

Kidney Transplantation: Mechanisms of Rejection and Acceptance

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Key Words

renal allograft, acute rejection, chronic rejection, antibody,
accommodation

Abstract

We describe the molecular and cellular mechanisms believed to be responsible for the rejection of renal allografts, including acute T cell–mediated rejection, acute antibody-mediated (humoral) rejection, rejection mediated by the innate immune system, and chronic rejection. We present mechanisms of graft acceptance, including accommodation, regulation, and tolerance. Studies in animals have replicated many pathologic features of acute and chronic rejection. We illuminate the pathogenesis of human pathology by reflection from experimental models.

IFN γ : interferon γ
TGF β :
transforming growth
factor β

INTRODUCTION

Long-term acceptance of renal allografts with minimal or no immunosuppression is the goal of clinical transplantation. The major obstacles are immunologically mediated injury on the one hand, and adverse effects of immunosuppression on the other. Here we describe the molecular and cellular mechanisms believed to be responsible for rejection of renal allografts, beginning with descriptive cellular and molecular pathology in humans. Relevant experimental evidence is reviewed, derived largely from heart and kidney transplantation in genetically defined mouse strains.

We concentrate on the effector phase of the alloimmune response and on how the mechanisms can be deciphered in tissue. For convenience, we divide rejection into acute and chronic processes, although these probably form a continuum. We consider T cell- and antibody-mediated mechanisms separately, knowing that they commonly occur together. The innate immune system and nonimmune graft injury are briefly discussed. Finally, we review new information regarding possible molecular mechanisms by which grafts resist rejection in a state of accommodation and the features associated with graft acceptance (tolerance).

ACUTE T CELL-MEDIATED REJECTION

Human Pathology

Patients with acute cellular rejection develop an abrupt rise in serum creatinine, fluid retention, and sometimes fever and graft tenderness. With current therapy, directed largely at T cells, the incidence of acute rejection is approximately 5%–10% in the first year in unsensitized patients. Pathologically, acute cellular rejection is manifested by the accumulation of mononuclear cells in the interstitium, accompanied by inflammation of the tubules and sometimes of the arteries (1). Mononuclear cells permeate the interstitial space around tubules and are com-

posed mostly of CD4⁺ and CD8⁺ T cells (1). T cells contain cytotoxic granules (perforin and granzyme A and B) or the cytotoxic effector ligand, FasL (2). Gene expression studies show an increase in mRNA for cytotoxic T cell (CTL)-associated transcripts (3): granzyme B, perforin, and FasL (4–6), as well as T-bet, a master transcription factor for effector T lymphocytes (Th1 and CTLs) (4, 7). Other cytokines and chemokines selectively expressed in acute rejection are interferon γ (IFN γ), tumor necrosis factor β (TNF β), TNF α , chemokine (C-C motif) ligand 5 (CCL5)/regulated upon activation, normal T cell expressed and secreted (RANTES), and CCL3/macrophage inflammatory protein (MIP-1 α) (4). Genes induced by IFN γ are also highly expressed in acute rejection (8, 9). Elevation of transforming growth factor β (TGF β) is variable and correlated with later development of fibrosis (10). In protocol biopsies, the genes that are differentially expressed in clinical versus subclinical rejection are T-bet, FasL, and CD152 (CTLA4) (4).

Tubulitis, invasion of the tubular epithelium by infiltrating T cells and macrophages, is a characteristic feature of acute cellular rejection (**Figure 1** and **Figure 2**) (1). In extreme cases, tubular basement membrane rupture occurs, causing the leakage of tubular protein into the interstitium, a feature that correlates with graft dysfunction and progressive tubular loss (11). Intratubular T cells with cytotoxic granules accumulate selectively in the tubules, compared with the interstitial infiltrate, accounting for 65% of the mononuclear cells in tubules compared with approximately 30% of the interstitial cells (12). Lymphocytes expressing perforin mRNA and perforin protein are closely associated with tubular epithelial cells (2, 13). Increased numbers of TUNEL⁺ tubular cells are present in acute rejection (12, 14). The degree of apoptosis correlates with the number of cytotoxic cells and macrophages in the infiltrate, suggesting a pathogenetic relationship (12, 15). Many lymphocytes in tubulitis lesions express proliferation markers and

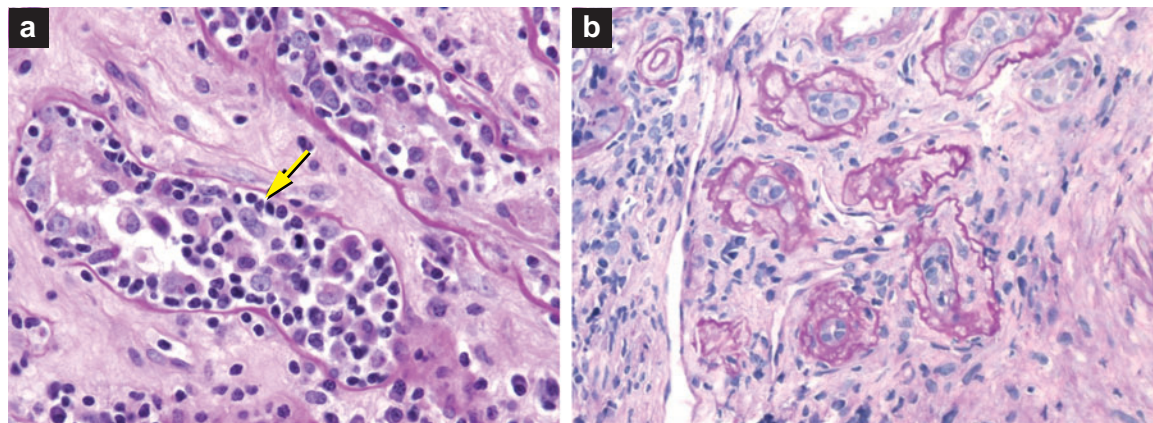


Figure 1

(a) In acute cellular rejection, mononuclear cells infiltrate the tubules (tubulitis, *arrow*), as in this striking example. (b) In late graft dysfunction from rejection, tubules are atrophic. (Periodic acid–Schiff stain.)

the integrin $\alpha E\beta 7$, which binds to E-cadherin on tubular epithelium (16). CD103(αE)⁺ cells are found exclusively in the tubules. Putative T regulatory cells (Tregs) expressing FOXP3⁺ and CD4⁺ are also concentrated in the tubules (17). Tubular epithelial cells upregulate intracellular adhesion molecule-1 (ICAM-1), CD80, and CD86, which are costimulatory molecules for T cells (18). Increased urine concentration of mRNA for CD103, perforin, granzyme B, and FOXP3 are found in acute rejection (19).

During acute rejection, a variety of chemokines are produced in the graft, including CXCL10, CCL2, CCL3, CCL4, CCL5, and CL1 (lymphotactin) (20, 21). Tubules are also a source of chemokines CCL2, CCL3, CCL4, CCL5, CXCL8 (IL-8), and CX3CL1 and cytokines TNF α , TGF β , and IL-6 (21–24). Epithelial activation is probably a response to local T cell production of IL-17 (25) and perhaps also to TNF α because tubular cells express TNFR2 (24). Heparan sulfate within the tubular basement membrane may provide binding sites for chemokines, such as CCL4, to create gradients (23). Infiltrating cells express several chemokine receptors, including CCR2, CCR5, CXCR3, and CX3CR1 (20). CCR5 is found mostly in diffuse infiltrates (20), whereas CXCR4 is in

nodular aggregates of mononuclear cells (22). The pattern of expression suggests a predominance of Th1 over Th2 cells (CCR5 and not CCR3 or CCR8) (20). CCR5 is probably important in the pathogenesis of rejection, as humans who are homozygous for inactive $\Delta 32$ form have a greater graft survival than those with the active form (26). Endarteritis (endothelialitis), characterized by subendothelial and intimal infiltrates of CD4⁺ and CD8⁺ T cells and macrophages (1), is a pathognomonic feature of cell-mediated rejection (**Figure 3** and **Figure 4**).

Endarteritis is detected in approximately 25%–40% of renal biopsies taken for acute rejection and rarely found in stable grafts (<0.5%) (27). Endarteritis responds poorly to steroids, but is reversible with anti-T-cell therapy (OKT3), arguing for a pathogenic role for T cells (1). Apoptosis of vascular endothelial cells (ECs) is present in sites of endarteritis (28). Endarteritis is not always associated with interstitial inflammation, arguing for a T cell pathway distinct from that of tubulointerstitial rejection.

Glomerulitis is occasionally a conspicuous feature of acute cellular rejection. The cells in the glomeruli are largely CD3⁺ T cells, with an admixture of CD68⁺ macrophages (29). In severe cases, the endothelium shows marked

ICAM: intracellular adhesion molecule

EC: endothelial cell

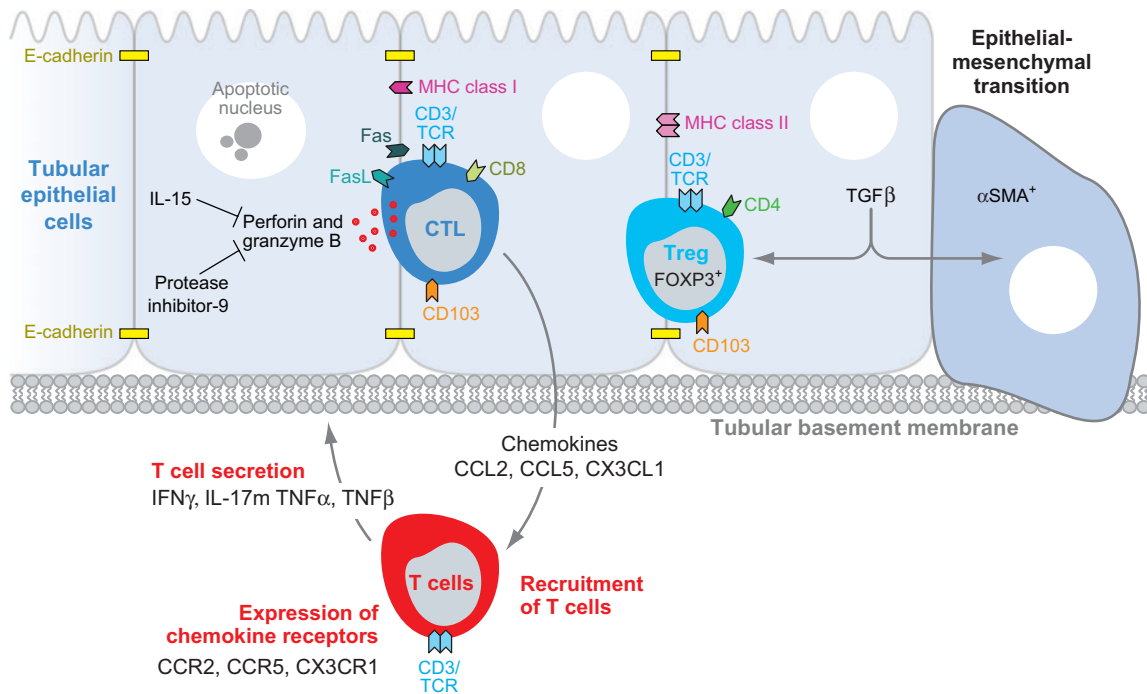


Figure 2

Diagram of postulated events in tubulitis. T cells are attracted into cortical tubules (proximal and distal), probably via chemokines (CCL2, CCL5, CX3CL1) from tubular cells, made in response to cytokines from the inflammatory cells [e.g., interleukin (IL)-17, tumor necrosis factor α (TNF α)]. These concentrate in the basement membrane matrix. T cells, especially cytotoxic T lymphocytes, enter between tubular cells and may cause apoptosis by releasing cytolytic granules containing granzymes and perforin or by exposure of FasL on the T cell surface. The integrin component CD103 is postulated to help retain T cells in the epithelial layer by binding to E-cadherin, expressed most strongly in the distal nephron. Cells with the phenotype of T regulatory cells (CD4⁺ FOXP3⁺) also accumulate in tubules; their function is unknown. Tubules produce TNF β , which promotes FOXP3 expression, and IL-15, which inhibits perforin production. Protease inhibitor-9 from tubules inhibits granzyme B (see text). Tubular cells chronically exposed to transforming growth factor β (TGF β) (e.g., from macrophages) may undergo epithelial-mesenchymal transition, an aberrant phenotype evidenced by epithelial cell expression of α -smooth muscle actin and loss of E-cadherin expression. These cells then may infiltrate the interstitium and contribute to fibrosis.

activation and swelling with mesangiolysis, and accumulation of webs of periodic acid-Schiff-stained material. Why the glomerulus becomes a target in a minority of cases is an unsolved mystery.

Experimental Studies

Proof of mechanisms in organ transplantation relies on *in vivo* models in animals. Mice and rats are particularly suitable to genetic and experimental manipulation, but some of the

models are highly artificial. Allograft studies in large animals, especially monkeys and pigs, have the advantage of greater preclinical relevance, but are limited by expense and availability.

Afferent alloimmune response. Space permits only a brief orientation to the nature of the afferent immune response to alloantigens, reviewed elsewhere in detail (31). The alloimmune response arises principally

against the genetically determined, highly polymorphic cell surface molecules, known as class I and class II major histocompatibility complex (MHC) antigens (HLA in humans, H-2 in mice), of the donor that are lacking in the recipient. These antigens are widely, and sometimes variably, expressed on tissue cells (e.g., endothelial and tubular cells), which may influence which graft cells become targets of the alloimmune response. The importance of MHC antigens in humans is evident by the observation that grafts from HLA-identical siblings survive considerably longer than those from HLA-nonidentical siblings (1). In mice, mutations affecting only 1–3 amino acids in a single class I or II molecule are sufficient to induce graft rejection. T cells recognize foreign MHC molecules either on the graft cells (direct pathway) or, after reprocessing, on the surface of recipient antigen-presenting cells (indirect pathway).

Patients normally do not have preexisting alloreactivity (presensitization) to MHC molecules, unless they have been exposed to foreign human cells through pregnancy, blood transfusion, or transplantation.

Other polymorphic antigens (known as minor or non-MHC antigens) and even autoantigens may also serve as a target of the T cell or antibody-mediated immune response to allografts. ABO blood group glycolipids expressed on endothelial and red blood cells are notable examples of non-MHC antigens. The spectrum and identity of other non-MHC antigens remain a long-standing topic of investigation.

A second signal (costimulatory signal) is ordinarily required for T cell activation (31). T cells then attack the graft cells by direct surface contact (cell-mediated cytotoxicity) or indirectly via cytokines and their effects on other cells. T cells stimulated by the indirect pathway can also provide help to B cells to expand and differentiate into plasma cells, producing antibodies to donor MHC molecules.

Costimulatory molecules. The second signals, or costimulatory molecules, most

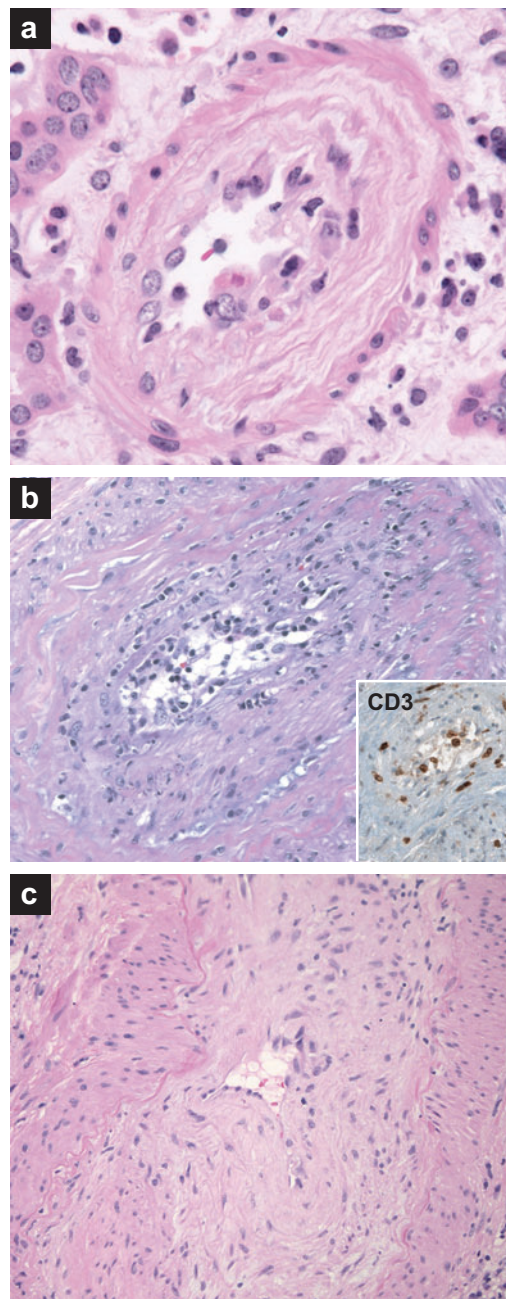


Figure 3

(a) Endarteritis, or mononuclear inflammatory cells under the arterial endothelium, in acute cellular rejection. (b) This artery demonstrates endarteritis and active early chronic transplant arteriopathy. The intima is thickened, fibrotic, and contains mononuclear cells, which stain for CD3 by immunohistochemistry (*inset*). (c) A later, and less active, chronic lesion, showing a thickened intima with fewer mononuclear cells. All panels are H&E stained.

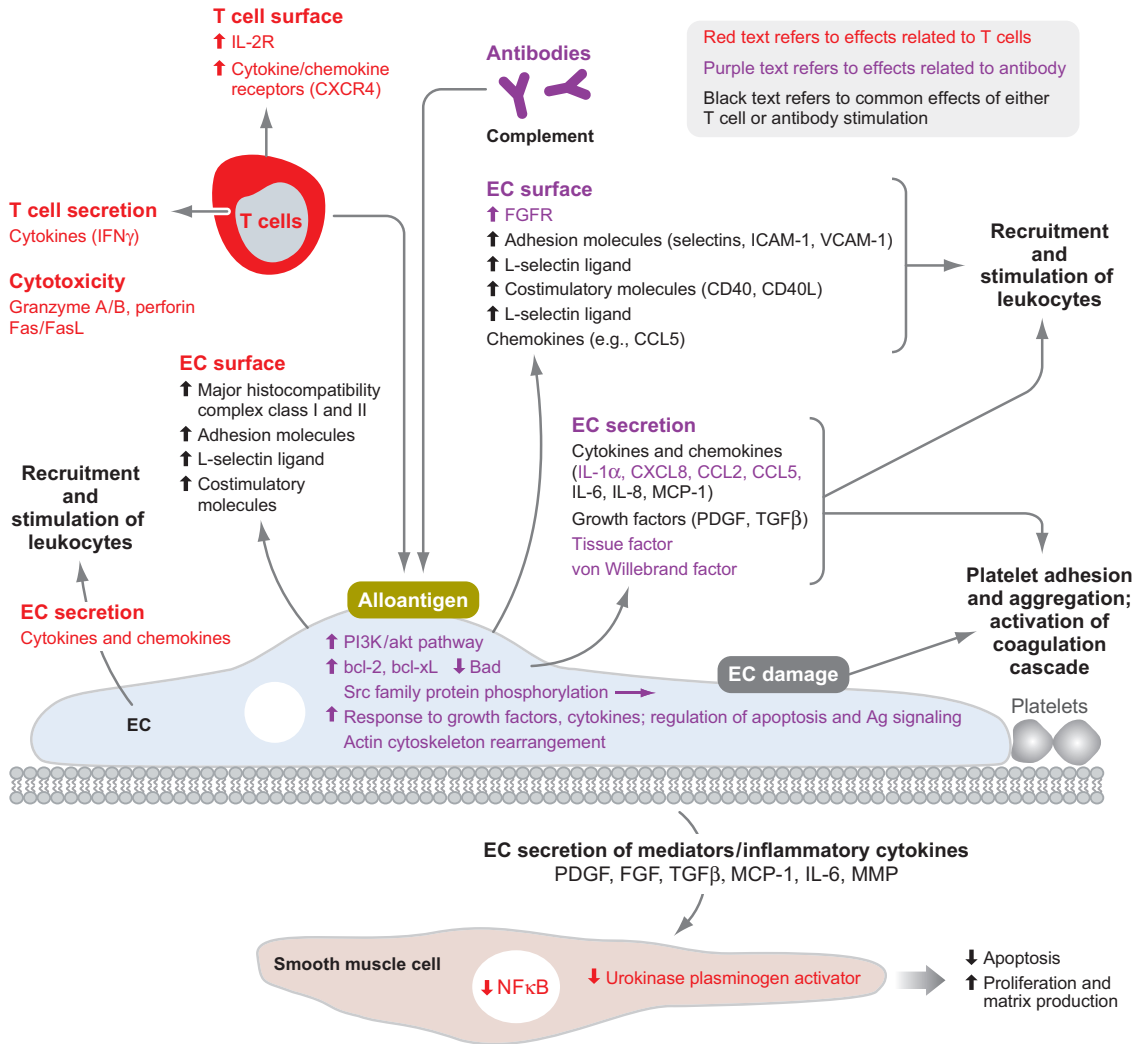


Figure 4

Diagram of postulated events in T cell- and antibody-mediated endothelial interactions in allografts. Both T cells and antibody may recognize antigen on target endothelium, leading to rejection and chronic changes in the endothelium and the underlying smooth muscle layer. T cells acquire an activated phenotype and may directly cause cytotoxicity of the target cells. Antigen recognition leads to a multiplicity of responses by the endothelial cell (EC), resulting in changes in EC surface molecule expression and EC secretion of various factors. These secreted factors activate the immune and coagulation systems and affect the neighboring cells. Although these effects are designated as T cell- or antibody-related effects, many effects may be common but have not been studied.

investigated for their role in graft rejection are the receptor ligand pairs: CD28/CTLA4 and CD80/CD86 (B7-1/B7-2), CD40 and CD154, ICOS and ICOSL, OX40 and

OX40L, and CD27 and CD70 (Table 1). Costimulatory blockade with antibodies or soluble receptors (CTLA4-Ig) or genetic deletion of single costimulatory molecules

Table 1 Effects of deficiency of costimulatory molecules and receptors on mouse cardiac allograft rejection

Molecule deficient	Recipient ¹	Donor ²	Acute rejection ³	Chronic rejection ⁴	Reference(s)
CD80 and CD86 (B7-1,-2)		DKO	–		(32)
CD80 and CD86 (B7-1,-2)	DKO		↓		(32)
B7 family	CTLA4-Ig		↓		(33–35)
CD28	KO		–		(33)
CD28	KO		↓		(36)
ICOS	KO		–		(37)
CD137 (4-1BB)	KO		↓		(38)
B7-3H	KO/rapamycin		↓	↓	(36)
CD40	KO	KO	↓	–	(39, 40)
CD40	KO		↓		(41)
CD40		KO	–		(41)
CD40L	KO		↓	–	(42)
CD40L		KO	–		(42)
CD40L	mAb/aCD4		↓	↓	(36, 43)
CD134L (OX40L)	KO		–		(44)
CD70	mAb		–		(45)
Combinations					
CD28 and CD40L	CTLA4-Ig/mAb		↓	↓	(46)
CD28 and CD70	KO/mAb		↓	↓	(45)
CD28 and CD137	DKO		↓		(38)
CD28 and B7-3H	DKO		↓		(36)
CD40L and B7-3H	mAb/KO		↓	↓	(36)

¹DKO, double knockout; CTLA4-Ig, soluble CTLA4 with Ig domain; KO, knockout deletion in recipient or donor; mAb, monoclonal antibody.

²Full MHC mismatch.

³↓, decreased acute rejection (improved graft survival); ↑, increased acute rejection; –, no effect; blank, not studied.

⁴↓, decreased chronic arteriopathy; ↑, increased chronic arteriopathy; –, no effect; blank, not studied.

generally prolongs acute rejection, but in general has little effect on chronic rejection. Prevention of chronic rejection has required interference with two or more separate pathways (e.g., B7 and CD40).

Antigen presenting cells. Dendritic cells (DCs) are critically important in antigen presentation and subsequent graft rejection. Indeed, depletion of donor DC prolongs survival of allografts (47). Immediately after transplantation, donor DCs migrate to recipient draining lymph nodes and spleen (48), and recipient DCs enter the graft. Thus, sensitization to the graft occurs in draining lymph

nodes (the dominant pathway) and probably in the graft as well. This two-way migration continues over ensuing weeks. Unique to the immune response to allografts is that DCs from both donor and recipient can provide activation signals to recipient T cells. Curiously, sensitization occurs even if the costimulatory molecules CD80/CD86 are absent from the donor DC and class II is absent from the recipient DC, arguing that the cells can act together (*in trans*) (49). The chemokines CCL19 and CCL21 are required for the migration of DCs to lymphoid tissue; absence of CCL19/CCL21, or their receptor CCR7, inhibits acute graft rejection (**Table 2**) (50).

Table 2 Effects of deficiency of chemokines and receptors on mouse cardiac allografts

Molecule deficient	Recipient ¹	Donor	Acute rejection	Chronic rejection	Reference(s)
CCL3 (MIP-1a)	KO		–		(59)
CCL5 (RANTES)	KO		–		(59)
CCL17	KO		↓		(60)
CCL19/CCL21	KO		↓		(50)
CCR1	KO		↓		(61)
CCR2	KO		–		(62)
CCR4	KO		–		(63)
CCR5	KO		–		(57)
CCR5	KO		↓		(59)
CCR5	KO+CNI		↓	↓	(59, 64)
CCR7		KO	↓		(65, 66)
CXCL9 (Mig)	pAb		↓		(67)
CXCL10 (IP-10)	KO		–		(68)
CXCL10 (IP-10)		KO	↓		(68)
CXCR3	KO		↓		(69)
CX3CR1 (fractalkine)	KO		–		(70)

¹Abbreviations as in **Table 1**; CNI, calcineurin inhibitor; pAb, polyclonal antibody.

With time, grafts can develop tertiary lymphoid organs (TLOs), as discussed below (51).

B cells can also present antigen by virtue of their costimulatory molecules, high levels of class II, and surface Ig, which concentrates antigens. Chimerically engineered mice with MHC class II deficiency confined to B cells show markedly prolonged cardiac allograft survival (>70 days), compared with controls (9.5 days). In these recipients, CD4⁺ T cells are less activated, and no alloantibody is formed (52). This study elegantly demonstrates the *in vivo* importance of B cell antigen presentation in the allograft response. Direct antigen presentation by graft cells can occur via tubular cells and ECs, which can process and present antigen to activated T cells *in vivo* and *in vitro* (53, 54), a process dependent upon CD80/CD86 expression (55). One study demonstrated that ECs can, via proteasome- and transporter-associated-with-antigen-processing-dependent pathways, process and present exogenously derived proteins in the context of MHC class I (54). Infiltrating

recipient macrophages can scavenge and present donor antigens from ECs and other donor cells (56).

Chemokines. Chemokines, a family of at least 48 small proteins, promote the migration of leukocytes expressing one or more of their corresponding receptors into tissue. In mouse heart allografts, acute rejection is delayed by a genetic deficiency in the recipient receptors CCR1, CCR5, or CXCR3, or ligands CCL17 and CCL19/CCL21 (**Table 2**). Surprisingly, CCR5 knockout mice showed high titers of alloantibody and *in situ* C3d deposition, perhaps related to the necessity of CCR5 for Treg infiltration of allografts (57, 58). Deficiency of chemokine receptor CCR7 or the ligand CXCL10 in the donor also decreases acute rejection. CXCL10 deficiency in the recipient had no effect, indicating that the relevant source is the graft. None of these individual deficiencies affects chronic rejection, although the addition of calcineurin inhibitors to CCR5 knockout mice inhibits chronic arteriopathy.

Effector T cells. Although T cells are essential for acute organ allograft rejection, the precise mechanisms by which they mediate graft injury are uncertain. The two theories are cell-mediated cytotoxicity of parenchymal cells (tubular, endothelial) and local cytokine release, analogous to a delayed-type hypersensitivity reaction. Cytokines may act directly on parenchymal cells or indirectly through effects on the endothelium and vascular supply.

Cytotoxicity. CD8⁺ class I reactive T cells kill target cells through perforin and granzyme A and B, or through the Fas/FasL cytolytic pathways (71). Perforin and granzyme A and B are proteins in granules that are directed into the extracellular space by exocytosis. FasL activates the death receptor Fas on the target cells. Both pathways induce caspase-mediated apoptosis of the target cell. Effector CD4⁺ T cells that can mediate class II-restricted cytotoxicity to minor antigens are also detectable (72). Analysis of CTLs has received much attention because the specificity of cytolysis can be used to account for the precise target cell selectivity of skin graft rejection. Class I mismatched heart allografts show prolonged survival in perforin knockout mice (73). Nevertheless, most available evidence with perforin and Fas/FasL knockouts indicates that any of these individual cytolytic pathways are dispensable, as acute rejection still occurs with these deficiencies in full mismatched combinations (Table 3).

Cytokines. Another pathway exists in which effector cells mediate cytotoxicity via secretion of TNF α and TNF β . These have local cytotoxic action on neighboring target cells with TNF receptors, as found on many cells. TNFR1 is expressed on ECs and TNFR2 on tubular cells in rejection (24). Cytotoxicity occurs through the apoptosis and the caspase pathways. Anti-TNF-blocking antibodies prolong survival of cardiac allografts in rats (76). In mice, knockout of both TNF receptors in the graft is required for prolongation of graft survival. Lack of TNFR1 in the recipient also prolongs graft survival, indicating that another role of TNF is communication between recipient cells (Table 4).

IFN γ , the prototypic Th1 helper cell cytokine, is strongly associated with rejection and graft-infiltrating cells and is required for the rejection of bm12 class II disparate skin grafts (91). However, studies in organ grafts have shown contradictory results. In mouse renal allografts, IFN γ has a paradoxical beneficial effect, inhibiting necrosis. Kidneys from IFN γ receptor knockout donors are rejected more quickly than wild-type donors. Allografts show fibrin thrombi and necrosis, reminiscent of hyperacute antibody-mediated rejection (81, 82). IFN γ or IFN γ R deficiency in the recipient also promotes accelerated graft necrosis. The mechanism is unclear, but may involve a lack of the normal inhibitory role of IFN γ on CTLs or a promotion of antibody production (92, 93).

Other cytokines can be deleted from the recipient with little or no effect on acute or

Table 3 Effects of deficiency of cytotoxic mediators on mouse cardiac and renal allografts

Molecule deficient	Recipient	Donor	Graft ¹	Acute	
				rejection	Reference(s)
Perforin	KO		K	–	(3)
Perforin	KO		H	–	(73)
Granzyme A and B	DKO		K	–	(3)
FasL	KO	KO	H	–	(74)
Fas		KO	H	–	(74)
Fas	KO		H	–	(75)

¹Abbreviations as in Tables 1 and 2; K, kidney allografts, fully MHC mismatched; H, heart.

Table 4 Effects of deficiency of cytokines and receptors on mouse cardiac and renal allografts¹

Molecule deficient	Recipient	Donor	Graft	Acute rejection	Chronic rejection	Reference(s)
IFN γ	KO		H	↑		(96–98)
IFN γ	Mab/ α CD4, -8		H		↓	(77, 78)
IFN γ	KO+CNI		H		↓	(79)
IFN γ R		KO	H	↓		(80)
IFN γ R	KO		H, K	↑		(81)
IFN γ R		KO	H, K	↑		(82)
IFN γ +IL2	DKO		H	–		(83)
IL-2	KO		H	–		(44)
IL-4	KO		H	–		(84)
IL-5	KO		H	↓		(85)
IL-9	KO		H	–		(86)
IL-10	KO		H	↑		(97, 108)
IL-12	KO		H	↑		(109, 110)
Migration inhibitory factor	KO	KO	H	–	–	(87)
TGF β	KO ^{-/+}		H	–	↑	(88)
TNFR1	KO		H	↓		(89)
TNFR1, -2	DKO		H	–		(90)
TNFR1, -2		DKO	H	–	↓	(90)

¹Abbreviations as in **Tables 1–3**; KO^{-/+}, heterozygous knockout; α CD4, -8, antibody to CD4 and CD8. All grafts fully MHC incompatible.

chronic graft rejection of a fully MHC mismatched heart, including IL-2, IL-4, IL-5, IL-9, and macrophage migration inhibitory factor. Deletion of IL-10 or IL-12 accelerates acute rejection, indicating these cytokines normally mute the immune response. Deletion of even one copy of the TGF β gene augments chronic rejection, indicating its importance for inhibition of the alloimmune response.

Pathologic lesions of rejection. Tubulitis, an important lesion in the diagnosis of rejection, is believed to contribute to the abrupt rise in creatinine during acute cellular rejection (**Figure 2**). This lesion is T cell dependent and occurs in the absence of B cells and alloantibody (94). CD103 cell depletion in rat kidney allografts reduces tubular CD8⁺ T cell infiltration and tubular injury (95). However, in mice, renal allograft rejection and tubulitis are similar between CD103 knock-

out mice and wild-type controls (3). Comparable losses of tubular epithelial area and E-cadherin occur, as do similar patterns of microarray transcriptomes and CTL-associated transcripts (3). Tubulitis and E-cadherin loss also occurred in perforin or granzyme A- and granzyme B-deficient recipients (3). Thus, in the nonimmunosuppressed mouse, CD103/E-cadherin and perforin/granzyme-mediated cytotoxicity are not necessary for acute tubular injury, but may contribute to the retention of T cells in tubules and the injury of the graft in other settings.

Endarteritis affects large and small arteries focally, both within and outside the parenchyma (**Figure 4**) (96). Endarteritis occurs without participation of antibody, as shown in B cell knockout mice (97). ECs undergo apoptosis in both acute and chronic rejection (28). In mouse cardiac allografts differing by class I MHC alone, apoptosis of ECs is dependent upon perforin (98). Inflammatory

cell adhesion and transendothelial migration begin when abluminal chemokines (CCL4, CCL5, CXCL8) are transcytosed to the luminal surface and are bound by luminal surface glycosaminoglycans (heparin sulfate) to create a chemotactic gradient and inflammatory cell migration (99). The infiltrating cells are mostly T cells and macrophages with an effector phenotype. ECs express chemokine receptors CCR2, CCR8, and CXCR1–4 so that the corresponding chemokines promote their activation and upregulation of adhesion molecules (ICAM-1, vascular cell adhesion molecule, PCAM) (99). Lack of ICAM-1 or Egr-1, a transcription factor controlling ICAM-1 expression, inhibits acute rejection (100, 101).

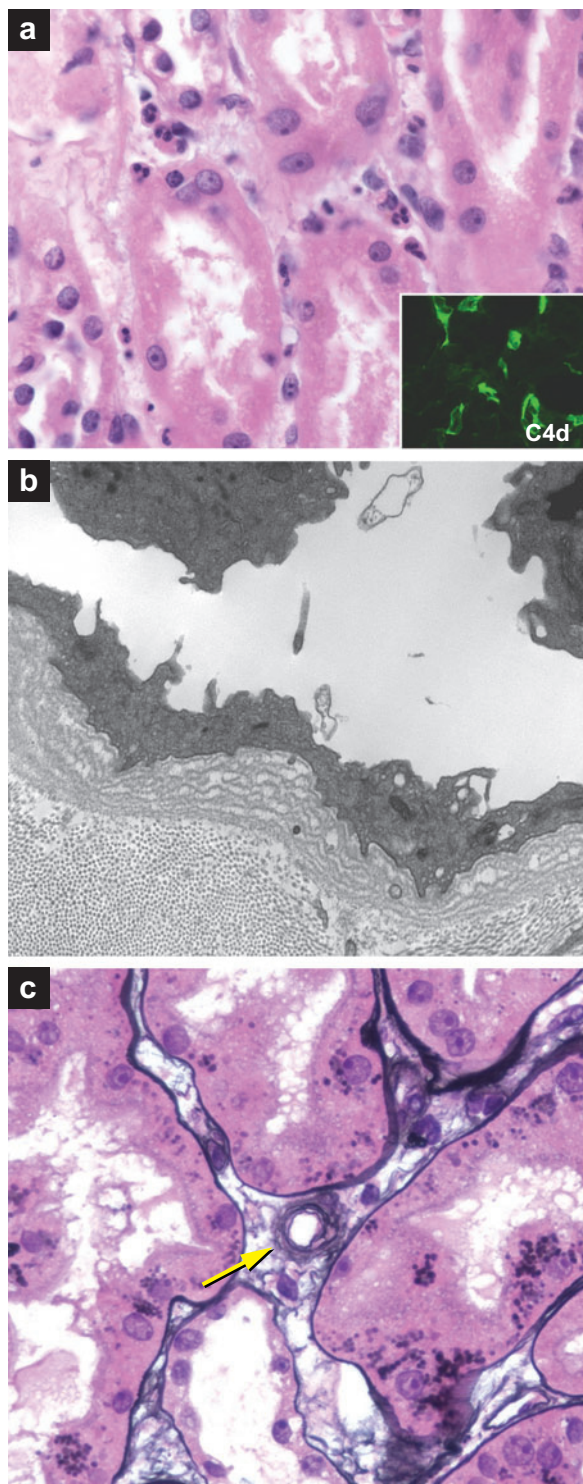
ACUTE ANTIBODY-MEDIATED REJECTION

Human Pathology

Acute antibody-mediated rejection, or acute humoral rejection (AHR), is now widely accepted as a distinct clinicopathologic entity (102). Approximately 25% of acute rejection episodes are due, at least in part, to antibody to donor HLA antigens. Risk factors include presensitization and decreased immunosuppression (e.g., noncompliance). AHR has occurred with all immunosuppression regimens, even with profoundly depleting T cell therapy (103). AHR may occur with or without a component of T cell-mediated rejection. Antigens other than HLA can serve as a target if expressed on ECs. Examples include ABO blood group antigens and the putative endothelial alloantigens, as suggested by the rare occurrence of AHR in HLA-identical sibling grafts (104). Even auto-antibodies have been implicated, such as those to angiotensin II type 1 receptors (105). Clinically, patients with AHR present with a rapid rise in serum creatinine, days to weeks or even years after transplantation. The clinical presentation is not distinctive from acute cellular rejection, and a renal biopsy is required for diagnosis.

The kidney typically shows an accumulation of neutrophils and monocytes in peritubular and glomerular capillaries (**Figure 5**) (29, 30). Peritubular capillaries are often dilated. Ultrastructurally, the endothelium of the peritubular and glomerular capillaries shows loss of fenestrations, detachment from the basement membrane, lysis, and apoptosis (106). The mononuclear infiltrate can be quite sparse. Tubulitis and endarteritis are generally minimal, unless a component of T cell-mediated rejection is present. Microthrombi, hemorrhage, necrosis of arterial walls, and infarction may occur in the more severe cases. Tregs (FOXP3⁺) are rarer in the infiltrate than in cell-mediated rejection, perhaps a factor in its poorer prognosis (17). None of the histologic features is specific for AHR (102). Furthermore, immunofluorescence microscopy does not reliably detect antibody deposition in the vessels.

Recognition of AHR has become substantially easier with the advent of the C4d stain pioneered by Feucht (107). Subsequent studies by others showed C4d to be highly correlated with histologic features of neutrophils or fibrinoid necrosis and circulating antidonor-specific antibody (108, 109). C4d is an inactive fragment of C4b of the classic complement pathway. C4b and C4d contain an occult sulfhydryl group that forms a covalent thioester bond with nearby proteins upon activation by antibody and C1. C4d per se has no known functional role, but remains bound in the tissue for several days after immunoglobulin and C1 have been released. C4d deposits in the majority of the peritubular capillaries as an intense ring pattern, using immunofluorescence microscopy with a monoclonal antibody in cryostat sections or immunohistochemistry with a polyclonal antibody in formalin-fixed paraffin-embedded tissues (110, 111). Immunoelectron microscopy demonstrates C4d on the surface and in intracytoplasmic vesicles of ECs (110). Antibodies to donor HLA class I or II antigens are present in 88%–95% of the patients who have C4d deposition (102).



However, AHR can occur in the absence of demonstrable circulating antibodies, which is probably due to the absorption of alloantibodies by the graft (112).

C3b, the component activated by C4b, theoretically should indicate more complete complement activation in tissue. Indeed, C3d is associated with acute inflammation (neutrophils) in ABO-incompatible grafts, a setting where C4d deposition is the rule, even in histologically normal grafts (113). In the usual, ABO-compatible graft, however, C3d provides no additional information beyond C4d. Lectin pathway components (e.g., H-ficolin), which activate C4 by binding to microbial carbohydrates, are sometimes detected in conjunction with C4d, but their significance is unknown (114).

Complement fixation is strongly associated with the ability of antibody to mediate AHR in humans (115). Recipients with antidonor HLA class I antibodies able to fix complement (C4d on FlowPRA beads) had more severe rejection, as measured by graft loss (115). Those with noncomplement-fixing antibodies had a prognosis similar to those without antibodies. Complement-fixing HLA class II antibodies did not affect graft survival, even though they were associated with C4d deposition. The potency of human IgG subclasses to activate the classical complement pathway decreases in the order $IgG3 > IgG1 > IgG2 > IgG4$. IgG3 antidonor antibodies were present in patients with acute rejection, but not in stable patients. The latter had a significant rise only in the IgG4 subclass (116).

←

Figure 5

(a) In acute humoral rejection, peritubular capillaries may show margination of neutrophils. C4d is detected in these capillaries by immunofluorescence (*inset*). (b) The endothelium characteristically appears activated and, as humoral rejection persists or recurs, the peritubular capillary basement membrane becomes multilaminated. (c) Occasionally, peritubular capillary multilamination may be seen by light microscopy (*arrow*). (a, H&E stained; b, electron microscopy; c, Jones methenamine silver.)

IgG4 does not fix complement and may inhibit immunologic injury.

Experimental Studies

Antibody-mediated rejection in general is difficult to demonstrate in skin grafts, in part because the skin revascularizes with recipient endothelium (117). However, antibody-mediated rejection has been widely studied in vascularized organ grafts, including the heart and kidney. Much of the knowledge comes from studies of acute xenograft rejection, which is mediated initially by naturally occurring antibodies to carbohydrate determinants, analogous to ABO-incompatible grafts in humans.

Complement. In animal studies, complement fixation is essential in promoting acute or hyperacute antibody-mediated rejection (118). Complement-fixing isotypes of monoclonal anti-H-2 class I antibodies are necessary for passively transferring the AHR of mouse cardiac allografts (119). Strong complement-fixing isotypes (IgG3) mediate acute antibody-mediated rejection independent of Fc receptors or NK cells (120). Passive transfer of complement-fixing isotypes in the mouse or rat leads to C4d deposition in the microvasculature, similar to that observed in renal and heart grafts in humans (121, 122). C4d is transient, disappearing after two weeks in mice after passive transfer ceases (121) and after five days in rat heart grafts retransplanted back into isogeneic recipients (122).

The acute effects of complement are well described and include chemoattraction of neutrophils and macrophages via C3a and C5a, vasospasm through the release of PGE2 from macrophages, and edema through the release of histamine from mast cells. C3a and C5a increase endothelial adhesion molecules, E-selectin, vascular cell adhesion molecule-1 and ICAM-1, and production of cytokines and chemokines such as IL-6, IL-1 α , CXCL8, and CCL5 (123). The membrane attack complex, C5b-9, causes lysis of ECs. The local pro-

duction of complement components, such as C6 by recipient macrophages, augments acute rejection. Elegant studies with bone marrow chimeras reveal that macrophages are a more important source of C6 than plasma for the AHR of rat hearts (124).

Protection from antibody-mediated rejection can be achieved by inhibition of the complement system, as shown by transgenic expression of the complement regulatory proteins CD46 (membrane cofactor), CD55 (decay-accelerating factor), and CD59 in pigs (125). High expression of transgenic human CD46 prevented hyperacute rejection and thrombotic manifestations (125). Similarly, expression of transgenic CD55 and CD59 in pigs can prevent hyperacute rejection, although a coagulopathy was still present in this system (126). Genetic absence of CD55 in mice increases the susceptibility of heart grafts to low levels of anti- α -gal antibodies (127). Finally, antibodies to C5 that block activation and cleavage and formation of the C5b-9 complex inhibit antibody-mediated rejection in rat to mouse heart grafts (128).

Complement-independent pathways. Antibodies can lyse target cells through the Fc γ RIIA (CD16) on NK cells and macrophages (antibody-dependent cellular cytotoxicity). One piece of evidence that supports this pathway in humans is that genetic variation in FcRIIA correlates with the risk of acute renal allograft rejection (129). Antibodies with limited ability to fix complement in vitro (e.g., IgG1 in the mouse) mediate acute antibody-mediated rejection against carbohydrate antigens in mouse hearts (120, 130). The efficacy of IgG1 antibody depended upon NK cells, Fc γ R, and the complement system. Inhibition of any one of these abrogated IgG1-mediated rejection. Further evidence of NK participation comes from studies of rat to mouse heart xenografts. During rejection, there is elevation of circulating IFN γ levels, probably produced by NK cells, which is inhibited by NK depletion with anti-asialo-GM-1 antibodies (131).

Antibodies without leukocyte or complement participation can activate ECs to produce chemokines and promote rejection in some models (119). Antibodies in vitro induce the expression of CD62E and CD106 in endothelium and promote adhesion and spreading of NK cells via Fc γ RIIA (132). Noncomplement-fixing antibodies to class I antigens can induce the expression of chemokines such as monocyte chemoattractant protein-1 in cultured endothelium, an effect augmented by TNF α (119). In low doses, antibodies induce a state of resistance to antibodies mediated through adenosine A2 receptors and induction of Bcl-xL (133). Curiously, the absence of IFN γ or IL-10 increases the susceptibility of the endothelium in murine heart xenografts to B cell-dependent antibody-mediated injury (92).

Coagulation. Pathologic features of antibody-mediated acute, hyperacute, or xenograft rejection all share the presence of microvascular thrombosis. In these conditions, von Willebrand factor is released from the endothelium, and platelet aggregation occurs (134). Clotting activation is a consequence of complement fixation, as shown in rats deficient in C6, in which release of von Willebrand factor and endothelial injury did not occur (134). Activation of endothelial protease-activated receptors by coagulation proteases, including thrombin (135), leads to the secretion of many proinflammatory cytokines. Depletion of fibrinogen with Ancrod delays antibody-mediated xenograft rejection in the mouse (131). Genetically engineered graft endothelial expression of a membrane-tethered form of tissue factor inhibitor and hirudin, a thrombin antagonist, inhibits xenograft rejection in the rat (136).

CHRONIC REJECTION

Human Pathology

Chronic rejection of renal allografts may occur by cellular or humoral mechanisms, or

both. Chronic changes can be seen in the glomeruli, vessels, tubules, and interstitium. Histologic features characteristic of chronic rejection are transplant glomerulopathy, peritubular capillaropathy, transplant arteriopathy, and, less specifically, interstitial fibrosis and tubular atrophy (1).

Transplant glomerulopathy. This lesion is characterized histologically by duplication or multilamination of the glomerular basement membrane (**Figure 6**). Electron microscopy reveals lamination of the basement membrane, loss of fenestrations of endothelial cells, and variable effacement of podocyte foot processes. Endothelial cells show evidence of increased vesicular transport by neo-expression of plasmalemmal vesicle-associated protein-1, a component of plasmalemmal vesicles (caveolae) (137). Glomerular basement membrane duplication may be caused by a number of insults to the allograft glomerulus, including recurrent or de novo immune complex glomerular disease, thrombotic microangiopathy, and chronic antibody-mediated injury (1). The majority of cases are associated with circulating antibody to donor MHC class II antigens (sometimes to class I antigens), and approximately 30%–50% of these have C4d deposition in the peritubular capillaries (102, 138). Transplant glomerulopathy, when accompanied by C4d deposition in peritubular capillaries and by circulating donor specific antibody, is diagnostic of chronic humoral rejection (CHR) (139). Among presensitized patients, prior episodes of AHR are a major risk factor for transplant glomerulopathy in later protocol biopsies (140), and protocol biopsies with subclinical AHR increase the risk of later graft dysfunction, fibrosis, and tubular atrophy (141).

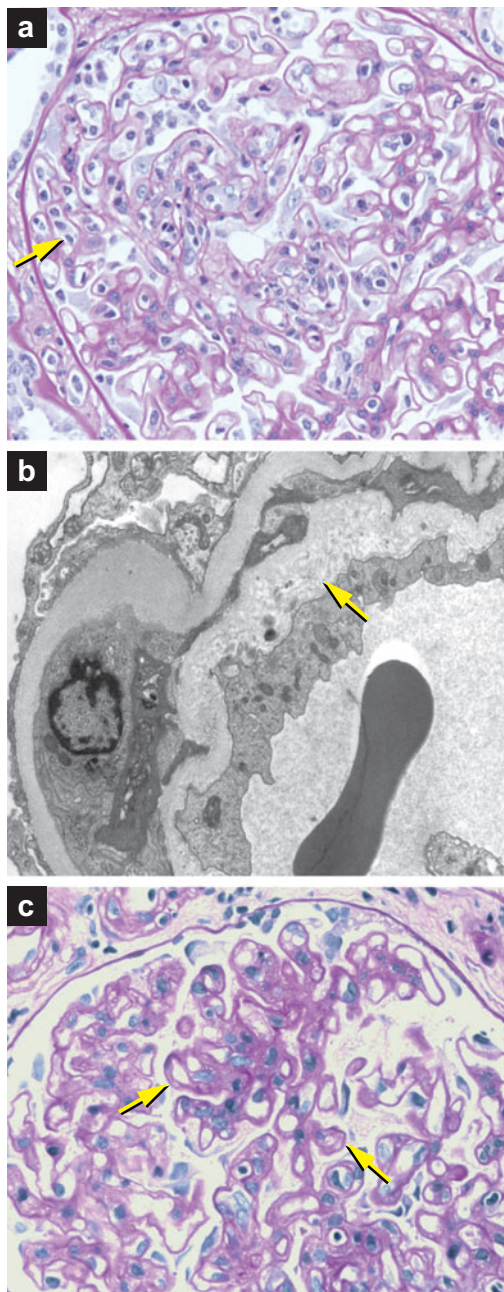
Peritubular capillaropathy. Just as basement membrane duplication or multilamination may be seen in glomeruli in transplant glomerulopathy, a similar finding may be seen in peritubular capillaries by electron

Figure 6

(a) Acute transplant glomerulitis, with mononuclear cells in many glomerular capillary loops (*arrow*). (b) As rejection becomes chronic, the glomerular basement membrane shows duplication or multilamination (*arrow*). These changes may be seen by electron microscopy before they are apparent by light microscopy. (c) Chronic allograft glomerulopathy, with widespread duplication of the glomerular basement membrane (*arrows*). (Periodic acid–Schiff stain; electron microscopy.)

microscopy (**Figure 5**), as has been studied extensively (1). When damaged endothelium repairs itself, it also forms a new basement membrane layer. The pathologic finding of basement membrane multilamination is thought to occur by repeated episodes of injury to the endothelium by antibody. Ivanyi et al. (142) have proposed that this peritubular capillaropathy be defined as one peritubular capillary with seven or more circumferential basement membrane layers, or three or more peritubular capillaries with five to six circumferential layers. Accumulation of mononuclear cells in peritubular capillaries is also characteristic feature of CHR and predicts later graft failure (143). What leads to episodic antibody injury in the graft endothelium is unknown, but one explanation is the fluctuation of antibody levels during the life span of the chronically rejected graft, as described in some patients followed longitudinally for donor-specific antibody (144). It follows that circulating antibody may be below the level of detection at a given time point, having left behind its pathologic lesions (**Figure 7**). In addition, the graft itself may show fluctuating levels of accommodation, injury, or repair to antibody (see below).

Transplant arteriopathy. Neointimal thickening with scattered mononuclear cells may be seen as a feature of either C4d-positive or C4d-negative chronic rejection. This lesion, also known as “transplant arteriopathy,” is characterized histologically by thickening



of the arterial intima without duplication of the elastica (in contrast to fibroelastic thickening seen in hypertension). Macrophages and CD3⁺ T cells may sometimes be demonstrated within the thickened intima, as evidence of cell-mediated immunologic activity.

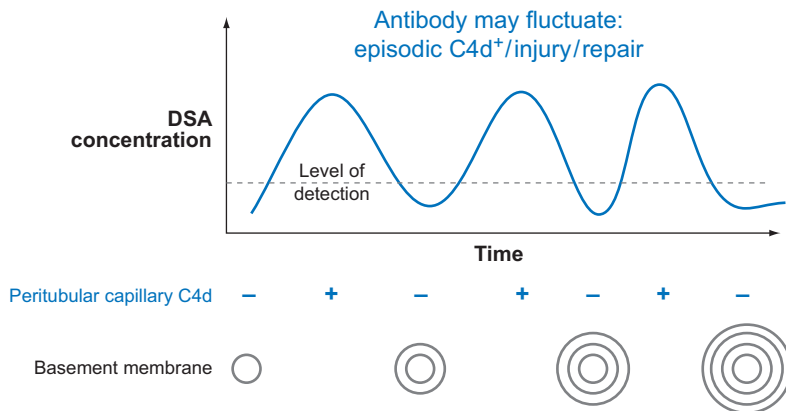


Figure 7

Hypothesized relation between donor-specific antibody (DSA) concentration in the serum, capillary C4d deposition, and multilamination of peritubular capillary basement membranes. At some time points, DSAs may be below the level of detection in patient serum, correlating to negative C4d deposition in the graft, whereas at other time points, DSA fixes complement and causes endothelial injury in the graft. Variation in the graft resistance to the effects of antibody and complement may also vary over time (not illustrated). The endothelium repairs itself, forming a new basement membrane layer. This cycle of antibody-mediated injury and endothelial activation and repair may explain the observation in tissue of basement membrane multilamination. Note that the remnants of previous antibody-mediated injury may be apparent on tissue examination, even when C4d or DSA is no longer detectable.

Interstitial fibrosis and tubular atrophy.

Although not specific for rejection, interstitial fibrosis with tubular atrophy is another important histologic feature of chronically rejected kidneys. The term chronic allograft nephropathy has been applied to this feature but is no longer a preferred designation in the Banff system (139). These changes may be associated with donor-specific antibody and C4d, as a manifestation of calcineurin inhibitor toxicity and as a residue of T cell-mediated rejection.

Epithelial-mesenchymal transition of tubular cells.

One putative mechanism of fibrosis is by epithelial-mesenchymal transition (EMT) of tubular cells to an activated myofibroblast that migrates into the interstitium. Steps in this conversion include loss of cell-cell adhesion, loss of E-cadherin, acquisition of α -smooth muscle actin, actin reorganization, tubular basement membrane disruption, cell migration, and production of profibrotic molecules. TGF β may play

an important role in the pathogenesis of fibrosis and EMT. Robertson et al. (145) sought evidence for EMT in human renal allograft biopsies by looking at tubular epithelial expression of S100A4, a human homolog of the mouse fibroblast-specific protein 1 (FSP1). These investigators found tubular expression of S100A4 near CD8⁺ T cells in tubules. Moreover, CD8⁺ T cells were in close proximity to S100A4-positive epithelial cells in remnant tubules in a fibrotic tissue. This study provides in vivo evidence for the relation of rejecting inflammatory cells contributing to the tubular atrophy with interstitial fibrosis seen in chronically rejected kidneys.

Lymphatic neogenesis. Increased lymphatic vessel formation has been documented in grafts, sometimes ones associated with nodular infiltrates that form structurally differentiated lymphoid tissue. Appreciation of this process has been enabled by new antibodies, such as to podoplanin and

LYVE-1, which identify lymphatic vessels. The prognostic and pathogenetic significance of lymphatic vessels and TLO in allografts remains to be determined. Kerjaschki et al. (146) demonstrated lymphatic neogenesis at the site of nodular inflammatory cell infiltrates in human renal allografts. Follicular DCs, CD23⁺ T cells, and B cells are present in kidney and heart grafts, with markers of active proliferation. Lymphatic neogenesis in biopsies taken for graft dysfunction was associated with a worse outcome (146). However, protocol biopsies of normally functioning grafts also show lymphatic vessels increase in areas with cellular infiltrates. In these grafts, increased lymphatic vessel density was associated with a better graft outcome (147).

Experimental Studies

The common model of chronic rejection in the mouse is the heterotopic cardiac allograft. This model has limitations because it is hemodynamically nonfunctional, although it does contract. These grafts show endarteritis acutely and later intimal fibrosis, especially in proximal coronaries that resemble the lesions in small arteries in human kidney allografts. Manipulation of various costimulatory factors, chemokines, and cytokines has been performed (Tables 1–3). In general, inhibition of more than one pathway is necessary for the reduction of chronic arteriopathy, which in mice may be initiated by either antibodies, T cells, or the innate immune system.

The best evidence that antibody is sufficient to initiate allograft arterial intimal fibrosis comes from murine models. A recent study used passive transfer of anti-MHC antibody into immunologically deficient mice [recombination activating gene (RAG-1) knockout, without functional T or B cells] bearing MHC class I mismatched cardiac allografts (121). These mice showed early C4d deposition in capillaries and later developed transplant arteriopathy, similar to the arteriopathy seen in human renal allografts. After treatment stopped, antibody and C4d disappeared,

leaving behind the arteriopathy. These observations may be relevant to the imperfect correlations between late arteriopathy and C4d/antibody in clinical studies. Using a male to female C57BL/6 heart transplant model (with intact RAG-1), this study also demonstrated that T cell-mediated immunity to minor antigens was sufficient to cause transplant arteriopathy, in the absence of a humoral component. A third pathway to arteriopathy in mice was shown to be mediated by NK cells, as noted above, using parent to F1 cardiac allografts (148).

IFN γ is a critical molecule in the pathogenesis of the chronic vascular injury. Antibodies to IFN γ or IFN γ knockout animals given transient immunosuppression show reduction of the chronic arteriopathy (Table 3), and synthesis of IFN γ by the T cells is necessary (149). Studies using a human to mouse arterial xenograft showed that IFN γ is sufficient to initiate intimal fibrosis (150).

Large animal models with functioning renal allografts replicate with considerable fidelity the pathological features of chronic rejection in human allografts. In a nonhuman primate model of chronic rejection, *Cynomolgus* monkeys received non-life-sustaining kidney transplants and were given suboptimal immunosuppression with cyclosporine (96). Transplant glomerulopathy and arteriopathy developed with infiltration of CD3⁺ T cells and CD68⁺ macrophages, in the intima, indicating an active cellular immune response. Focal expression of α -smooth muscle actin⁺ myofibroblasts were found in arteries with acute endarteritis. Myofibroblasts, which produce extracellular matrix proteins such as collagens, may represent an early stage in the development of transplant arteriopathy, such that repeated episodes of treated or attenuated acute rejection leads to the intimal fibrosis of chronic rejection. An evolution from endarteritis to arteriopathy was evident in single cross sections, which showed fibrotic areas nearest the internal elastica and the most cellular areas under the endothelium. Arteriopathy developed even in the 40% that had

no C4d deposition, arguing that complement-independent mechanisms were sufficient.

A nonhuman primate model of tolerance induction for renal allografts has been developed using simultaneous kidney and donor bone marrow transplantation. These animals undergo total body irradiation and, in some cases, splenectomy. They initially receive immunosuppressive medications, which are discontinued after one month. In this model, most animals do not reject their kidney allografts and show no evidence of alloantibody or C4d, representing a state of tolerance to the graft. Despite the conditioning regimen, a significant minority develops CHR, with alloantibody, transplant glomerulopathy, and C4d deposition (151). The glomerulopathy is progressive and leads to renal failure. This study identified a sequence of four stages of CHR, beginning with antibody production without C4d deposition in grafts, then deposition of C4d with normal graft histology, followed by pathologic changes, and finally a fourth stage, with graft dysfunction and renal failure. A similar sequence has been observed in humans (R.B. Colvin et al., unpublished data), but the evidence is still anecdotal and the inevitability of progression has not been proven.

EMT has been clearly demonstrated in cultured tubular epithelial cells, in which TGF β promotes transition to fibroblasts and bone morphogenic protein-7 can reverse this process (152). Using genetic markers for prior epithelial differentiation, epithelial mesenchyme transition was elegantly proved to occur in the mouse kidney in vivo (153). Rat kidney allografts lose E-cadherin expression and increase smooth muscle actin in areas of fibrosis (154). The overall contribution of EMT to fibrosis is probably small.

Lymphatic neogenesis has also been studied in a murine allograft model. Baddoura et al. (51) investigated a special type of lymphatic neogenesis, termed TLOs, in mouse cardiac allografts. TLOs are defined as containing high endothelial venules, naïve T and B cell regions, and follicular DCs, as reminiscent of lymph node architecture. TLO-

like structures with high endothelial venules develop in 78% of chronically rejected allografts. Chronic rejection TLOs may thus serve as a site of local immune activation, analogous to autoimmune diseases or chronic infections (51). Local production of alloantibody has been demonstrated in the lymphoid nodules of aortic allografts in rats (155).

INNATE IMMUNITY

New roles for components of the innate immune system in acute and chronic rejection have been recently appreciated in experimental studies (156). At the time of engraftment, the graft undergoes ischemia-reperfusion injury with activation of Toll-like receptors of the innate immune system, leading to cytokine release of TNF α and IL1. These proinflammatory mediators induce tubular epithelial-cell-derived CXCL8, which attracts neutrophils by activating CXCR2. Toll-like receptor activation of the innate immune system also affects DCs and their maturation, leading the transition to the adaptive or antigen-specific phase of transplantation immunity.

Local synthesis of the complement protein C3, but not C4, promotes T cell-mediated rejection of renal allografts in mice (157). Kidneys from mice genetically unable to produce C3 survive more than 60 days, compared with controls that are rejected in <20 days. Tubules are the major site of synthesis, and it is common to find C3 deposited along the TBM in human kidneys with acute or chronic rejection. However, it may be the lack of C3 on donor DCs that is principally responsible, as DCs from C3 knockout mice are deficient in antigen presentation (158). Activation of C3 leads to C3b covalently bound to nearby acceptor molecules, which may include the antigen or cell surface receptors. Even after C3b is inactivated by factor I and by proteases, residual C3d remains bound locally on the acceptor site. Unlike C4d, C3d is a powerful adjuvant and stimulatory ligand for its receptor CD21 on B cells (159).

NK cells have long been implicated in the rejection of bone marrow, but only recently in the response to organ allografts (156). NK cells have a potent cytolytic capacity and are major sources of mediators, such as IFN γ . NK cell depletion inhibited acute rejection of murine heart grafts only in animals deficient in T cell costimulatory molecules (CD28) (160), arguing that the NK cells play a supportive role in the interaction of T cells with DCs, probably by promoting the maturation of the latter (161). The major problem in deciphering the role of NK cells is the lack of a unique marker that can be used in tissue.

NK cells recognize foreign cells by virtue of the missing self, that is, class I MHC molecules, for which NK cells have an array of stimulatory and inhibitory receptors. When parental strain bone marrow is placed into an F1 recipient, the marrow is rejected, a phenomenon termed hybrid resistance. This phenomenon has been observed in solid organ transplants as well: The parent of F1 heart grafts in mice developed florid arterial intimal thickening, similar in time course and appearance to that in MHC mismatched grafts (148). In this setting, T cells would be expected to be tolerant to self and not contribute to the allogeneic response.

MECHANISMS OF GRAFT ACCEPTANCE

Accommodation

Recipients may have circulating antigraft antibodies, yet their grafts are functional and appear normal by light microscopy, a state termed accommodation. Accommodation needs to be distinguished from a state of tolerance, in which the graft also functions without rejection: In classic tolerance, antigraft antibodies are not produced, and in vitro assays of alloreactivity are reduced. Accommodation may develop as an alteration in the host response (e.g., change in antibody titer, avidity, or Fc functionality) or as an alteration in the graft. Graft ECs express factors that

protect against antibody- and complement-mediated damage to the graft (162). Evidence for this mechanism of accommodation exists, primarily in the xenograft model, where xenograft survival has been associated with increased graft expression of antiapoptotic (bcl-2, bcl-xL, heme-oxygenase-1, and A20) and complement regulatory factors (162, 163). Mouse cardiac allografts overexpressing bcl-2 develop less transplant arteriopathy (164). Mice treated with anti-CD4 and anti-CD40L antibodies showed increased expression of HO-1, Bcl-xL, and A20, and transplant arteriopathy was prevented (43).

In human renal allografts, increased expression of bcl-xL was associated with the development of antidonor antibody (165). Duffy antigen receptor for chemokines, a pseudoreceptor for chemokines, is increased in acute rejection and may inhibit CCR5-dependent T cell recruitment (166). Tubules secrete proteins that inhibit effector T cells (**Figure 2**). Protease inhibitor-9, the only known inhibitor of granzyme B, is synthesized by tubules in acute rejection (167), and IL-15 produced by tubular cells inhibits expression of perforin (168). We have found increased expression of the complement regulatory factor protectin (CD59) in peritubular capillaries in acute and chronic renal allograft rejection and decay-accelerating factor (CD55) in a subset of CHR cases (169). We have hypothesized that chronic rejection may be a manifestation of acute rejection that has been attenuated, albeit incompletely, by mechanisms of accommodation (123).

ABO-blood-group-incompatible transplantation is the classic setting in which accommodation has been described. After pretransplant regimens to remove antibody, circulating ABO antibodies return and fix complement in the graft, yet grafts function normally (170). Eighty percent of ABO-incompatible grafts showed C4d deposition in protocol biopsies (113). Accommodation to blood group antigens, which are carbohydrates, probably works differently from that toward protein antigens (MHC). In a

human renal allograft biopsy study using gene expression analysis, accommodated ABO-incompatible allografts showed increased expression of Smad proteins, which are part of a complex pathway involved in TGF β signaling, as well as increased expression of protein tyrosine kinases and associated molecules and mucin-1, and decreased expression of TNF α . In contrast to what has been found in some HLA-disparate human allografts and in xenografts, this study found no upregulation of HO-1, bcl-2, and bcl-xL (171). The α -1,3-galactosyltransferase (α -gal) knockout mouse model should help understand accommodation and tolerance to carbohydrate antigens. In this model, deletion of anti-gal-producing B cells can occur (172).

Regulation/Tolerance

Tregs, the subject of recent increased attention, express the transcription factor FOXP3 and typically express surface CD4 and CD25. Mouse studies have shown that CD4⁺ CD25⁺ Tregs can induce both CD8⁺ and CD4⁺ T cell hyporesponsiveness to donor target cells. Tregs that are able to transfer specific non-responsiveness to alloantigens accumulate in skin grafts (173). In mouse cardiac allografts, Tregs can attenuate rejection, which is dependent upon antigen-specific engagement of the TCR (174) and CCR5 (58).

It is well established in mice that different organs with the same MHC disparity are accepted spontaneously at different rates, in the order liver > kidney > heart or intestine (175). Renal allografts in mice across an MHC disparity go through a transient episode of acute cellular rejection, with graft dysfunction, that resolves spontaneously (176). This observa-

tion suggests that an allograft kidney has some ability to modulate the immune response. In both humans and in other large animals, a beneficial effect, with reduction of rejection episodes, of cotransplantation of two organs has been observed. In pigs, a donor kidney was required not only to induce but also to maintain tolerance in porcine heart recipients (177). In this same study, adoptive transfer of CD25⁺ cells from heart-kidney recipients to heart-only recipients produced short-term, but not long-term, tolerance. These findings suggest that there is ongoing education of Tregs in particular anatomic locations (in this case, the kidney).

Detection of Tregs in tissue is enabled by immunohistochemical demonstration of the specific transcription factor FOXP3. In acute cellular rejection, FOXP3⁺ cells infiltrate the graft and account for approximately 4% of the infiltrating CD4⁺ cells (17). The vast majority of FOXP3⁺ cells are CD4⁺ (96%), but a minority express CD8 and a few have neither CD4 nor CD8 (17). Few FOXP3 cells are found in humoral rejection, probably accounting for the correlation of low FOXP3 mRNA in the urine with adverse prognosis of acute rejection (19). It is notable that CD4⁺FOXP3⁺ cells are concentrated most in the tubules, where 15% of CD4⁺ cells are FOXP3⁺ (Treg tubulitis). The anatomic location of FOXP3⁺ cells in renal allografts may provide clues as to how Tregs are educated.

Much remains to be learned about the mechanisms and the diagnosis of graft acceptance, which will depend not only upon continued study of relevant animal models, but also upon careful analysis of protocol biopsies and immunological function in patients with stable or accepted grafts.

SUMMARY POINTS

1. Graft rejection is not a single process, but caused by different mechanisms, related to antibody, complement, T cells, and other cell types. A variety of target cells in the graft are affected by these mediators, particularly endothelial and tubular cells.

2. Studies in inbred strains of mice using organ allografts identify the central role of costimulatory molecules and chemokines in acute rejection. Surprisingly little proof of the necessity of cytotoxic mechanisms is available. Some inflammatory mediators, e.g., IFN γ , can alter the graft's susceptibility to injury.
3. Chronic rejection is a major obstacle to transplantation success. Recent evidence points to a major role for alloantibodies to MHC molecules, as evidenced by circulating antibodies and C4d deposition in the graft. C4d deposition is the most practical way to infer antibody interaction with endothelial cells. The major targets of antibodies are ECs of glomeruli, peritubular capillaries, and arteries.
4. Experimental studies support three mechanisms of chronic transplant arteriopathy: antibodies, T cells, and NK cells. It remains to be established which of these apply to humans and what will be the appropriate treatment.
5. Grafted organs induce molecules that resist or mitigate the pathological effects of T cells or antibodies (accommodation). In addition, recent evidence points to the potentially important role of local immunoregulatory events in the graft, as manifested by infiltration of FOXP3⁺ Tregs and lymphoid neogenesis.

FUTURE ISSUES

1. Control of antibody production is a major obstacle for long-term graft survival. New approaches to control B cell tolerance and plasma cells are needed. Production of antibodies to the donor occurs even in patients and animals undergoing various regimens to induce tolerance.
2. The significance and stability of the putative state of accommodation recognized in stable patients with circulating antibodies and/or C4d deposition in the graft needs to be determined. Methods for increasing the strength of accommodation to T cells or antibody-mediated injury might be a new approach to mitigate chronic rejection.
3. The early stages and pathogenesis of late graft loss remain enigmatic. Considerable insight is expected through systematic pathological and molecular study of protocol biopsies taken while the process is active but not clinically evident.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

1. Colvin RB, Nickleit V. 2006. Renal transplant pathology. In *Heptinstall's Pathology of the Kidney*, ed. JC Jennette, JL Olson, MM Schwartz, FG Silva, pp. 1347–490. Philadelphia: Lippincott-Raven
2. Robertson H, Wheeler J, Kirby JA, Morley AR. 1996. Renal allograft rejection: in situ demonstration of cytotoxic intratubular cells. *Transplantation* 61:1546–49
3. Einecke G, Fairhead T, Hidalgo LG, Sis B, Turner P, et al. 2006. Tubulitis and epithelial cell alterations in mouse kidney transplant rejection are independent of CD103, perforin or granzymes A/B. *Am. J. Transplant.* 6:2109–20
4. Hoffmann SC, Hale DA, Kleiner DE, Mannon RB, Kampen RL, et al. 2005. Functionally significant renal allograft rejection is defined by transcriptional criteria. *Am. J. Transplant.* 5:573–81
5. Desvaux D, Schwarzingler M, Pastural M, Baron C, Abtahi M, et al. 2004. Molecular diagnosis of renal-allograft rejection: correlation with histopathologic evaluation and antirejection-therapy resistance. *Transplantation* 78:647–53
6. Sarwal M, Chua MS, Kambham N, Hsieh SC, Satterwhite T, et al. 2003. Molecular heterogeneity in acute renal allograft rejection identified by DNA microarray profiling. *N. Engl. J. Med.* 349:125–38
7. Glimcher LH, Townsend MJ, Sullivan BM, Lord GM. 2004. Recent developments in the transcriptional regulation of cytolytic effector cells. *Nat. Rev. Immunol.* 4:900–11
8. Akalin E, Hendrix RC, Polavarapu RG, Pearson TC, Neylan JF, et al. 2001. Gene expression analysis in human renal allograft biopsy samples using high-density oligoarray technology. *Transplantation* 72:948–53
9. Flechner SM, Kurian SM, Head SR, Sharp SM, Whisenant TC, et al. 2004. Kidney transplant rejection and tissue injury by gene profiling of biopsies and peripheral blood lymphocytes. *Am. J. Transplant.* 4:1475–89
10. Eikmans M, Sijpkens YW, Baelde HJ, de Heer E, Paul LC, et al. 2002. High transforming growth factor- β and extracellular matrix mRNA response in renal allografts during early acute rejection is associated with absence of chronic rejection. *Transplantation* 73:573–79
11. Bonsib SM, Abul-Ezz SR, Ahmad I, Young SM, Ellis EN, et al. 2000. Acute rejection-associated tubular basement membrane defects and chronic allograft nephropathy. *Kidney Int.* 58:2206–14
12. Meehan S, McCluskey R, Pascual M, Anderson P, Schlossman S, et al. 1997. Cytotoxicity and apoptosis in human renal allografts: identification, distribution, and quantitation of cells with a cytotoxic granule protein GMP-17 (TIA-1) and cells with fragmented nuclear DNA. *Lab. Invest.* 76:639–49
13. Einecke G, Melk A, Ramassar V, Zhu LF, Bleackley RC, et al. 2005. Expression of CTL associated transcripts precedes the development of tubulitis in T-cell mediated kidney graft rejection. *Am. J. Transplant.* 5:1827–36
14. August C, Schmid KW, Dietl KH, Heidenreich S. 1999. Prognostic value of lymphocyte apoptosis in acute rejection of renal allografts. *Transplantation* 67:581–85
15. Noronha IL, Oliveira SG, Tavares TS, Di Petta A, Dominguez WV, et al. 2005. Apoptosis in kidney and pancreas allograft biopsies. *Transplantation* 79:1231–35
16. Robertson H, Wong WK, Talbot D, Burt AD, Kirby JA. 2001. Tubulitis after renal transplantation: demonstration of an association between CD103⁺ T cells, transforming growth factor β 1 expression and rejection grade. *Transplantation* 71:306–13

17. Veronese F, Rotman S, Smith RN, Pelle TD, Farrell ML, et al. 2007. Pathological and clinical correlates of FOXP3 cells in renal allografts during acute rejection. *Am. J. Transplant.* 7:914–22
18. Niemann-Masanek U, Mueller A, Yard BA, Waldherr R, van der Woude FJ. 2002. B7-1 (CD80) and B7-2 (CD 86) expression in human tubular epithelial cells in vivo and in vitro. *Nephron* 92:542–56
19. Muthukumar T, Dadhania D, Ding R, Snopkowski C, Naqvi R, et al. 2005. Messenger RNA for FOXP3 in the urine of renal-allograft recipients. *N. Engl. J. Med.* 353:2342–51
20. Segerer S, Cui Y, Eitner F, Goodpaster T, Hudkins KL, et al. 2001. Expression of chemokines and chemokine receptors during human renal transplant rejection. *Am. J. Kidney Dis.* 37:518–31
21. Robertson H, Morley AR, Talbot D, Callanan K, Kirby JA. 2000. Renal allograft rejection: β -chemokine involvement in the development of tubulitis. *Transplantation* 69:684–87
22. Cockwell P, Chakravorty SJ, Girdlestone J, Savage CO. 2002. Fractalkine expression in human renal inflammation. *J. Pathol.* 196:85–90
23. Ali S, Malik G, Burns A, Robertson H, Kirby JA. 2005. Renal transplantation: Examination of the regulation of chemokine binding during acute rejection. *Transplantation* 79:672–79
24. Al-Lamki RS, Wang J, Skepper JN, Thiru S, Pober JS, et al. 2001. Expression of tumor necrosis factor receptors in normal kidney and rejecting renal transplants. *Lab. Invest.* 81:1503–15
25. Van Kooten C, Boonstra JG, Paape ME, Fossiez F, Banchereau J, et al. 1998. Interleukin-17 activates human renal epithelial cells in vitro and is expressed during renal allograft rejection. *J. Am. Soc. Nephrol.* 9:1526–34
26. Hoffmann S, Park J, Jacobson LM, Muehrer RJ, Lorentzen D, et al. 2004. Donor genomics influence graft events: the effect of donor polymorphisms on acute rejection and chronic allograft nephropathy. *Kidney Int.* 66:1686–93
27. Mengel M, Gwinner W, Schwarz A, Bajeski R, Franz I, et al. 2007. Infiltrates in protocol biopsies from renal allografts. *Am. J. Transplant.* 7:356–65
28. Cailhier JF, Laplante P, Hebert MJ. 2006. Endothelial apoptosis and chronic transplant vasculopathy: recent results, novel mechanisms. *Am. J. Transplant.* 6:247–53
29. Tinckam KJ, Djurdjev O, Magil AB. 2005. Glomerular monocytes predict worse outcomes after acute renal allograft rejection independent of C4d status. *Kidney Int.* 68:1866–74
30. Fahim T, Bohmig GA, Exner M, Huttary N, Kerschner H, et al. 2007. The cellular lesion of humoral rejection: predominant recruitment of monocytes to peritubular and glomerular capillaries. *Am. J. Transplant.* 7:385–93
31. Dooms H, Abbas AK. 2006. Control of CD4⁺ T-cell memory by cytokines and costimulators. *Immunol. Rev.* 211:23–38
32. Szot GL, Zhou P, Sharpe AH, He G, Kim O, et al. 2000. Absence of host B7 expression is sufficient for long-term murine vascularized heart allograft survival. *Transplantation* 69:904–9
33. Szot GL, Zhou P, Rulifson I, Wang J, Guo Z, et al. 2001. Different mechanisms of cardiac allograft rejection in wildtype and CD28-deficient mice. *Am. J. Transplant.* 1:38–46
34. Mandelbrot DA, Oosterwegel MA, Shimizu K, Yamada A, Freeman GJ, et al. 2001. B7-dependent T-cell costimulation in mice lacking CD28 and CTLA4. *J. Clin. Invest.* 107:881–87

35. Sayegh MH, Zheng XG, Magee C, Hancock WW, Turka LA. 1997. Donor antigen is necessary for the prevention of chronic rejection in CTLA4Ig-treated murine cardiac allograft recipients. *Transplantation* 64:1646–50
36. Wang L, Fraser CC, Kikly K, Wells AD, Han R, et al. 2005. B7-H3 promotes acute and chronic allograft rejection. *Eur. J. Immunol.* 35:428–38
37. Ozkaynak E, Gao W, Shemmeri N, Wang C, Gutierrez-Ramos JC, et al. 2001. Importance of ICOS-B7RP-1 costimulation in acute and chronic allograft rejection. *Nat. Immunol.* 2:591–96
38. Lee EA, Kim JE, Seo JH, Kwon BS, Nam SH, et al. 2006. 4-1BB (CD137) signals depend upon CD28 signals in alloimmune responses. *Exp. Mol. Med.* 38:606–15
39. Raisky O, Spriewald BM, Morrison KJ, Ensminger S, Mohieddine T, et al. 2003. CD8⁺ T cells induce graft vascular occlusion in a CD40 knockout donor/recipient combination. *J. Heart Lung Transplant.* 22:177–83
40. Ensminger SM, Spriewald BM, Sorensen HV, Witzke O, Flashman EG, et al. 2001. Critical role for IL-4 in the development of transplant arteriosclerosis in the absence of CD40-CD154 costimulation. *J. Immunol.* 167:532–41
41. Bingaman AW, Ha J, Durham MM, Waitze SY, Tucker-Burden C, et al. 2001. Analysis of the CD40 and CD28 pathways on alloimmune responses by CD4⁺ T cells in vivo. *Transplantation* 72:1286–92
42. Shimizu K, Schonbeck U, Mach F, Libby P, Mitchell RN. 2000. Host CD40 ligand deficiency induces long-term allograft survival and donor-specific tolerance in mouse cardiac transplantation but does not prevent graft arteriosclerosis. *J. Immunol.* 165:3506–18
43. Hancock WW, Buelow R, Sayegh MH, Turka LA. 1998. Antibody-induced transplant arteriosclerosis is prevented by graft expression of antioxidant and antiapoptotic genes. *Nat. Med.* 4:1392–96
44. Dai Z, Konieczny BT, Baddoura FK, Lakkis FG. 1998. Impaired alloantigen-mediated T cell apoptosis and failure to induce long-term allograft survival in IL-2-deficient mice. *J. Immunol.* 161:1659–63
45. Yamada A, Salama AD, Sho M, Najafian N, Ito T, et al. 2005. CD70 signaling is critical for CD28-independent CD8⁺ T cell-mediated alloimmune responses in vivo. *J. Immunol.* 174:1357–64
46. Larsen CP, Elwood ET, Alexander DZ, Ritchie SC, Hendrix R, et al. 1996. Long-term acceptance of skin and cardiac allografts after blocking CD40 and CD28 pathways. *Nature* 381:434–38
47. Lechler RI, Batchelor JR. 1982. Immunogenicity of retransplanted rat kidney allografts. Effect of inducing chimerism in the first recipient and quantitative studies on immunosuppression of the second recipient. *J. Exp. Med.* 156:1835–41
48. Larsen CP, Morris PJ, Austyn JM. 1990. Migration of dendritic leukocytes from cardiac allografts into host spleens. A novel pathway for initiation of rejection. *J. Exp. Med.* 171:307–14
49. Mandelbrot DA, Kishimoto K, Auchincloss HJ, Sharpe AH, Sayegh MH. 2001. Rejection of mouse cardiac allografts by costimulation in trans. *J. Immunol.* 167:1174–78
50. Colvin BL, Wang Z, Nakano H, Wu W, Kakiuchi T, et al. 2005. CXCL9 antagonism further extends prolonged cardiac allograft survival in CCL19/CCL21-deficient mice. *Am. J. Transplant.* 5:2104–13
51. Baddoura FK, Nasr IW, Wrobel B, Li Q, Ruddle NH, et al. 2005. Lymphoid neogenesis in murine cardiac allografts undergoing chronic rejection. *Am. J. Transplant.* 5:510–16

52. Noorchashm H, Reed AJ, Rostami SY, Mozaffari R, Zekavat G, et al. 2006. B cell-mediated antigen presentation is required for the pathogenesis of acute cardiac allograft rejection. *J. Immunol.* 177:7715–22
53. Hagerty DT, Allen PM. 1992. Processing and presentation of self- and nonself foreign antigens by the renal proximal tubule. *J. Immunol.* 126:2324–32
54. Bagai R, Valujskikh A, Canaday DH, Bailey E, Lalli PN, et al. 2005. Mouse endothelial cells cross-present lymphocyte-derived antigen on class I MHC via a TAP1- and proteasome-dependent pathway. *J. Immunol.* 174:7711–15
55. Kreisel D, Krupnick AS, Balsara KR, Riha M, Gelman AE, et al. 2002. Mouse vascular endothelium activates CD8⁺ T lymphocytes in a B7-dependent fashion. *J. Immunol.* 169:6154–61
56. Xu H, Dhanireddy KK, Kirk AD. 2006. Human monocytes as intermediaries between allogeneic endothelial cells and allospecific T cells: a role for direct scavenger receptor-mediated endothelial membrane uptake in the initiation of alloimmunity. *J. Immunol.* 176:750–61
57. Amano H, Bickerstaff A, Orosz CG, Novick AC, Toma H, et al. 2005. Absence of recipient CCR5 promotes early and increased allospecific antibody responses to cardiac allografts. *J. Immunol.* 174:6499–508
58. Lee I, Wang L, Wells AD, Dorf ME, Ozkaynak E, et al. 2005. Recruitment of Foxp3⁺ T regulatory cells mediating allograft tolerance depends on the CCR4 chemokine receptor. *J. Exp. Med.* 201:1037–44
59. Gao W, Faia KL, Csizmadia V, Smiley ST, Soler D, et al. 2001. Beneficial effects of targeting CCR5 in allograft recipients. *Transplantation* 72:1199–205
60. Alferink J, Lieberam I, Reindl W, Behrens A, Weiss S, et al. 2003. Compartmentalized production of CCL17 in vivo: strong inducibility in peripheral dendritic cells contrasts selective absence from the spleen. *J. Exp. Med.* 197:585–99
61. Gao W, Topham PS, King JA, Smiley ST, Csizmadia V, et al. 2000. Targeting of the chemokine receptor CCR1 suppresses development of acute and chronic cardiac allograft rejection. *J. Clin. Invest.* 105:35–44
62. Abdi R, Means TK, Ito T, Smith RN, Najafian N, et al. 2004. Differential role of CCR2 in islet and heart allograft rejection: tissue specificity of chemokine/chemokine receptor function in vivo. *J. Immunol.* 172:767–75
63. Huser N, Tertilt C, Gerauer K, Maier S, Traeger T, et al. 2005. CCR4-deficient mice show prolonged graft survival in a chronic cardiac transplant rejection model. *Eur. J. Immunol.* 35:128–38
64. Luckow B, Joergensen J, Chilla S, Li JP, Henger A, et al. 2004. Reduced intragraft mRNA expression of matrix metalloproteinases Mmp3, Mmp12, Mmp13 and Adam8, and diminished transplant arteriosclerosis in Ccr5-deficient mice. *Eur. J. Immunol.* 34:2568–78
65. Hopken UE, Droese J, Li JP, Joergensen J, Breitfeld D, et al. 2004. The chemokine receptor CCR7 controls lymph node-dependent cytotoxic T cell priming in alloimmune responses. *Eur. J. Immunol.* 34:461–70
66. Beckmann JH, Yan S, Luhrs H, Heid B, Skubich S, et al. 2004. Prolongation of allograft survival in CCR7-deficient mice. *Transplantation* 77:1809–14
67. Miura M, Morita K, Kobayashi H, Hamilton TA, Burdick MD, et al. 2001. Monokine induced by IFN- γ is a dominant factor directing T cells into murine cardiac allografts during acute rejection. *J. Immunol.* 167:3494–504
68. Hancock WW, Gao W, Csizmadia V, Faia KL, Shemmeri N, et al. 2001. Donor-derived IP-10 initiates development of acute allograft rejection. *J. Exp. Med.* 193:975–80

69. Hancock WW, Lu B, Gao W, Csizmadia V, Faia K, et al. 2000. Requirement of the chemokine receptor CXCR3 for acute allograft rejection. *J. Exp. Med.* 192:1515–20
70. Haskell CA, Hancock WW, Salant DJ, Gao W, Csizmadia V, et al. 2001. Targeted deletion of CX₃CR1 reveals a role for fractalkine in cardiac allograft rejection. *J. Clin. Invest.* 108:679–88
71. Barry M, Bleackley RC. 2002. Cytotoxic T lymphocytes: All roads lead to death. *Nat. Rev. Immunol.* 2:401–9
72. Zorn E, Miklos DB, Floyd BH, Mattes-Ritz A, Guo L, et al. 2004. Minor histocompatibility antigen DBY elicits a coordinated B and T cell response after allogeneic stem cell transplantation. *J. Exp. Med.* 199:1133–42
73. Schulz M, Schuurman HJ, Joergensen J, Steiner C, Meerloo T, et al. 1995. Acute rejection of vascular heart allografts by perforin-deficient mice. *Eur. J. Immunol.* 25:474–80
74. Larsen CP, Alexander DZ, Hendrix R, Ritchie SC, Pearson TC. 1995. Fas-mediated cytotoxicity. An immunoeffector or immunoregulatory pathway in T cell-mediated immune responses? *Transplantation* 60:221–24
75. Li XC, Li Y, Dodge I, Wells AD, Zheng XX, et al. 1999. Induction of allograft tolerance in the absence of Fas-mediated apoptosis. *J. Immunol.* 163:2500–7
76. Imagawa DK, Millis JM, Seu P, Olthoff KM, Hart J, et al. 1991. The role of tumor necrosis factor in allograft rejection. III. Evidence that anti-TNF antibody therapy prolongs allograft survival in rats with acute rejection. *Transplantation* 51:57–62
77. Russell PS, Chase CM, Colvin RB. 1995. Coronary atherosclerosis in transplanted mouse hearts. IV Effects of treatment with monoclonal antibodies to intercellular adhesion molecule-1 and leukocyte function-associated antigen-1. *Transplantation* 60:724–29
78. Nagano H, Libby P, Taylor MK, Hasegawa S, Stinn JL, et al. 1998. Coronary arteriosclerosis after T-cell-mediated injury in transplanted mouse hearts: role of interferon- γ . *Am. J. Pathol.* 152:1187–97
79. Raisanen-Sokolowski A, Glysing-Jensen T, Koglin J, Russell ME. 1998. Reduced transplant arteriosclerosis in murine cardiac allografts placed in interferon- γ knockout recipients. *Am. J. Pathol.* 152:359–65
80. Wiseman AC, Pietra BA, Kelly BP, Rayat GR, Rizeq M, et al. 2001. Donor IFN- γ receptors are critical for acute CD4⁺ T cell-mediated cardiac allograft rejection. *J. Immunol.* 167:5457–63
81. Halloran PF, Miller LW, Urmson J, Ramassar V, Zhu LF, et al. 2001. IFN- γ alters the pathology of graft rejection: protection from early necrosis. *J. Immunol.* 166:7072–81
82. Halloran PF, Afrouzian M, Ramassar V, Urmson J, Zhu LF, et al. 2001. Interferon- γ acts directly on rejecting renal allografts to prevent graft necrosis. *Am. J. Pathol.* 158:215–26
83. Zand MS, Li Y, Hancock W, Li XC, Roy-Chaudhury P, et al. 2000. Interleukin-2 and interferon- γ double knockout mice reject heterotopic cardiac allografts. *Transplantation* 70:1378–81
84. Raisanen-Sokolowski A, Mottram PL, Glysing-Jensen T, Sato A, Russell ME. 1997. Heart transplants in interferon- γ , interleukin 4, and interleukin 10 knockout mice. Recipient environment alters graft rejection. *J. Clin. Invest.* 100:2449–56
85. Braun MY, Desalle F, Le Moine A, Pretolani M, Matthys P, et al. 2000. IL-5 and eosinophils mediate the rejection of fully histoincompatible vascularized cardiac allografts: regulatory role of alloreactive CD8⁺ T lymphocytes and IFN- γ . *Eur. J. Immunol.* 30:1290–96
86. Poulin LF, Richard M, Le Moine A, Kiss R, McKenzie AN, et al. 2003. Interleukin-9 promotes eosinophilic rejection of mouse heart allografts. *Transplantation* 76:572–77

87. Demir Y, Chen Y, Metz C, Renz H, Heeger PS. 2003. Cardiac allograft rejection in the absence of macrophage migration inhibitory factor. *Transplantation* 76:244–47
88. Koglin J, Glysing-Jensen T, Raisanen-Sokolowski A, Russell ME. 1998. Immune sources of transforming growth factor- β 1 reduce transplant arteriosclerosis: insight derived from a knockout mouse model. *Circ. Res.* 83:652–60
89. McKee CM, Defina R, He H, Haley KJ, Stone JR, et al. 2002. Prolonged allograft survival in TNF receptor 1-deficient recipients is due to immunoregulatory effects, not to inhibition of direct antigraft cytotoxicity. *J. Immunol.* 168:483–89
90. Suzuki J, Cole SE, Batirel S, Kosuge H, Shimizu K, et al. 2003. Tumor necrosis factor receptor-1 and -2 double deficiency reduces graft arterial disease in murine cardiac allografts. *Am. J. Transplant.* 3:968–76
91. Ring GH, Saleem S, Dai Z, Hassan AT, Konieczny BT, et al. 1999. Interferon-gamma is necessary for initiating the acute rejection of major histocompatibility complex class II-disparate skin allografts. *Transplantation* 67:1362–65
92. Wang H, DeVries ME, Deng S, Khandaker MH, Pickering JG, et al. 2000. The axis of interleukin 12 and γ interferon regulates acute vascular xenogeneic rejection. *Nat. Med.* 6:549–55
93. Hidalgo LG, Urmson J, Halloran PF. 2005. IFN- γ decreases CTL generation by limiting IL-2 production: a feedback loop controlling effector cell production. *Am. J. Transplant.* 5:651–61
94. Jabs WJ, Sedlmeyer A, Ramassar V, Hidalgo LG, Urmson J, et al. 2003. Heterogeneity in the evolution and mechanisms of the lesions of kidney allograft rejection in mice. *Am. J. Transplant.* 3:1501–9
95. Yuan R, El-Asady R, Liu K, Wang D, Drachenberg CB, et al. 2005. Critical role for CD103⁺CD8⁺ effectors in promoting tubular injury following allogeneic renal transplantation. *J. Immunol.* 175:2868–79
96. Wieczorek G, Bigaud M, Menninger K, Riesen S, Quesniaux V, et al. 2006. Acute and chronic vascular rejection in nonhuman primate kidney transplantation. *Am. J. Transplant.* 6:1285–96
97. Russell PS, Chase CM, Colvin RB. 1997. Alloantibody- and T cell-mediated immunity in the pathogenesis of transplant arteriosclerosis: lack of progression to sclerotic lesions in B cell-deficient mice. *Transplantation* 64:1531–36
98. Choy JC, Kerjner A, Wong BW, McManus BM, Granville DJ. 2004. Perforin mediates endothelial cell death and resultant transplant vascular disease in cardiac allografts. *Am. J. Pathol.* 165:127–33
99. Middleton J, Patterson AM, Gardner L, Schmutz C, Ashton BA. 2002. Leukocyte extravasation: chemokine transport and presentation by the endothelium. *Blood* 100:3853–60
100. Zhang QW, Kish DD, Fairchild RL. 2003. Absence of allograft ICAM-1 attenuates alloantigen-specific T cell priming, but not primed T cell trafficking into the graft, to mediate acute rejection. *J. Immunol.* 170:5530–37
101. Okada M, Wang CY, Hwang DW, Sakaguchi T, Olson KE, et al. 2002. Transcriptional control of cardiac allograft vasculopathy by early growth response gene-1 (Egr-1). *Circ. Res.* 91:135–42
102. Colvin RB. 2007. Antibody-mediated renal allograft rejection: diagnosis and pathogenesis. *J. Am. Soc. Nephrol.* 18:1046–56
103. Lorenz M, Regele H, Schillinger M, Exner M, Rasoul-Rockenschaub S, et al. 2004. Risk factors for capillary C4d deposition in kidney allografts: evaluation of a large study cohort. *Transplantation* 78:447–52

104. Collins AB, Chicano SL, Cornell LD, Tolkoﬀ-Rubin N, Goes NB, et al. 2006. Putative antibody-mediated rejection with C4d deposition in HLA-identical, ABO-compatible renal allografts. *Transplant. Proc.* 38:3427–29
105. Dragun D, Muller DN, Brasen JH, Fritsche L, Nieminen-Kelha M, et al. 2005. Angiotensin II type 1-receptor activating antibodies in renal-allograft rejection. *N. Engl. J. Med.* 352:558–69
106. Liptak P, Kemeny E, Morvay Z, Szederkenyi E, Szenohradszky P, et al. 2005. Peritubular capillary damage in acute humoral rejection: an ultrastructural study on human renal allografts. *Am. J. Transplant.* 5:2870–76
107. Feucht HE, Schneeberger H, Hillebrand G, Burkhardt K, Weiss M, et al. 1993. Capillary deposition of C4d complement fragment and early renal graft loss. *Kidney Int.* 43:1333–38
108. Mauiyiyedi S, Crespo M, Collins AB, Schneeberger EE, Pascual MA, et al. 2002. Acute humoral rejection in kidney transplantation: II. Morphology, immunopathology, and pathologic classification. *J. Am. Soc. Nephrol.* 13:79–87
109. Collins AB, Schneeberger EE, Pascual MA, Saidman SL, Williams WW, et al. 1999. Complement activation in acute humoral renal allograft rejection: diagnostic significance of C4d deposits in peritubular capillaries. *J. Am. Soc. Nephrol.* 10:2208–14
110. Regele H, Bohmig GA, Habicht A, Gollowitz D, Schillinger M, et al. 2002. Capillary deposition of complement split product C4d in renal allografts is associated with basement membrane injury in peritubular and glomerular capillaries: a contribution of humoral immunity to chronic allograft rejection. *J. Am. Soc. Nephrol.* 13:2371–80
111. Nadasdy GM, Bott C, Cowden D, Pelletier R, Ferguson R, et al. 2005. Comparative study for the detection of peritubular capillary C4d deposition in human renal allografts using different methodologies. *Hum. Pathol.* 36:1178–85
112. Martin L, Guignier F, Bocrice O, D’Athis P, Rageot D, et al. 2005. Detection of anti-HLA antibodies with flow cytometry in needle core biopsies of renal transplants recipients with chronic allograft nephropathy. *Transplantation* 79:1459–61
113. Haas M, Rahman MH, Racusen LC, Kraus ES, Bagnasco SM, et al. 2006. C4d and C3d staining in biopsies of ABO- and HLA-incompatible renal allografts: correlation with histologic findings. *Am. J. Transplant.* 6:1829–40
114. Imai N, Nishi S, Alchi B, Ueno M, Fukase S, et al. 2006. Immunohistochemical evidence of activated lectin pathway in kidney allografts with peritubular capillary C4d deposition. *Nephrol. Dial. Transplant.* 21:2589–95
115. Wahrmann M, Exner M, Schillinger M, Haidbauer B, Regele H, et al. 2006. Pivotal role of complement-fixing HLA alloantibodies in presensitized kidney allograft recipients. *Am. J. Transplant.* 6:1033–41
116. Gao ZH, McAlister VC, Wright JRJ, McAlister CC, Peltekian K, et al. 2004. Immunoglobulin-G subclass antidonor reactivity in transplant recipients. *Liver Transplant.* 10:1055–59
117. Jooste SV, Colvin RB, Winn HJ. 1981. The vascular bed as the primary target in the destruction of skin grafts by antiserum. II. Loss of sensitivity to antiserum in long-term xenografts of skin. *J. Exp. Med.* 154:1332–41
118. Wasowska BA, Qian Z, Cangello DL, Behrens E, Van Tran K, et al. 2001. Passive transfer of alloantibodies restores acute cardiac rejection in IgKO mice. *Transplantation* 71:727–36
119. Rahimi S, Qian Z, Layton J, Fox-Talbot K, Baldwin WM, et al. 2004. Non-complement- and complement-activating antibodies synergize to cause rejection of cardiac allografts. *Am. J. Transplant.* 4:326–34
120. Yin D, Zeng H, Ma L, Shen J, Xu H, et al. 2004. Cutting Edge: NK cells mediate IgG1-dependent hyperacute rejection of xenografts. *J. Immunol.* 172:7235–38

121. Uehara S, Chase CM, Cornell LD, Madsen JC, Russell PS, et al. 2007. Chronic cardiac transplant arteriopathy in mice: relationship of alloantibody, C4d deposition and neointimal fibrosis. *Am. J. Transplant.* 7:57–65
122. Minami K, Murata K, Lee CY, Fox-Talbot K, Wasowska BA, et al. 2006. C4d deposition and clearance in cardiac transplants correlates with alloantibody levels and rejection in rats. *Am. J. Transplant.* 6:923–32
123. Colvin RB, Smith RN. 2005. Antibody-mediated organ-allograft rejection. *Nat. Rev. Immunol.* 5:807–17
124. Qian Z, Wasowska BA, Behrens E, Cangello DL, Brody JR, et al. 1999. C6 produced by macrophages contributes to cardiac allograft rejection. *Am. J. Pathol.* 155:1293–302
125. Loveland BE, Milland J, Kyriakou P, Thorley BR, Christiansen D, et al. 2004. Characterization of a CD46 transgenic pig and protection of transgenic kidneys against hyperacute rejection in nonimmunosuppressed baboons. *Xenotransplantation* 11:171–83
126. Cowan PJ, Aminian A, Barlow H, Brown AA, Chen CG, et al. 2000. Renal xenografts from triple-transgenic pigs are not hyperacutely rejected but cause coagulopathy in non-immunosuppressed baboons. *Transplantation* 69:2504–15
127. Shimizu I, Smith NR, Zhao G, Medof E, Sykes M. 2006. Decay-accelerating factor prevents acute humoral rejection induced by low levels of anti- α Gal natural antibodies. *Transplantation* 81:95–100
128. Wang H, Rollins SA, Gao Z, Garcia B, Zhang Z, et al. 1999. Complement inhibition with an anti-C5 monoclonal antibody prevents hyperacute rejection in a xenograft heart transplantation model. *Transplantation* 68:1643–51
129. Yuan FF, Watson N, Sullivan JS, Biffin S, Moses J, et al. 2004. Association of Fc γ receptor IIA polymorphisms with acute renal-allograft rejection. *Transplantation* 78:766–69
130. Xu H, Yin D, Naziruddin B, Chen L, Stark A, et al. 2003. The in vitro and in vivo effects of antigalactose antibodies on endothelial cell activation and xenograft rejection. *J. Immunol.* 170:1531–39
131. Chen D, Weber M, Lechler R, Dorling A. 2006. NK-cell-dependent acute xenograft rejection in the mouse heart-to-rat model. *Xenotransplantation* 13:408–14
132. Hauzenberger E, Klominek J, Holgersson J. 2004. Anti-Gal IgG potentiates natural killer cell migration across porcine endothelium via endothelial cell activation and increased natural killer cell motility triggered by CD16 cross-linking. *Eur. J. Immunol.* 34:1154–63
133. Delikouras A, Dorling A. 2003. Transplant accommodation. *Am. J. Transplant.* 3:917–18
134. Ota H, Fox-Talbot K, Hu W, Qian Z, Sanfilippo F, et al. 2005. Terminal complement components mediate release of von Willebrand factor and adhesion of platelets in arteries of allografts. *Transplantation* 79:276–81
135. Camerer E, Huang W, Coughlin SR. 2000. Tissue factor- and factor X-dependent activation of protease-activated receptor 2 by factor VIIa. *Proc. Natl. Acad. Sci. USA* 97:5255–60
136. Chen D, Weber M, McVey JH, Kembell-Cook G, Tuddenham EG, et al. 2004. Complete inhibition of acute humoral rejection using regulated expression of membrane-tethered anticoagulants on xenograft endothelium. *Am. J. Transplant.* 4:1958–63
137. Yamamoto I, Horita S, Takahashi T, Tanabe K, Fuchinoue S, et al. 2007. Glomerular expression of plasmalemmal vesicle-associated protein-1 in patients with transplant glomerulopathy. *Am. J. Transplant.* 7:1954–60
138. Sis B, Campbell PM, Mueller T, Hunter C, Cockfield SM, et al. 2007. Transplant glomerulopathy, late antibody-mediated rejection and the ABCD tetrad in kidney allograft biopsies for cause. *Am. J. Transplant.* 7:1743–52

139. Solez K, Colvin RB, Racusen L, Sis B, Halloran PH, et al. 2007. Banff '05 meeting report: differential diagnosis of chronic injury and elimination of chronic allograft nephropathy ("CAN") in the Banff schema. *Am. J. Transplant.* 7:518–26
140. Gloor JM, Cosio FG, Rea DJ, Wadei HM, Winters JL, et al. 2006. Histologic findings one year after positive crossmatch or ABO blood group incompatible living donor kidney transplantation. *Am. J. Transplant.* 6:1841–47
141. Haas M, Montgomery RA, Segev DL, Rahman MH, Racusen LC, et al. 2007. Subclinical acute antibody-mediated rejection in positive crossmatch renal allografts. *Am. J. Transplant.* 7:576–85
142. Ivanyi B, Kemeny E, Szederkenyi E, Marofka F, Szenohradzky P. 2001. The value of electron microscopy in the diagnosis of chronic renal allograft rejection. *Mod. Pathol.* 14:1200–8
143. Lerut E, Naesens M, Kuypers DR, Vanrenterghem Y, Van Damme B. 2007. Subclinical peritubular capillaritis at 3 months is associated with chronic rejection at 1 year. *Transplantation* 83:1416–22
144. Terasaki PI, ed. 2007. *Clinical Transplant 2006*. Los Angeles: Terasaki Found. Lab.
145. Robertson H, Ali S, McDonnell BJ, Burt AD, Kirby JA. 2004. Chronic renal allograft dysfunction: the role of T cell-mediated tubular epithelial to mesenchymal cell transition. *J. Am. Soc. Nephrol.* 15:390–97
146. Kerjaschki D, Regele HM, Moosberger I, Nagy-Bojarski K, Watschinger B, et al. 2004. Lymphatic neoangiogenesis in human kidney transplants is associated with immunologically active lymphocytic infiltrates. *J. Am. Soc. Nephrol.* 15:603–12
147. Stult S, Gwinner W, Franz I, Schwarz A, Jonigk D, et al. 2007. Lymphatic neoangiogenesis in human renal allografts: results from sequential protocol biopsies. *Am. J. Transplant.* 7:377–84
148. Uehara S, Chase CM, Kitchens WH, Rose HS, Colvin RB, et al. 2005. NK cells can trigger allograft vasculopathy: the role of hybrid resistance in solid organ allografts. *J. Immunol.* 175:3424–30
149. Furukawa Y, Cole SE, Shah RV, Fukumoto Y, Libby P, et al. 2004. Wild-type but not interferon- γ -deficient T cells induce graft arterial disease in the absence of B cells. *Cardiovasc. Res.* 63:347–56
150. Tellides G, Tereb DA, Kirkiles-Smith NC, Kim RW, Wilson JH, et al. 2000. Interferon- γ elicits arteriosclerosis in the absence of leukocytes. *Nature* 403:207–11
151. Smith RN, Kawai T, Boskovic S, Nadazdin O, Sachs DH, et al. 2006. Chronic antibody mediated rejection of renal allografts: pathological, serological and immunologic features in nonhuman primates. *Am. J. Transplant.* 6:1790–98
152. Zeisberg M, Hanai J, Sugimoto H, Mammoto T, Charytan D, et al. 2003. BMP-7 counteracts TGF- β 1-induced epithelial-to-mesenchymal transition and reverses chronic renal injury. *Nat. Med.* 9:964–68
153. Iwano M, Plieth D, Danoff TM, Xue C, Okada H, et al. 2002. Evidence that fibroblasts derive from epithelium during tissue fibrosis. *J. Clin. Invest.* 110:341–50
154. Djamali A, Reese S, Yracheta J, Oberley T, Hullett D, et al. 2005. Epithelial-to-mesenchymal transition and oxidative stress in chronic allograft nephropathy. *Am. J. Transplant.* 5:500–9
155. Thauinat O, Field AC, Dai J, Louedec L, Patey N, et al. 2005. Lymphoid neogenesis in chronic rejection: evidence for a local humoral alloimmune response. *Proc. Natl. Acad. Sci. USA* 102:14723–28
156. Kitchens WH, Uehara S, Chase CM, Colvin RB, Russell PS, et al. 2006. The changing role of natural killer cells in solid organ rejection and tolerance. *Transplantation* 81:811–17

157. Pratt JR, Basheer SA, Sacks SH. 2002. Local synthesis of complement component C3 regulates acute renal transplant rejection. *Nat. Med.* 8:582–87
158. Peng Q, Li K, Patel H, Sacks SH, Zhou W. 2006. Dendritic cell synthesis of C3 is required for full T cell activation and development of a Th1 phenotype. *J. Immunol.* 176:3330–41
159. Fearon DT, Carroll MC. 2000. Regulation of B lymphocyte responses to foreign and self-antigens by the CD19/CD21 complex. *Annu. Rev. Immunol.* 18:393–422
160. Maier S, Tertilt C, Chambron N, Gerauer K, Huser N, et al. 2001. Inhibition of natural killer cells results in acceptance of cardiac allografts in CD28^{-/-} mice. *Nat. Med.* 7:557–62
161. Gerosa F, Baldani-Guerra B, Nisii C, Marchesini V, Carra G, et al. 2002. Reciprocal activating interaction between natural killer cells and dendritic cells. *J. Exp. Med.* 195:327–33
162. Bach FH, Ferran C, Hechenleitner P, Mark W, Koyamada N, et al. 1997. Accommodation of vascularized xenografts: expression of “protective genes” by donor endothelial cells in a host Th2 cytokine environment. *Nat. Med.* 3:196–204
163. Tabata T, de Perrot M, Keshavjee S, Liu M, Downey GP, et al. 2003. Accommodation after lung xenografting from hamster to rat. *Transplantation* 75:607–12
164. Tanaka M, Nakae S, Terry RD, Mokhtari GK, Gunawan F, et al. 2004. Cardiomyocyte-specific Bcl-2 overexpression attenuates ischemia-reperfusion injury, immune response during acute rejection, and graft coronary artery disease. *Blood* 104:3789–96
165. Salama AD, Delidakis A, Pusey CD, Cook HT, Bhargal G, et al. 2001. Transplant accommodation in highly sensitized patients: a potential role for Bcl-xL and alloantibody. *Am. J. Transplant.* 1:260–69
166. Segerer S, Bohmig GA, Exner M, Colin Y, Cartron JP, et al. 2003. When renal allografts turn DARC. *Transplantation* 75:1030–34
167. Rowshani AT, Florquin S, Bemelman F, Kummer JA, Hack CE, et al. 2004. Hyperexpression of the granzyme B inhibitor PI-9 in human renal allografts: a potential mechanism for stable renal function in patients with subclinical rejection. *Kidney Int.* 66:1417–22
168. Wong WK, Robertson H, Carroll HP, Ali S, Kirby JA. 2003. Tubulitis in renal allograft rejection: role of transforming growth factor- β and interleukin-15 in development and maintenance of CD103⁺ intraepithelial T cells. *Transplantation* 75:505–14
169. Cornell L, Della Pelle P, Brousaides N, Collins A, Colvin R. 2004. Endothelial response to rejection: enhanced expression of complement regulatory proteins decay accelerating factor (DAF, CD55) and protectin (CD59) in human renal allografts. *Mod. Pathol.* 17:A285
170. Aikawa A, Yamashita M, Hadano T, Ohara T, Arai K, et al. 2003. ABO incompatible kidney transplantation: immunological aspect. *Exp. Clin. Transplant.* 1:112–18
171. Park WD, Grande JP, Ninova D, Nath KA, Platt JL, et al. 2003. Accommodation in ABO-incompatible kidney allografts, a novel mechanism of self-protection against antibody-mediated injury. *Am. J. Transplant.* 3:952–60
172. Galili U. 2004. Immune response, accommodation, and tolerance to transplantation carbohydrate antigens. *Transplantation* 78:1093–98
173. Graca L, Cobbold SP, Waldmann H. 2002. Identification of regulatory T cells in tolerated allografts. *J. Exp. Med.* 195:1641–46
174. Sanchez-Fueyo A, Sandner S, Habicht A, Mariat C, Kenny J, et al. 2006. Specificity of CD4⁺CD25⁺ regulatory T cell function in alloimmunity. *J. Immunol.* 176:329–34
175. Zhang Z, Zhu L, Quan D, Garcia B, Ozcay N, et al. 1996. Pattern of liver, kidney, heart, and intestine allograft rejection in different mouse strain combinations. *Transplantation* 62:1267–72

176. Russell PS, Chase CM, Colvin RB, Plate JM. 1978. Kidney transplants in mice. An analysis of the immune status of mice bearing long-term, H-2 incompatible transplants. *J. Exp. Med.* 147:1449–68
177. Mezrich J, Yamada K, Sachs DH, Madsen JC. 2004. Regulatory T cells generated by the kidney may mediate the beneficial immune effects of combining kidney with heart transplantation. *Surgery* 135:473–78



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