The liver is unique among transplanted organs with respect to its interaction with the host immune system. There is evidence, both anecdotal and documented, that some liver recipients who cease taking immunosuppressive drugs maintain allograft function, suggesting robust tolerance is in place. Moreover, recipients of human liver allografts require less immunosuppression than do other organ recipients, and liver transplants confer protection on other organ grafts from the same donor. Hence, the liver shows features of immune privilege. Still, the liver can display destructive immunologic processes such as rejection in approximately one quarter of patients. The understanding of the cellular and molecular mechanisms operant in tolerance vs allograft rejection is important for developing new agents to improve long-term outcome, minimize infectious complications (including recurrence of hepatotropic viruses), and deliver immunosuppression without long-term toxicity. This review describes the unique aspects of the hepatic immune response, the pathways involved in T-cell activation and alloantigen recognition, effector cells and pathways mediating liver allograft rejection, the role of regulatory T cells, and targets of current and future immunosuppressive agents.

Liver transplantation is the definitive treatment of choice for patients with end-stage liver disease. Recipients of human liver allografts require less immunosuppression than do other organ recipients, and liver transplants confer protection on other organ grafts from the same donor. Nonetheless, a significant subset of patients does develop allograft rejection. For the most part, early rejection episodes do not negatively affect long-term graft survival. A number of animal models show spontaneous, lifetime tolerance to liver allografts without therapeutic manipulation; however, the frequency and extent to which drug weaning can be accomplished in human liver recipients has yet to be defined. It is widely accepted that after organ transplantation, hematopoietic donor cells are cotransferred into the recipient and may survive for prolonged periods. The size of the liver and its high content in hematopoietic cells (both resident and circulating) may in part explain why liver transplantation is associated with a significant number of donor hematopoietic cells in the peripheral blood of recipients and permissiveness with regards to HLA incompatibility. Donor leukocytes could mediate tolerance by functioning as immature antigen-presenting cells (APCs) or could act as surrogate targets of rejection, thus protecting the graft.

Hematopoietic microchimerism is defined by the persistence of less than 1% circulating donor cells in a recipient (macrochimerism denotes >1%). A recent study using nested polymerase chain reaction of donor-specific HLA DR allelic analysis showed that all liver recipients showed microchimerism within the first 3 months after transplantation. However, in accord with prior studies by Hisanaga et al and Devlin et al, microchimerism did not correlate with freedom from rejection or ability to tolerate staged immunosuppressive drug withdrawal. Taken together, these data are consistent with the notion put forth by Starzl and Zinkernagel that microchimerism is a necessary prerequisite for, but is not synonymous with, tolerance. Clearly, mechanisms mediating graft tol-

Abbreviations used in this paper: APC, antigen-presenting cell; CNI, calcineurin inhibitor; CTLA-4, cytotoxic T lymphocyte–associated antigen 4; DC, dendritic cell; IL, interleukin; MHC, major histocompatibility complex; NK, natural killer; PD-1, programmed death-1; TCR, T-cell receptor; TNF, tumor necrosis factor; Treg, regulatory T cell.
erance are more complex than simply induction of anergy by transferred donor cells.

This review covers the unique aspects of the hepatic immune response; the pathways involved in T-cell activation and expansion; basic mechanisms of non-self-alloantigen recognition, effector cells, and pathways operant in allograft rejection; the role of regulatory T cells; and targets of current and future immunosuppressive agents.

The Liver Is a Specialized Immune Organ

The liver is among the most interesting and potent immunologic organs, rivaled only by what are considered the true immune organs, such as thymus and lymph nodes. The liver is exposed continuously to a diverse and large antigenic load, including pathogens, toxins, and tumor cells, as well as dietary and commensal proteins. The liver must be actively immunocompetent and simultaneously control inappropriate inflammatory responses to dietary and other harmless antigens encountered in the portal circulation, thus being able to selectively induce immunity or tolerance to antigens. Pathogen invasion is sensed through various pattern-recognition receptors including Toll-like receptors (TLRs), which are expressed on immune cells as well as on some parenchymal cells. For example, Toll-like receptor 4, the receptor for lipopolysaccharide, is expressed on immune cells, as well as hepatocytes, endothelial cells, and stellate cells.

Lymphocytes are broadly divided into B cells, T cells, and natural killer (NK) cells. Although B and T cells possess clonotypic antigen receptors that confer specificity for diverse antigenic structures, NK cells possess multiple receptors that can detect conserved antigens or danger signals that mediate major histocompatibility complex (MHC)-unrestricted cytolysis of susceptible tumor and virus-infected cells. Numerous groups have documented that the liver contains an unusually high percentage of unconventional lymphoid cells that rarely are present in the blood, including NK cells, NK T cells expressing the γδ T-cell receptor (TCR), and the invariant Vα24Jβ8 TCR. For example, NK (CD3−CD16−CD56+) cells represent 5%–15% of the mononuclear cell population, but in the liver can comprise up to 45% of the lymphocyte population. It has been suggested that these cells deliver a death signal to circulating recipient-derived T cells that migrate through the liver after transplantation, contributing to tolerance. Moreover, significantly higher proportions of hepatic T cells express TCR γδ than matched peripheral blood T cells. The hepatic αβ TCR+ population contains more than twice as many CD8+ cells and 4 times as many double-negative cells (T cells that express neither CD8 nor CD4 coreceptors) as the corresponding population in matched peripheral blood. The liver also hosts the largest population of tissue macrophages (Kupffer cells) in the body and different types of dendritic cells (DCs).

DCs are professional APCs that are present in virtually all organs and provide a critical link between innate and adaptive immunity. The ability of DCs to process and present various types of antigen (Ag) is unmatched in the human body. In particular, DCs can display Ag processed exogenously on MHC class I (cross-presentation or cross-priming) or complete MHC-peptide complexes acquired externally from dead cells (cross-dressing) to CD8+ T cells. Immature DCs express low levels of MHC class II adhesion and costimulatory molecules, but their expression is up-regulated dramatically during maturation in response to inflammatory stimuli. There is expanding evidence that liver-derived DCs can downregulate immune responses, hence inducing and maintaining peripheral T-cell tolerance.

Intrinsic differences between liver DCs and other tissue-resident DCs have been noted and may have important biologic implications. For example, human monocytes differentiated into DCs when cocultured with rat liver epithelial cells or liver-conditioned media secrete interleukin (IL)-10 but not IL-12p70, thus skewing the immune response towards T helper type 2 rather than Th1. Thus, the hepatic microenvironment may support the generation and survival of regulatory T cells (described later). Furthermore, it has been suggested that monitoring of DC subsets and their activation status or cytokine production may provide a useful tool for predicting allograft outcome, including successful withdrawal of immunosuppression. Independent of the type or extent of immunosuppressive therapy, circulating plasmacytoid DCs are increased relative to myeloid DCs in clinically tolerant pediatric liver transplant recipients compared with those on maintenance immunosuppression.

The context in which antigen is presented to T cells determines whether the responding T cell is activated or tolerized; critical variables include the nature of the APC, the presence or absence of costimulatory molecules, and the cytokine microenvironment. Recent provocative data indicate that Ito (stellate) cells, known primarily for mediating hepatic fibrogenesis, are APCs that can present antigen as efficiently as DCs. Liver sinusoidal endothelial cells have a unique immune phenotype, expressing markers typical of cells of myeloid lineage (CD1, CD4, CD11c) even though data suggest that these cells differentiate from hepatocyte progenitors. CD4+ T cells primed by antigen-presenting liver sinusoidal endothelial cells or hepatocytes (which lack costimulatory molecules) fail to differentiate toward effector T helper type 1 cells but, instead, express high levels of immune-suppressive IL-10. Moreover, antigen presentation to CD8 T cells by liver sinusoidal endothelial cells in vivo leads to their inability to respond to specific antigen on restimulation. Thus, liver sinusoidal endothelial cells—the only cells to have direct contact with immune cells passing
through the liver—may play an important role in establishing tolerance after liver transplantation.14

Costimulatory Pathways and Transplantation

Because T cells are essential for allograft rejection and many immunosuppressive drugs target T-cell signaling, it is important to understand the multistep processes by which they become activated. T lymphocytes must receive 2 distinct coordinated signals to achieve optimal activation and expansion. The first signal is delivered by the TCR after recognition of peptide-MHC complexes on APCs and the second signal is provided by the interaction of costimulatory molecules on the T cells and their cognate ligands on APCs.39 Figure 1A shows this 2-signal model, including the wide array of heretofore described receptor-ligand pairs.40 Although the CD28/B7 and CD40/CD154 costimulatory pathways have garnered most of the attention in transplantation, there is emerging evidence for the importance of other molecules in alloimmune responses,14 including critical negative second signals that down-regulate or terminate T-cell responses (Figure 1B). CD28 (the prototypical T-cell costimulatory molecule) is expressed constitutively on the surface of T cells; the ligands for CD28 (B7-1 [CD80] and B7-2 [CD86]) are found on a variety of APCs including DCs, B cells, and macrophages. In naive T cells, CD28 costimulation enhances cell-cycle entry, potently stimulating expression of IL-2 and induction of anti-apoptotic proteins.41 In contrast, cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), which has approximately 20-fold higher affinity for both B7-1 and B7-2 than does CD28, is up-regulated after T-cell activation and inhibits T-cell responses, regulating peripheral T-cell tolerance.42 A recent analysis of CTLA-4 single-nucleotide polymorphisms in 483 liver transplant recipients showed that the CTLA-4+49A/+6230G haplotype, which is associated with reduced soluble CTLA-4 production, is a co-dominant risk factor for acute rejection.43

Programmed death-1 (PD-1) also is induced after activation of T cells, shares sequence homology with CTLA-4, and contains an immunoreceptor tyrosine-based inhibitory motif in the cytoplasmic tail, characteristic of cellular receptors that deliver a negative signal.14 As shown in Figure 1B, when PD-1 is engaged by its ligands or CTLA-4 binds to B7 and displaces CD28, the activation and phosphorylation of SHP-2 (SRC homology 2–domain-containing protein tyrosine phosphatase 2) that deactivates downstream signal transducers resulting to the PD-1 tail results.44 SHP-2–mediated dephosphorylation inactivates multiple signaling molecules, resulting in down-regulation of cytokine messenger RNA synthesis and inhibition of T-cell proliferation.44 A recent preliminary analysis of liver perfusates collected from donor livers before allograft implantation indicated a significantly higher expression of PD-1 on CD8+ T cells from the liver relative to peripheral blood and suggested this may be an important mechanism imparting tolerogeneity.45

Several members of the tumor necrosis factor (TNF)/TNF receptor families also can provide alternate costimulatory signals to T lymphocytes. These receptor/ligand pairs include CD40/CD154, 4-1BB/4-1BBL (CD137), OX-40/OX-40L (CD134/CD134L), and CD30-CD30L (CD153).14 Of these, CD40/CD154 is the best characterized in alloimmune responses. CD40 is expressed constitutively on APCs including B cells, monocytes, macrophages, and DCs, but also can be expressed on nonimmune cells including endothelial cells, mast cells, platelets, and epithelial cells. In contrast, CD154 is expressed on CD4 T cells after activation, and to a lesser extent on NK cells, B cells, and CD8 T cells. Unlike the CD28/B7 costimulatory pathway that primarily has been defined in the context of effects on T-cell function, CD40/CD154 ligation enhances APC function as measured by up-regulation of class II, CD80, and CD86 expression and production of cytokines including IL-12.14,46 The sum effect of these changes to the APC is to significantly augment B- and T-cell responses to alloantigen. Molecular and immunohistochemical analyses of CD80, CD86, and CD154 expression in biopsy specimens of liver recipients showed an association between increased expression of CD86 and CD154 (but not CD80) in the graft during severe acute cellular rejection.47 CD154 also was detected on Kupffer cells and sinusoidal macrophages in livers during chronic rejection, but not in stable liver allografts or normal livers.48 Thus, the ultimate fate of cellular immune responses is determined by the balance between positive and negative signals delivered by costimulatory molecules to T cells; undoubtedly, an expanding list of molecules will continue to be studied as possible therapeutic targets to prevent and treat allograft rejection.

Basic Aspects of T-Cell Recognition of Alloantigen

The term allore cognition refers to T-cell recognition of genetically encoded polymorphisms between members of the same species.49 The primary targets are the MHC molecules present on donor cells. The recognition of allograft MHC antigen is the primary event ultimately leading to graft rejection. Recipient T lymphocytes can recognize donor alloantigen through 2 distinct but not mutually exclusive pathways. In the direct pathway, host T lymphocytes recognize native MHC molecules expressed on graft-associated APCs. In the indirect pathway, host T lymphocytes recognize donor alloantigen-derived peptides in the context of self MHC molecules expressed on recipient APCs (Figure 2).50 Evidence for both pathways exists in liver transplantation. It is likely that the direct pathways predominate early posttransplant and is a major factor in acute rejection because graft-derived APCs expressing donor alloantigen rapidly egress from the graft and enter secondary lymphoid tis-
Figure 1. The 2-signal model for positive and negative costimulation of T-cell activation. (A) T-cell activation requires 2 signals: signal 1, TCR engagement with MHC–peptide complex, and signal 2, ligation of costimulatory molecules on T cells with their respective ligands on APC. T cells that receive both signal 1 and positive costimulation show proliferation, secretion of cytokines, and differentiation into effector cells. The prominent costimulatory molecules delivering positive costimulation signals are CD28 and CD40L. Under certain circumstances, ICOS, CD134, CD30, 4-1BB, CD27, and CD70 also can deliver positive T-cell costimulation. Some costimulatory molecules, such as CTLA-4 and PD-1, can lead to negative T-cell signaling, resulting in decreased cell proliferation and cytokine production and cellular anergy. Reprinted from Gao et al.40 (B) Negative regulatory signaling of T cells. PD-1 and CTLA-4 are negative regulators of TCR signaling. Engagement of PD-1 by its ligands (PD ligand-1 or PD ligand-2) or binding of CTLA-4 to B7 (displacing CD28) is followed by the recruitment of the phosphatase SHP-2 to the immunoreceptor tyrosine-based inhibitory motifs in the cytoplasmic tails of PD-1 and CTLA-4. Then SHP-2 dephosphorylates the CD3ζ chains and other signaling pathways, which results in inhibition of TCR signaling and downstream events associated with T-cell activation. Reprinted with permission from Mak and Saunders.44
Host T cells primed through the direct pathway have the ability to engage the allograft directly and, thus, to efficiently mediate effector functions. Further, the proportion of host T cells that can respond to native alloantigen is substantially greater than the proportion of host T cells that can respond through the indirect pathway to donor-derived peptides presented in the context of self MHC. The indirect pathway probably emanates from donor alloantigen that is shed from damaged graft tissue, or perhaps in the case of the liver, from soluble MHC molecules that are picked up and presented by self APCs, in particular DCs. Therefore, the direct pathway may be important in initiating the classic form of acute rejection.
because donor-derived passenger leukocytes have a limited lifespan, the indirect pathway may predominate later by sustaining a response fueled by epitope spreading as a variety of allopeptides are presented successively by self APC. Accordingly, T cells with specificity for allopeptides have been detected readily in liver recipients undergoing chronic rejection. At the same time, it is important to recognize that the indirect pathway may be involved in immune regulation because T cells with allopeptide specificity were shown to have regulatory function through inhibition of interferon γ production in other organ recipients. The contribution of the indirect pathway to hepatic allograft tolerance recently was confirmed.

In addition to graft rejection and tolerance, these allore cognition pathways likely are important in recurrence of viral or autoimmune liver diseases. In this regard, liver transplantation is performed with no regard to specific matching of donor-recipient HLA alleles; this may serve as a barrier to the development of protective (ie, antiviral) cell-mediated immunity directed against infected cells within the allograft. CD8+ T cells are the primary effector lymphocytes for provision of protective immunity against intracellular pathogen infection of parenchymal cells. Recognition of the infected allograft could occur either via recipient-derived T cells or via those derived from the donor. For the recipient-derived T cells, recognition could occur either through use of shared HLA molecules, or could occur through the expansion of recipient-derived T cells that are uniquely restricted by the HLA molecules of the donor liver. A study from our group has shown the generation of new hepatitis C virus (HCV)-specific T cells that are restricted by donor HLA alleles, yet derived from the recipient’s original T-cell pool (Figure 3). For the purposes of this study, we selected HLA A2-negative recipients of HLA A2+ grafts; HLA-A2 was selected as the restricting allele because a large number of HCV HLA-A2 binding peptides have been described as targets of HCV-specific CTLs.

As shown in Figure 3A, recipient HCV-specific CTL clones cultured with HLA A2+ lymphoid cell lines that had been pulsed with cognate peptide (NS31466-1475, KLVALGINAV) but not irrelevant HCV core peptide (core35-44, YLLPRRGPRL) elicited a strong immune response by interferon γ enzyme-linked immunospot. Moreover, when these recipient CTLs were cultured with recipient-derived (syngeneic) lymphoid cell lines and cognate peptide, there was no appreciable immune response. These data suggest that these HCV-specific CTLs circulate within the recipient and only display functional antiviral activity (ie, secrete interferon γ and show cytotoxicity; Figure 3B), when they encounter allograft-derived HLA molecules and viral peptide. Furthermore, these CTLs can recognize endogenously processed viral Ag expressed by the HLA molecule of the donor graft (Figure 3C). It is likely that the microenvironment (eg, lymphoid aggregates in the portal tracts or secondary lymphoid tissues) in which this immune response was initiated contained DCs that engulfed HCV-infected hepatocytes. These results underscore the plasticity of the TCR, as well as the need to assess both allograft- and recipient-restricted CTLs (and thus direct and indirect pathways) to develop a comprehensive understanding of protective immunity to HCV after liver transplantation.

**Effector Pathways of Graft Injury**

Acute cellular rejection occurred historically in 50% to 75% of liver allograft recipients, although recent advances in immunosuppression have yielded lower rates (ie, <30% according to Scientific Registry of Transplant Recipients data; http://www.ustransplant.org/glossary). The majority of episodes occur within 90 days of transplant surgery, and the majority of cases respond to high-dose corticosteroids. Three main types of allograft rejection are described: the extremely rare hyperacute rejection characterized by endothelial injury and mediated by deposition of antibody and fibrin but no lymphocytic infiltration or bile duct injury; acute rejection characterized by the diagnostic triad of portal inflammation, bile duct damage, and venular endothelial inflammation; chronic rejection characterized by loss of small bile ducts (ie, ductopenic rejection) and an obliterative vasculopathy affecting large- and medium-sized arteries and the portal microvasculature.

For both acute and chronic rejection, the targets of mature effector T cells are donor-derived bile duct epithelial cells and vascular endothelium, whereas direct involvement of hepatocytes is uncommon. Cytotoxic lymphocytes kill cholangiocytes via either perforin-dependent pathways or activation of members of the TNF receptor superfamily (particularly Fas) (Figure 4). The interaction between CTL and target cell is facilitated by integrins, notably LFA-1, which help form an immunologic synapse containing the secretory domain. In addition, as shown in Figure 4B, other integrins such as very late antigen-4 may enhance adhesion and provide survival signals for effector cells.

Recent data indicate that bile ducts constitutively express the chemokine CCL19, which attracts DCs and promotes their localization at the biliary epithelium where they are ideally situated to respond to Ag. Although human cholangiocytes lack CD80 and CD86, minimizing their ability to activate naive T cells, the majority of T cells that come into contact with activated cholangiocytes are already primed cells, and therefore, cholangiocytes may promote the local proliferation and survival of such cells. Given the finding that the majority of lymphocytes infiltrating human liver allografts in rejection express functional chemokine receptors CXCR3, CXCR4, and CCR5 indicates that therapeutic strategies in the future may aim to prevent lymphocyte infiltration of liver allografts by inhibiting chemokine ligand/receptor-mediated pathways.
Figure 3. Recipient-derived T cells that recognize HCV peptides in the context of donor HLA molecules (HLA A2). Taken together, these results suggest that T cells circulate in liver transplant recipients and are not activated until they encounter donor alleles containing HCV peptides. (A) Enzyme-linked immunospot assay was performed with 1000 T cells, lymphoid cell lines (LCLs) expressing A2 allele (top row), A3 allele (middle row), or syngeneic (recipient-derived) LCLs (bottom row) co-cultured with no peptide, cognate peptide (NS31406–1415, KLVALGINAV), or irrelevant HCV core peptide (core35–44, YLLPRRGPRL). (B) Cytotoxic activity of HCV-specific tetramer+CD8+ T cells. T-cell clones were assayed for peptide-specific cytotoxicity using the fluorometric assessment of T lymphocyte Ag-specific lysis assay, which determines the percentage of labeled target cells surviving after a 5-hour incubation with effector cells; effector:target ratio is 50:1 with 1 × 10^6 target cells. The target cells are selected by gating on the PKH-26high population and show the reduction in carboxyfluorescein diacetate succinimidyl ester (CFSE) fluorescence after incubation with NS31406-specific CTL, cognate peptide (KLVALGINAV), and HLA-A2 LCLs (a), compared with no peptide (b), irrelevant peptide NS52594–2602 (ALYDVVTKVL) (c), and no effector cells (d). The percentage killing of syngeneic LCLs was not different after incubation in the presence (e) or absence (f) of cognate peptide. FITC, fluorescein isothiocyanate. (C) NS31406-specific clones (HCV1–HCV4, represented by bars) recognize endogenously processed Ag. T2 cells were pulsed with HCV1406–1415 peptide for 2 hours. Cell lines were transduced with either empty retroviral vector or with retroviral vector containing the HCV minigene that encodes NS31406–1415 peptide. Cytopathic effect assay. Cytomegalovirus-specific T cells were used as a control. Interferon-γ secretion (mean + SD) was assessed by enzyme-linked immunosorbent assay. Reprinted with permission from Rosen et al.55
The perforin pathway is mediated by granzymes and perforin stored in specialized secretory lysosomes that are released into the immunologic synapse after engagement with a target cell. Granzymes cleave specific substrates, including caspases and Bcl2-interacting domain, resulting in the induction of apoptosis. There is strong circumstantial evidence that the granzyme/perforin pathway is activated in human allograft rejection. Cross-linking of Fas with FasL leads to caspase-dependent apoptosis. A wide range of cells (including macrophages) express TNF ligands, implicating them as potential contributors to bile duct injury. CD40 does not directly activate caspases but instead increases the expression of FasL via nuclear factor κB-dependent pathways, resulting in autocrine or juxtacrine apoptosis via Fas activation. Although ICAM-1 and LFA-1 are critical for the formation of the immunologic synapse, other integrins such as very late antigen-4 (VLA-4) may enhance adhesion and provide survival signals for effector cells. Reprinted with permission from Adams and Afford.

Figure 4. Cholangiocyte apoptosis is mediated by CTLs via activation of TNF receptors or the granzyme/perforin pathway. (A) CTLs are attracted to target cells by the release of chemokines, secreting specialized granules that contain proteinases and activate the caspase cascade and apoptosis in the target cell. Inflamed cholangiocytes express increased MHC class I and intercellular cell adhesion molecules (ICAM-1), promoting antigen recognition and cell–cell adhesion. On CTL activation, perforin- and granzyme-containing secretory granules are mobilized to the cell surface where membrane fusion occurs and granzymes are delivered into the target cell. Granzyme A makes nicks in single-stranded DNA, resulting in direct apoptosis; granzyme B activates Bcl2-interacting domain and caspase-3–dependent apoptosis. (B) CTLs also kill cholangiocytes by the activation of Fas and other TNF receptors (TNFRs). Fas ligand is contained in the secretory granules and with other members of the TNF family is concentrated in the immunologic synapse. Cross-linking of Fas with FasL leads to caspase-dependent apoptosis. A wide range of cells (including macrophages) express TNF ligands, implicating them as potential contributors to bile duct injury. CD40 does not directly activate caspases but instead increases the expression of FasL via nuclear factor κB-dependent pathways, resulting in autocrine or juxtacrine apoptosis via Fas activation. Although ICAM-1 and LFA-1 are critical for the formation of the immunologic synapse, other integrins such as very late antigen-4 (VLA-4) may enhance adhesion and provide survival signals for effector cells. Reprinted with permission from Adams and Afford.
linking of Fas (ubiquitously expressed on lymphoid and nonlymphoid tissue including the liver) with trimerized FasL or agonist antibodies leads to formation of the death-inducing signaling complex in target cells, activation of caspase 8, and propagation of a death signal that culminates in apoptosis.\textsuperscript{57} The Fas/FasL pathway has been shown convincingly to play critical roles in a variety of hepatic pathologies,\textsuperscript{62,63} and there is evidence that this pathway also is active during liver allograft rejection.\textsuperscript{64,65} A wide range of cells (including macrophages) express TNF ligands, implicating them as potential contributors to bile duct injury.\textsuperscript{57} Other TNF receptors likely involved in acute and chronic rejection (and thus potential targets for pharmacologic intervention) are shown in Figure 4B. CD40 ligation on cholangiocytes results in up-regulation of FasL; thus, although CD40 does not directly activate caspasases, it enhances the expression of FasL via nuclear factor k\textsuperscript{B}-dependent pathways, resulting in autocrine or juxtacrine apoptosis via Fas activation.\textsuperscript{57}

**Regulatory T Cells and Transplantation**

The balance of effector (proinflammatory) responses and regulatory responses may ultimately determine whether rejection or tolerance occurs. Several subsets of CD4\textsuperscript{+} T cells with suppressive properties have been described, including naturally occurring CD4\textsuperscript{+}CD25\textsuperscript{+} regulatory T cells (Treg), T regulatory type 1, and T helper type 3 cells.\textsuperscript{66} CD4\textsuperscript{+}CD25\textsuperscript{+} Treg suppress the response of conventional T cells via a cell contact-dependent manner, whereas T helper type 3 and T regulatory type 1 cells produce immunosuppressive cytokines (eg, IL-10 and transforming growth factor-\textbeta\textsuperscript{}). Although immunoregulatory activity specific for donor alloantigens is enriched in the CD4\textsuperscript{+} T-cell populations, CD8\textsuperscript{+} T cells, double-negative (CD4\textsuperscript{-}CD8\textsuperscript{-}) T cells, as well as NK T cells also have shown regulatory activities in different situations.\textsuperscript{67} Treg cells also may mediate their effects by modifying the functions of other T cells, either directly or indirectly through APCs.\textsuperscript{67}

CD25 (the \(\alpha\)-subunit of the IL-2 receptor) is used as a marker for Tregs, but is by no means a definitive or specific marker. Further, CD4\textsuperscript{+}CD25\textsuperscript{high} Tregs have been characterized by the constitutive expression of CD62L, intracellular expression of CTLA-4, forhead transcription factor 3 (FoxP3, the most specific marker for Tregs), lymphocyte activation gene-3 (LAG-3), and, most recently, the downregulation of IL-7 receptor (CD127) expression.\textsuperscript{68–71} Treg frequency in the peripheral blood\textsuperscript{23} and intrahepatic FoxP3\textsuperscript{+} cells\textsuperscript{73} are reduced in liver allograft recipients with acute rejection, and T regulatory type 1 cells are increased in recipients developing spontaneous graft tolerance. A recent study showed that donor CD4\textsuperscript{+}CD25\textsuperscript{+} Tregs originating from the liver graft are able to suppress responder T cells from both recipient and donor.\textsuperscript{74} These findings indicate that Treg can suppress across an MHC barrier, congruent with previous observations that Tregs suppress Ag nonspecifically once activated through their TCR. Thus, chimerism of donor Treg may contribute to suppression of the direct pathway alloresponse, which, as described earlier, is the dominant Ag presentation mechanism early after liver transplantation.\textsuperscript{74}

**How Immunosuppressive Agents Work**

This section builds on the understanding of basic mechanisms outlined earlier to explain how current immunosuppressive agents work (Table 1) and to describe potential new targets for immune manipulation. The reader is referred to excellent reviews that additionally describe important drug–drug interactions and side effects of these medications.\textsuperscript{75–77}

In general, immunosuppression can be attained by blocking lymphocyte response pathways, depleting lymphocytes, or diverting lymphocyte traffic.\textsuperscript{76} Calcineurin inhibitors (CNIs), cyclosporine and tacrolimus, remain the backbone of immune suppression in liver transplantation. Cyclosporine engages the immunophilin cyclophilin, forming a complex that then engages calcineurin.\textsuperscript{76} This complex in turn inhibits transcription of several genes, including IL-2, critical to T-cell activation. Tacrolimus engages a different immunophilin, FK506-binding protein 12, to create a complex that also inhibits calcineurin but with greater molecular potency.\textsuperscript{76} Thus, both CNIs interfere with signal 1 T-cell signal transduction (Figures 1 and 5). Rapamycin (sirolimus) is a macrolide antibiotic structurally related to tacrolimus but with very different mechanisms of action. Rapamycin binds to the immunophilin, FK506-binding protein 12, but rather than inhibiting cytokine gene transcription in T cells, it blocks signals transduced from a variety of growth factor receptors to the nucleus by acting on phosphatidylinositol kinases called mammalian targets of RAPA.\textsuperscript{77,78} Moreover, it also inactivates 70-kD-S6 kinase, which results in selective inhibition of the synthesis of new ribosomal proteins, prolonging cell-cycle progression from the G\textsubscript{1} to the S phase.\textsuperscript{77} Thus, CNIs and sirolimus differentially affect IL-2: CNIs inhibit IL-2 production whereas sirolimus blocks the IL-2 signaling pathway at a later stage after receptor binding.\textsuperscript{77} Interestingly, these differences are manifested by the fact that CNIs (which inhibit IL-2 secretion essential for effector cells and Treg development) prevent activation-induced cell death of effector cells, whereas sirolimus does not have the same effect.\textsuperscript{67} Moreover, CNIs are associated with depletion in the peripheral blood of CD4\textsuperscript{+}CD25\textsuperscript{+}FoxP3\textsuperscript{+} Tregs after renal transplantation, whereas sirolimus is associated with preservation of Tregs,\textsuperscript{79} in keeping with in vitro data.\textsuperscript{80}

Corticosteroids are the most frequently used non-CNI agents. Their effects are primarily transcriptional through DNA binding and protein–protein interactions of the steroid-receptor complex, which target transcription factors such as activator protein 1 and nuclear factor-\textk\textsuperscript{B},\textsuperscript{76} abrogating expression of multiple cytokines, including IL-1, IL-2, IL-3, and IL-6.\textsuperscript{75} In addition, corti-
corticosteroids suppress eicosanoid production, down-regulate adhesion molecules, and increase the expression of transforming growth factor-β. Antimetabolites used in transplantation include azathioprine, mycophenolate mofetil, and mycophenolic acid. All 3 antagonize purine synthesis, which blocks differentiation and proliferation of T and B lymphocytes.75 Mycophenolate mofetil and mycophenolic acid inhibit synthesis of guanosine monophosphate nucleotides, whereas azathioprine converts 6-mercaptopurine to tissue inhibitor of metalloproteinase, which is converted to thioguanine nucleotides.76

Antibodies used in transplantation target a wide variety of different pathways. Antithymocyte globulin is a polyclonal antibody preparation that targets multiple different epitopes on T cells (CD2, CD3, CD4, CD8, CD28) and NK cells (CD16).75 Muromonab-CD3 (OKT3) is a murine-derived antibody that exerts its activity by binding to the CD3 antigen on the surface of T lymphocytes, inactivating the adjacent TCR (signal 1). Two currently licensed IL-2–receptor antibodies, basiliximab (chimeric) and daclizumab (humanized), bind to the IL-2 receptor α-chain (CD25 antigen), depleting activated T cells and inhibiting IL-2-induced T-cell proliferation.75 Although short-term treatment with CD25-specific antibodies is effective at reducing the incidence of allograft rejection, theoretically, it also might compromise the subsequent development of immunoregulation to donor alloantigens.67 Alemtuzumab or campath-1H is a humanized, recombinant monoclonal antibody that targets antigen CD52 on T, B, NK cells, monocytes, and macrophages, causing lysis and depletion.

### Table 1. Mechanisms of Action of Immunosuppressive Agents Used in Liver Transplantation

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<thead>
<tr>
<th>Drug</th>
<th>Mechanisms of action</th>
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<tbody>
<tr>
<td>Small-molecule immunosuppressive agents</td>
<td>Binds to cyclophilin; complex inhibits calcineurin phosphatase and T-cell activation (inhibit signal 1 T-cell signal transduction) Inhibits IL-2 synthesis and release</td>
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<tr>
<td>Cyclosporine (CyA)</td>
<td>Binds to FKBP12; complex inhibits calcineurin phosphatase and T-cell activation (inhibit signal 1 T-cell signal transduction) Inhibits IL-2 synthesis and release</td>
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<tr>
<td>Tacrolimus (FK506)</td>
<td>Binds to FKBP12 (but not cyclophilin); complex inhibits target of rapamycin and IL-2 driven T-cell proliferation Relative to CyA and FK506, blocks IL-2 signalling pathway at a later stage after receptor binding Blocks signals transduced from a variety of growth factor receptors to the nucleus by acting on phosphatidyl inositol kinases called mammalian targets of RAPA (mTOR) Inactivates 70 kilodalton S6 kinase, which results in selective inhibition of the synthesis of new ribosomal proteins</td>
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<tr>
<td>Siroliums (rapamycin)</td>
<td>Inhibits synthesis of guanosine monophosphate nucleotides, blocks purine synthesis, preventing proliferation of T and B cells</td>
</tr>
<tr>
<td>Mycophenolate mofetil (MMF) and mycophenolic acid (MPA)</td>
<td>Inhibits synthesis of guanosine monophosphate nucleotides, blocks purine synthesis, preventing proliferation of T and B cells</td>
</tr>
<tr>
<td>Azathioprine (AZA)</td>
<td>Converts 6-mercaptopurine to tissue inhibitor of metalloproteinase, which is converted to thioguanine nucleotides, interfering with DNA synthesis; thioguanine derivatives may inhibit purine synthesis</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>Preserves Tregs in vitro and in vivo Inhibits B-cell stimulation, decreasing antibody production Target transcription factors such as activator protein 1 and nuclear factor-κB, abrogating expression of multiple cytokines, including IL-1, IL-2, IL-3, and IL-6 Suppress eicosanoid production</td>
</tr>
<tr>
<td>Mycophenolate mofetil (MMF) and mycophenolic acid (MPA)</td>
<td>Down-regulate adhesion molecules Inhibit proliferation and differentiation of B and T cells</td>
</tr>
<tr>
<td>Azathioprine (AZA)</td>
<td>Inhibits activation of T and B lymphocytes</td>
</tr>
<tr>
<td>Protein immunosuppressive drugs</td>
<td>Polyclonal antibody preparation that targets multiple different epitopes on T cells (CD2, CD3, CD4, CD8, CD28) and NK cells (CD16), functionally altering and depleting different cell types</td>
</tr>
<tr>
<td>Antithymocyte globulin</td>
<td>Binds to the CD3 antigen on the surface of T lymphocytes, inactivating the adjacent TCR (signal 1), ultimately leading to T-cell depletion</td>
</tr>
<tr>
<td>Muromonab-CD3 (OKT3)</td>
<td>IL-2–receptor antibodies, basiliximab (chimeric) and daclizumab (humanized), bind to the IL-2 receptor α-chain (CD25 antigen), depleting activated T cells and inhibiting IL-2–induced T-cell proliferation</td>
</tr>
<tr>
<td>Basiliximab (chimeric) and daclizumab (humanized)</td>
<td>Humanized, recombinant monoclonal antibody that targets antigen CD52 on T, B, NK cells, monocytes, and macrophages, causing lysis and depletion</td>
</tr>
<tr>
<td>Alemtuzumab (campath-IH)</td>
<td>Chimeric monoclonal antibody that binds to CD20 on B cells, mediating lysis</td>
</tr>
<tr>
<td>Rituximab</td>
<td>CTLA-4 fusion protein immunoglobulin that binds to B7 on T cells, preventing CD28 signaling</td>
</tr>
</tbody>
</table>

Adapted from Halloran.76

*Demonstrated efficacy in renal and islet cell transplantation (no published data in liver transplantation).*
depletion of circulating lymphocytes for approximately 1 month, and the rationale for its initial use is to facilitate lower doses of maintenance immunosuppression.77 Rituximab is a chimeric monoclonal antibody against CD20 on B cells; its use in liver transplantation has been limited to patients who develop posttransplant lymphoproliferative disorders81 and in recipients of ABO-incompatible grafts.82 A new generation of biological agents that block the interaction between CD80/86 and CD28 costimulatory pathways (signal 2) includes belatacept (LEA29Y), a CTLA-4 fusion protein immunoglobulin, which has shown efficacy in recipients of kidney76,83 and islet cell allografts,84 but has not yet been studied in liver recipients. Blockade of T-cell/APC costimulatory pathways mediated via CD40–CD154 has been accomplished using the anti-CD154 monoclonal antibody, but studies have been halted because of reports of thromboembolic phenomena, perhaps related to the expression of CD154 on activated platelets.77 In summary, based on our understanding of cellular and molecular mechanisms, the immune strategies currently used in liver transplantation target a number of mechanisms (eg, signal 1 and 2 transduction, lymphocyte depletion). The imminent future likely will witness an even greater expansion in our armamentarium of immunosuppressive agents than in the past decade. Our limitations remain very similar to those of the past: long-term toxicity remains a concern and we have very few biomarkers to measure the induction of tolerance85 and fine-tune immunosuppression based on an individual patient’s characteristics.

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