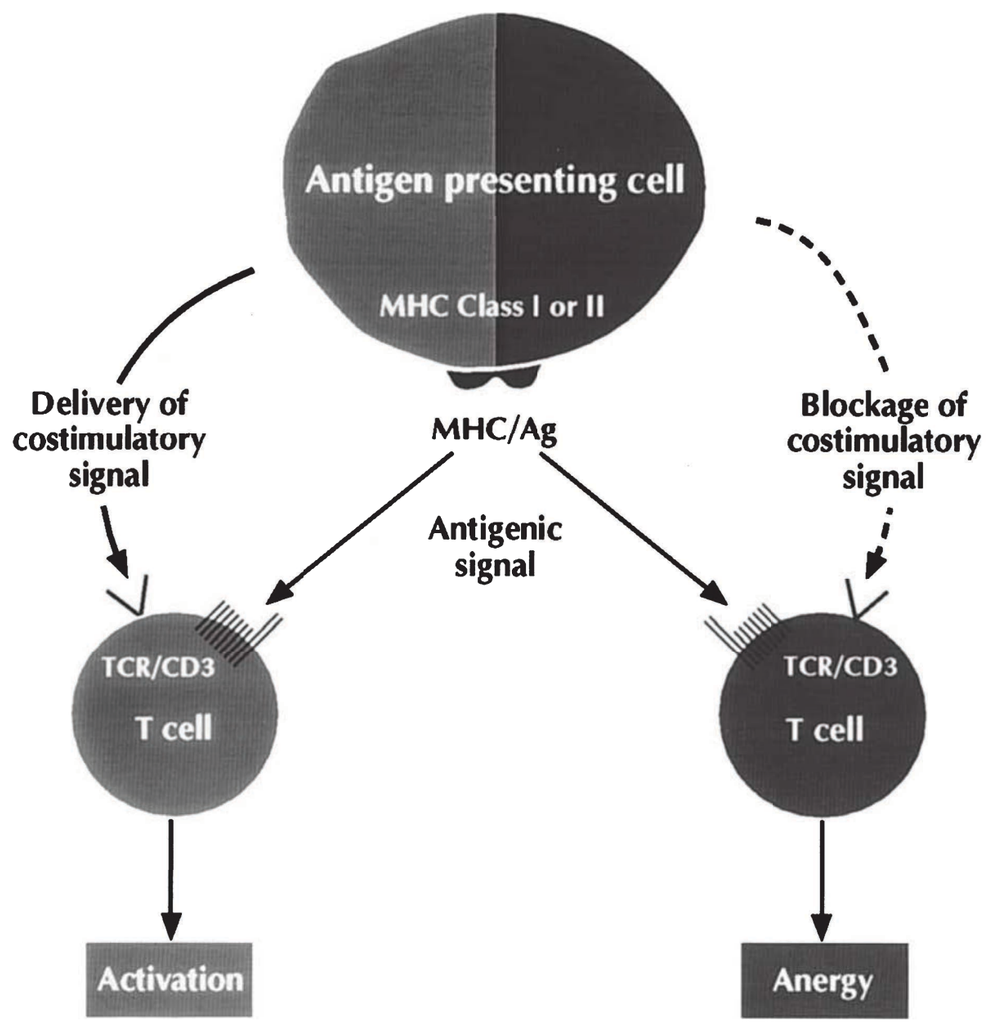

FIGURE 81.5 T cell activation/anergy decision points. Several potential sites for the regulation of T cell signaling are shown. The antigenic peptide displayed by major histocompatibility complex (MHC) (*site 1*), costimulatory signals (*site 2*), T cell antigen receptor (TCR) (*site 3*), and cytokine 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. (From Suthanthiran M. Transplantation tolerance: fooling mother nature. *Proc Natl Acad Sci USA.* 1996;93:12072.)

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 (Tregs), has been identified to mediate potent suppressive activity.[151,152] There are two major types of CD4+CD25+ T regs: naturally occurring CD4+CD25+Foxp3+ T-regs (nTregs) that arise from the thymus and induced CD4+CD25+Foxp3+ T-regs (iTregs) that originate in the periphery. IL-2 and TGF-β, a prototypic anti-inflammatory cytokine, are important for the maintenance of nTregs and TGF-β can differentiate CD4+CD25-Foxp3–T cells into CD4+CD25+Foxp3+ T cells. IL-6, a pro-inflammatory cytokine, inhibits the generation of Tregs and in the presence of TGF-β induces naïve T cells to differentiate into Th17 cells. Th 17 cells are a newly discovered effector T helper cell subset that produce IL-17, a proinflammatory cytokine, which activates the NF-κB and mitogen-activated protein kinases pathways.[153] Although not completely proven, Th 17 cells may contribute to acute allograft rejection that is resistant to suppression by Tregs.[154]

At least four distinct mechanisms have been advanced to explain the cellular basis for suppression:

1. An anti-idiotypic regulatory mechanism in which the idiotype of the TCR of the original antigen-responsive T cells functions as an immunogen and elicits an anti-idiotypic response. The elicited anti-idiotypic regulatory cells, in turn, prevent the further responses of the idiotype-bearing cells to the original sensitizing stimulus.
2. 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.
3. Immune deviation, a shift in CD4+ T cell programs away from Th1-type (IL-2, IFN-γ expressing) toward the Th2-type (IL-4, IL-10 expressing) program.
4. 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[155]). The process leading to full tolerance is infectious. Tolerant T cells recruit nontolerant T cells into the tolerant state.[149] The 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.[149] Tolerance is clearly a multistep process.[148-150]

It is very likely that more than one mechanism is operative in the induction of tolerance (Fig. 81.5). 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.[156] 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.

Clinical Trials in Transplant Tolerance

Small and large animal studies have successfully demonstrated the concept of "mixed chimerism" in achieving allograft tolerance. In these models, transplanting the donor's hematopoietic stem cells in tandem with the allograft create a bone marrow lymphohematopoietic chimera in which the donor and recipient hematopoiesis coexist thereby allowing the acceptance of the allograft. In a landmark trial, following a pretransplant nonmyeloablative-conditioning regimen, a total of five patients underwent combined bone marrow and kidney transplants from HLA single-haplotype mismatched living-related donors.[23] All five patients developed transient chimerism with one allograft failure due to irreversible humoral rejection and four patients achieving tolerance after discontinuation of all immunosuppressive regimens at 240, 244, 272, and 422 days after transplantation. Analysis of kidney allograft biopsy specimens from tolerant patients revealed the presence of high levels of the regulatory T-cell signature, FOXP3 mRNA, and the absence of the cellular rejection biomarker, granzyme B mRNA.

In a study of 25 tolerant kidney transplant patients who were off immunosuppressive medications for at least a year, unique B cell signatures were identified from peripheral whole blood specimens using gene microarrays and urinary cell sediments using real-time quantitative polymerase chain reaction (PCR) assays.[156] The predictive genes for tolerance (IGKV4-1, IGLLA, IGKV1D-13) were important for B cell differentiation and activation. They encode lambda and kappa light chains, which were increased during transition from pre- to mature B cells and during class switching and receptor editing. The study also showed that in tolerant patients, there was an increase in transitional B cells (CD38+CD24+) producing IL-10 cytokine.

Tolerance-inducing protocols and transplant tolerance trials are very likely to be tested in the clinic as novel conditioning immunosuppressive regimens become available to the transplant community.

CONCLUSION

Successful organ transplantation represents the fruition of the dedicated efforts of basic scientists, clinicians, and allied personnel. An excellent paradigm for the effective application of knowledge gained by basic research to the alleviation of life-threatening illness, renal transplantation also affords marvelous opportunities for the investigation of the systemic basis for renal disease independent of organ-specific mechanisms. Synergistic therapeutic protocols that target discrete steps in antigen recognition, signal transduction, and effector immunity are being explored in the clinic. The ultimate prize of transplantation would be that the basic principles learned would facilitate the prevention of the disease that necessitated transplantation in the first place.

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