Effector Mechanisms of Rejection

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Organ transplantation appears today to be the best alternative to replace the loss of vital organs induced by various diseases. Transplants can, however, also be rejected by the recipient. In this review, we provide an overview of the mechanisms and the cells/molecules involved in acute and chronic rejections. T cells and B cells mainly control the antigen-specific rejection and act either as effector, regulatory, or memory cells. On the other hand, nonspecific cells such as endothelial cells, NK cells, macrophages, or polymorphonuclear cells are also crucial actors of transplant rejection. Last, beyond cells, the high contribution of antibodies, chemokines, and complement molecules in graft rejection is discussed in this article. The understanding of the different components involved in graft rejection is essential as some of them are used in the clinic as biomarkers to detect and quantify the level of rejection.

DIFFERENT TYPES OF REJECTION

Several types of rejection of vascularized organs can be defined according to their underlying mechanisms and tempos, the major types being hyperacute, acute, and chronic rejection. In allogeneic context and in the absence of preformed antidonor antibodies, cells and tissues are mainly rejected by acute cellular rejection mechanisms.

Hyperacute rejection appears in the first minutes following transplantation and occurs only in vascularized grafts. This very fast rejection is characterized by vessels thrombosis leading to graft necrosis. Hyperacute rejection is caused by the presence of antidonor antibodies existing in the recipient before transplantation. These antibodies induce both complement activation and stimulation of endothelial cells to secrete Von Willebrand procoagulant factor, resulting in platelet adhesion and aggregation. The result of these series of reactions is the generation of intravascular thrombosis leading to lesion formation and ultimately to graft loss. Today, this type of rejection is avoided in most cases by checking for ABO compatibility and by excluding the presence of antidonor human leukocyte antigen (HLA) antibodies by crossmatch techniques between donor graft cells and recipient sera. This type of rejection is also observed in models of xenotransplantation of vascularized organs between phylogenetically distant species when no immunosuppressive treatment is given to the recipients.

Acute rejection is caused by an immune response directed against the graft and occurs between 1 week and several months after transplantation. Acute rejection is diagnosed on histological analysis of a graft biopsy according to an international classification system, the Banff

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classification for the kidney (Mengel et al. 2012). Acute rejection is thought to result from two immunological mechanisms that may act alone or in combination: (1) a T-cell-dependent process that corresponds to acute cellular rejection, and (2) a B-cell-dependent process that generates the acute humoral rejection. With current immunosuppressive treatment, acute rejection occurs in less than 15% of the transplants (Port et al. 2004) in nonsensitized patients.

Chronic rejection, on the other hand, is now the leading cause of graft rejection. Chronic rejection can be mediated by either humoral or cellular mechanisms linked to memory/plasma cells and antibodies. The presence of tertiary lymphoid organs in the graft is a characteristic of this form of rejection.

INNATE AND ADAPTIVE IMMUNE RESPONSES

Two major immunological mechanisms occur during allograft rejection: the nonspecific innate response that predominates in the early phase of the immune response, and the donor-specific adaptive response that results from alloantigen recognition by host T cells.

The Innate Response and Allograft Rejection

Although the adaptive response plays a central role in the mechanisms of allograft rejection, early proinflammatory signals (arising before the initiation of the T-cell response) are also considered as important factors of graft rejection. Inflammation is caused by the innate immune response induced independently of the adaptive response (Christopher et al. 2002; He et al. 2002, 2003; Land 2005). In fact, it was shown that 1 day after a heart transplant, the expression of genes coding for molecules linked to inflammation (proinflammatory cytokines, chemokines, components of the cellular infiltrate) was similar in normal mice and in mice deficient for T and B cells, but with normal NK and myeloid compartments (*Rag1* or *Rag2* knock-out mice) (He et al. 2003). These investigators also showed that the innate response is antigen independent, develops early after transplantation, and conditions the development of the adaptive response (He et al. 2003).

Innate immune responses are the consequence of several events associated with clinical transplantation, such as ischemia-reperfusion injury and infections, and lead to the release of damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) (Chong and Alegre 2012). DAMPs and PAMPs are recognized by so-called pattern-recognition receptors (PRRs) expressed by hematopoietic cells.

The specificity of PRRs is genetically determined and several subgroups can be classified based on their structure. The transmembrane group of PRRs includes several families of molecules such as toll-like receptors (TLRs), C-type lectins, RAGE (receptor for advanced glycation endproducts), complement receptors, scavenger receptors, and mannose receptors. The cytosolic PRR group includes retinoic acid-inducible gene-I-like receptors and nucleotide-binding domain and leucine-rich repeat-containing receptors (Iwasaki and Medzhitov 2010).

Immediately after transplantation, PRR-mediated danger signals activate dendritic cells (DCs) (LaRosa et al. 2007) leading to antigen-presenting cell (APC) maturation, up-regulation of costimulatory molecules, and secretion of proinflammatory cytokines. In this context, donor APCs migrate to the T cell areas of secondary lymphoid organs and induce the activation and differentiation of alloreactive naïve T cells into effector T helper cells. These effector cells migrate into the graft where they activate macrophages and granulocytes (neutrophils, eosinophils, and basophils) that have infiltrated the graft in response to inflammatory stimuli. The latter cells contribute to lesion formation, either directly or through the production of proinflammatory cytokines and chemokines. They also help boost and maintain the adaptive immune T-cell response. Another cell type involved in innate immunity is the NK cell. In a proinflammatory context, NK cells become activated and are able to kill target cells (Pratschke et al. 2009).

The complement system plays a central role in the effector mechanisms occurring during the innate response. The three complement path-

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ways (classical, alternative, and mannose lectin) can be activated by DAMPs as well as by the inflammatory environment that develops during ischemia reperfusion injury (Castellano et al. 2010). Moreover, the intermediate product of complement, C5a, is able to bind to APC receptors, leading to Th1 activation and development of the adaptive immunity (Zhou et al. 2000).

Initiation of the Adaptive Response and Allograft Rejection

The adaptive immune response appears later than the innate response, its main characteristic being antigen specific. The initiation of the adaptive response is made possible by the presentation of alloantigens by APCs, mainly DCs and their allorecognition by recipient T cells. Three main pathways of allorecognition are described in the literature. The first is the direct pathway, in which donor DCs present in the graft act as passenger leukocytes. In the context of a proinflammatory environment, these cells mature and migrate to secondary lymphoid organs where they prime host T cells. The maturation of these cells is induced by proinflammatory signals such as IL-1β, TNF-α, and CD40. The second is the indirect allorecognition pathway in which recipient DCs capture, process, and present alloantigens as peptides on their host MHC molecules, and then prime T cells (Ochando et al. 2006). As dendritic cells express both MHC class I and class II, donor antigens can be presented to either $CD4^+$ or $CD8^+$ T cells. The third pathway is that of semidirect allorecognition, characterized by the dual ability of recipient DCs: (1) to present intact donor major histocompatibility complex (MHC) molecules acquired by cell-to-cell contact or fusion with donor exosomes, and (2) to internalize and process donor MHC as peptides on recipient MHC molecules. In this pathway, alloreactive $CD8^+$ and $CD4^+$ T cells are stimulated by direct and indirect allorecognition (Herrera et al. 2004; Smyth et al. 2006).

Using the two-photon microscopy technique, a recent elegant study by Bousso's group (Celli et al. 2011) provided a better understanding of the adaptive immune response by clarifying several different issues that until then had remained hypothetical. In a murine model of skin transplantation, they showed that donor dermal DCs disappeared rapidly from the graft and migrated to draining lymph nodes (LNs). However, these donor DCs were found to be dead in the secondary lymphoid organs. This is consistent with a previous study demonstrating that donor DCs are eliminated by NK cells (Garrod et al. 2010). It has been suggested that these dying cells could be a source of alloantigens for recipient APCs present in the draining LNs that stimulate T cells by the indirect pathway. Moreover, these experiments showed that host inflammatory monocytes and DCs act as antigen-transporting cells. These recipient graft-infiltrating cells indeed have the ability to reach the draining LNs and cross-prime $CD8^+$ T cells by the indirect pathway (Celli et al. 2011).

Initiation of the innate and adaptive immune response leads to acute and chronic graft rejection. Cellular and humoral responses, but also chemokines and innate cells, are present in both types of rejection and are described in this article.

ACUTE REJECTION

The Components of Acute Rejection

Chemokines

Chemokines are chemoattractant cytokines that influence immune cell migration. In transplantation settings, chemokines act during four key phases: (1) recruitment of mononuclear cells to the site of inflammation, (2) migration of APCs to draining LNs, (3) interaction between unprimed T cells and APCs in LNs, and (4) migration of primed alloreactive T cells to the graft (Belperio and Ardehali 2008). The roles of some chemokines in allograft rejection have been investigated directly by analyzing chemokine expression by immune cells as described in Figure 1. Furthermore, the function of these chemokine receptors and ligands has been shown using knockout (KO) mice or blocking antibodies.

One example is that of CCR5, which was shown to be expressed by mononuclear cells,

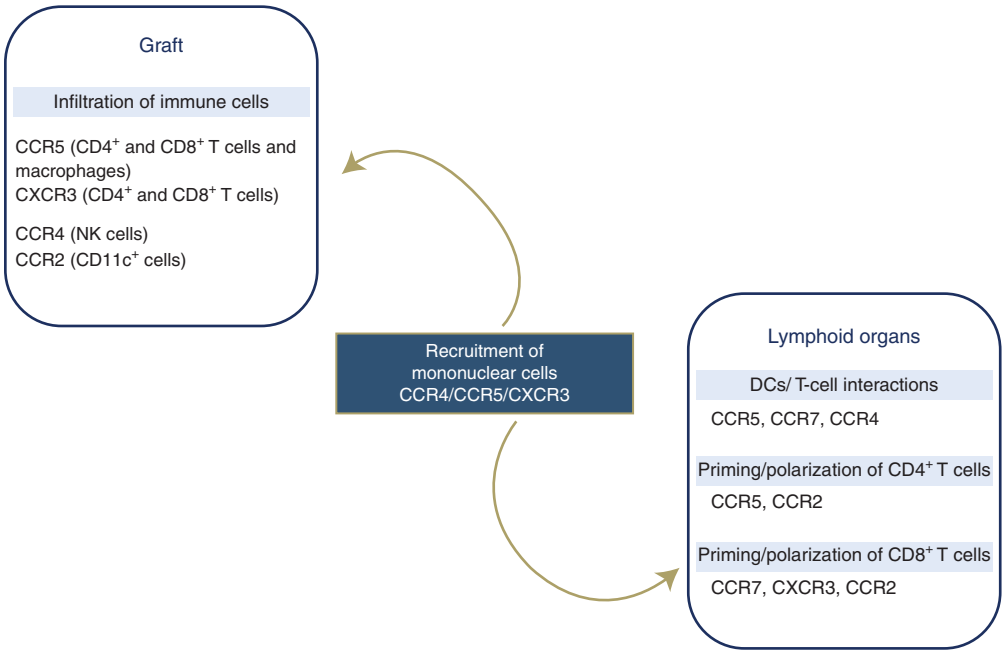

Figure 1. Chemokines: Homing of immune cells and role in acute rejection. Mononuclear cells are able to migrate into the graft or into the lymphoid organs in response to chemokine gradients. Experiments on animal models of transplantation and analysis of human samples from transplant recipients allow a better understanding of the involvement of chemokines in graft rejection.

suggesting that this chemokine helps their migration to inflammatory sites. Prolongation of cardiac allograft survival was obtained in $CCR5^{-/-}$ mice, demonstrating a major role for this chemokine (Gao et al. 2001). However, more recently, in a similar heart transplant model, Nozaki et al. (2007) only detected a modest effect of CCR5 on graft rejection using $CCR5^{-/-}$ mice. Involvement of CCR5 in graft rejection was also shown in murine models of pancreatic islet transplantation (Abdi et al. 2002). More recently, in a cardiac transplantation model in rhesus monkeys, cotreatment with a CCR5 antagonist and cyclosporine A was able to prolong heart survival. This prolongation was associated with a delay in alloantibody response, a lower graft infiltration, and a decrease in alternatively activated macrophages (Li et al. 2011). In addition to the recruitment of mononuclear cells to the graft, CCR5 polarizes T cells toward a type 1 response, and promotes the trafficking of T cells to LNs and favors DC/T-cell interaction within the LNs (Amano et al. 2005). Furthermore, Y-box protein-1, a molecule that acts as a transcription regulator of CCL5, one CCR5 ligand also called RANTES, expression in T cells, and monocytes/macrophages, was shown to be up-regulated during rejection in human kidney transplant biopsies (Raffetseder et al. 2009). Contrary to these studies, in a rat model of kidney transplant tolerance following CD28 blockade, CCL5 recruited $CD4^+$ Tregs to the graft via a graft-to-periphery CCL5 gradient (Dilek et al. 2012).

Another well-described chemokine involved in graft rejection is CXCR3. In a murine model, absence of CXCR3 in graft recipients was shown to prolong cardiac transplant survival (Hancock et al. 2000). However, as for CCR5, a more recent study obtained controversial results showing no delay in graft rejection between controls and CXCR3 KO mice (Kwun et al. 2008). Analysis of CXCR3 and its ligands, CXCL9 and CXCL10, was performed in biopsies from transplant patients undergoing acute rejection. Fahmy et al. (2003) showed an up-regulation

of CXCL10 in endomyocardial biopsies, whereas an increase in CXCL10 was detected in macrophages of lung biopsies (Agostini et al. 2001). Furthermore, the number of $CXCR3^+$ cells present in human kidney transplant biopsies was reported to increase with graft rejection and to decrease with immunosuppressive treatment, suggesting that CXCR3 might be a good target to treat rejection (Hoffmann et al. 2006). Other recent studies have highlighted the role of chemokines as biomarkers of clinical rejection and are described in Table 1.

In mouse models of cardiac and pancreatic islet transplantation, expression of CXCR3 was detected in Tregs, memory $CD4^+$ and $CD8^+$ T cells, NKT, and NK cells (Uppaluri et al. 2008). Zhai et al. (2006) showed that CXCR3 is involved in the recruitment of $CXCR3^+CD4^+$ T cells following ischemia-reperfusion injury in a model of rat syngeneic orthotopic liver transplantation. Similarly, expression of CXCR3 by Tregs resulted in their recruitment to peripheral sites of inflammation, and a high number of circulating $CXCR3^+$ Tregs was detected in kidney transplant patients treated with an mTOR inhibitor as their immunosuppressive therapy (Hoerning et al. 2011). In addition to the role of CXCL9/CXCL10 in the recruitment of T cells to the allograft, Rosenblum et al. (2010) recently showed that these chemokines regulate donor-specific $CD8^+$ T-cell priming. As such, in a murine model of cardiac transplantation using KO mice for either the CXCL9 or CXCL10 gene, CXCL9 promoted, whereas CXCL10 inhibited the differentiation of IFN-γ-producing donor-specific $CD8^+$ T cells.

A new molecule called TAK-779 was identified as a blocking agent for both CCR5 and CXCR3. This molecule was shown to prevent cardiac allograft rejection in mice (Akashi et al. 2005) as well as kidney rejection in rats (Kakuta et al. 2012). In the latter study, prolongation of graft survival correlated with a decrease in macrophage infiltration (Kakuta et al. 2012). Last, coexpression of CXCL10 and fractalkine or CX3CL1 by endothelial cells was shown to be involved in effector memory T-cell recruitment (Manes et al. 2007; Manes and Pober 2008). Fractalkine acts as a chemoattractant and adhesion molecule for cells that express CX3CR1, including certain immune cells such as monocytes/macrophages, NK cells, and T cells (Imai et al. 1997; Fong et al. 1998; Foussat et al. 2000).

Some studies have also been performed to analyze other chemokine receptors. For example, CCR2 was shown to be an important chemoattractant receptor for mononuclear cells. This is because CCR2 induced mobilization of monocytes from bone marrow to blood and an accumulation of $CD11c^+$ cells in the allograft in a mouse model of lung allotransplantation (Gelman et al. 2010). Interaction of CCR2 with its ligand (CCL2) also caused T-cell clonal expansion and differentiation (Lee et al. 2003). In $CCR2^{-/-}$ mice, an increase in graft survival was observed after pancreatic islet transplantation (compared to control mice) and associated with the absence of $CD8^+$ effector T cells in these mice (Abdi et al. 2004).

CCR4 is an important chemokine for homing of memory T cells (Campbell et al. 1999) and was shown to be crucial for the recruitment of $FoxP3^+$ Tregs in a model of tolerance induced by donor-specific transfusion/CD154 therapy (46) as no tolerance was achieved in CCR4-deficient mice (Lee et al. 2005). Inhibition of CCR4 and its ligands led to an increase in heart graft survival associated with a decreased in DC/T-cell interactions and an inhibition of monocytes and NK cells present in the graft (Alferink et al. 2003; Huser et al. 2005). Last, CCR7 is known to be involved in the localization of both DCs and T cells in the T-cell-rich zones of the LNs, indicating a role for CCR7 in T-cell homing and priming (Hopken et al. 2004). Recently, Liu et al. (2011) showed that a lack of CCR7 prevented tolerance induction in a cardiac murine model of transplantation. This impairment of tolerance was partially rescued by adoptive transfer of wild-type plasmacytoid DCs to the CCR7-deficient mice (Liu et al. 2011).

As discussed before, some of these chemokines and chemokine receptors might be useful as markers of acute rejection. In the case of renal transplantation, rejection could be monitored via noninvasive urine sampling. The most well-known urine marker is IP-10 or CXCL10. An increase in IP-10 mRNA and protein in the

Table 1. Use of chemokines, cytokines, miRNA, and cells as biomarkers of graft rejection in clinical studies

Type of rejection	Molecules/cells	Transplant organ	Samples of analysis	Significance	References
Acute	CXCL10	Heart	Blood, serum	Higher serum level of CXCL10 in transplanted patients versus control ones. Among transplanted patients, high expression of CXCL10 is correlated with acute rejection.	Crescioli et al. 2009
	C reactive protein (CRP)	Heart	Blood, serum	Significant differential expression of CRP between rejected and nonrejected heart transplant recipient. In the first year postheart transplantation, CRP was the most useful parameter for noninvasive screening of acute cellular rejection.	Martinez-Dolz et al. 2009
	Granzyme B CTLA-4 IL-6 TNF-α TGF-β	Heart	Biopsies	Up-regulation in mRNA expression of all analyzed genes but the strongest increase is seen for granzyme B, TGF-β, and TNF-α genes. Correlation between the expression analysis of these three genes and acute rejection.	Ramsperger-Gleixner et al. 2011
	CD69	Heart	Blood, T lymphocytes	Overexpression of CD69 in T lymphocytes of patients with acute rejection. Relationship between increased expression by T lymphocytes and acute rejection is statistically more pronounced for $CD8^+$ T cells than for $CD4^+$ T cells. Presence of $CD69^+/CD8^+$ cells exceeding 15% in patients with acute rejection of all T lymphocytes is correlated with vigorous rejection.	Schowengerdt et al. 2000
	Functional markers of $CD4^+$ T-cell subsets: Th1 Th17 $FoxP3^+$ T cells	Heart	Blood and biopsies	Higher mRNA expression of the functional markers in patients with acute cardiac rejection in blood and biopsies samples (but not for Th2 mRNA cytokines). Higher percentage of Th1, Th17, and $FoxP3^+CD4^+$-circulating T cells. Graft infiltration by Th1 and Th17 cells. Kinetic changes of these $CD4^+$ T-cell subsets after cardiac allograft.	Wang et al. 2011
	CCL3L1	Liver	Blood, PBMC	Analysis of copy number variation in genes. Higher number of CCL3L1 gene in patients with acute rejection.	Li et al. 2012

Acute	CCL2 CXCL8 CCL5 CXCL9 CXCL10	Liver	Blood, serum	Chemokines screening using Luminex multiplex assay. Higher expression of molecules correlated with an up-regulation of NF-κB: CCL2, CCL5, and CXCL8. Higher expression of molecules correlated with T-cell immunity: CXCL9 and CXCL10. Biomarkers of early allograft dysfunction.	Friedman et al. 2012
	Granzyme B Perforin	Liver	Biopsies	Analysis by immunohistochemistry. Higher frequencies of perforin and granzyme B expression in acute rejection patients. In most of the biopsies, perforin and granzyme B are simultaneously expressed.	Ji et al. 2004
	Granzyme B	Liver	Biopsies	Higher granzyme B mRNA expression in the biopsies of the acute rejection group.	Krams et al. 1995
	Three subsets of T cells: $CD4^+CD7^+$ $CD8^+CD38^+$ $CD4^+CD25^+$	Liver	Biopsies and blood	Higher number of $CD4^+CD7^+$ and $CD8^+CD38^+$ cells in blood and liver of liver recipient with acute rejection. Decreased expansion of Treg cells ($CD4^+CD25^+$) in blood and liver in patients with acute rejection.	Perrella et al. 2006
	Hepatocyte-derived miRNAs (HDmiRs)	Liver	Blood, serum	Serum level of HDmiRs (miR-122, miR-148a, and miR194) are elevated up to 20-fold in patients with acute rejection. HDmiRs are promising candidates as biomarkers rejection, but also as hepatic injury, after liver transplantation.	Farid et al. 2012
	CCL4	Lung	Blood, PBMC	Analysis of copy number variation in genes. Higher number of CCL4 gene in patients with acute rejection.	Colobran et al. 2009
	Treg and cytotoxic markers	Lung	Bronchoalveolar lavage and blood	Higher mRNA expression for all of the markers in patients with acute rejection, but the expression is significantly increased only for CTLA-4, FoxP3, and granzyme B mRNA. But no difference have been observed in blood samples.	Madsen et al. 2010
	$CD4^+/CD8^+$ T cells NK NK-like T cells B cells Tregs Invariant receptor NK-T cells (iNKT)	Lung	Bronchoalveolar lavage fluid	Reduction of CD4:CD8 T-cell ratio in patients with acute lung rejection. Higher frequency of $CD4^+$ T cells expressing CD38 during rejection. Proportions of NK and Treg, as a frequency of total $CD3^+$ T cells, trended toward being reduced in rejection. Proportions of B cells, NK-like T cells, and the percentage of $CD4^-/CD8^-$ iNKT cells trended toward being increased in rejection.	Gregson et al. 2008

Continued

Table 1. *Continued*

Type of rejection	Molecules/cells	Transplant organ	Samples of analysis	Significance	References
Acute	$CD4^+/CD8^+$ T cells B cells Memory T cells	Lung	Bronchus-associated lymphoid tissues	Reduction of CD4:CD8 T-cell ratio is correlated with elevated acute rejection episode for patients in the first year of transplantation.	Shenoy et al. 2012
	CXCR1 CXCR2	Kidney	SNP genotype	Donor CXCR1-2668 GA/AA genotype is associated with acute rejection in kidney transplantation.	Ro et al. 2011
	CXCL10 CXCL13	Kidney	Blood, PBMC	Overexpression of CXCL10 and CXCL13 in PBMC of patients with acute rejection.	Mao et al. 2011
	Granzyme B Perforin FasL	Kidney	Blood, T lymphocytes	Significant higher perforin mRNA expression in patients with acute rejection. No consistent pattern of granzyme B and FasL expression.	Shin et al. 2005
	CD69 expression	Kidney	Blood, T lymphocytes	Higher percentages of $CD69^+$ cells in the $CD3^+$ and $CD8^+$ T-cell subsets of patients with acute rejection. Correlation between $CD69^+CD8^+$ T cells and acute rejection is the more clinically useful test (based on receiver-operator characteristics).	Posselt et al. 2003
	CXCL10	Kidney	Blood, serum	High serum level of CXCL10 is associated with acute risk of graft failure.	Rotondi et al. 2004
	CXCL9	Kidney	Blood, serum	High level of CXCL9 pretransplantation in recipient patients is a marker of high risk of acute rejection.	Rotondi et al. 2010
	Pattern of cytokines Granzyme B Perforin	Kidney	Blood, PBMC	High level of proinflammatory cytokines (IL-4, IL-5, IL-6, and IFN-γ) and cytotoxic molecules (perforin and granzyme B) are associated with acute rejection in kidney transplant recipient.	Dugre et al. 2000
	CXCL9 CXCL10	Kidney	Urine	Increased expression of CXCL9 and CXCL10 in urine of patients with acute rejection.	Jackson et al. 2011a
	Granzyme B Perforin	Kidney	Urine	Increased mRNA expression of granzyme B and perforin in urine of patients with acute rejection.	Li et al. 2001

Acute	Perforin Granzyme B FasL PI-9 FoxP3	Kidney	Urine	Increased mRNA signature expression in urine of patients with acute rejection. FoxP3 mRNA level is the most accurate and predictive for acute rejection with a sensibility and specificity of 100%.	Aquino-Dias et al. 2008
	miRNAs miR-210	Kidney	Urine	Down-regulation of miR-10b and miR-210 in patients with acute rejection (compared with controls). Up-regulation of miR-10a in patients with acute rejection (compared with controls). Expression of miR-210 is only one that changes before and after rejection episode. Low expression of miR-210 is correlated with acute rejection and prediction of long-term of kidney function.	Lorenzen et al. 2011
	CXCR3	Kidney	Biopsies	Increase number of CXCR3-positive cells in biopsies from patients with acute rejection and decrease with immunosuppressive treatment.	Hoffmann et al. 2006
	CX3CR1	Kidney	Biopsies	CX3CR1 intragraft staining is associated with worst outcome. CX3CR1 is expressed by macrophage and dendritic cells in acute rejection.	Hoffmann et al. 2010
	Pattern of chemokines	Kidney	Biopsies	Increase of inflammatory chemokines and chemokine receptors transcripts during acute rejection: CCL3/CCL5/CXCL9/CXCL10/CXCL11 and CCR5/CCR7/CXCR3. Rejection associated with Th1 polarization.	Lo et al. 2011
	miRNAs	Kidney	Biopsies	Up-regulation of seven miRNA and down-regulation of 10 miRNA samples of patients with acute rejection. Three samples (overexpressed) are predictive with a high accurancy of acute rejection: miR-142-5p, miR-155, and miR-223.	Anglicheau et al. 2009
Chronic	CCR4	Lung	Blood, PBMC	Reduction of $CCR4^+$ expression on $CD4^+$ memory T cells in patients with chronic rejection.	Paantjens et al. 2011
	CXCL9 CXCL10	Lung	Bronchoalveolar lavage fluid	Cumulative expression of CXCL9 and CXCL10 in bronchoalveolar liquid is a marker of graft failure.	Neujahr et al. 2012

Continued

Table 1. *Continued*

Type of rejection	Molecules/cells	Transplant organ	Samples of analysis	Significance	References
Chronic	HNPs (human neutrophil peptides 1–3)	Lung	Bronchoalveolar lavage fluid	High level of HNPs peptides by mass spectrometry in patients who have developed bronchiolitis obliterans syndrome (marker of chronic lung rejection). Validation by enzyme-linked immunosorbent assay (ELISA). Monitoring levels of HNPs can predate the clinical onset of disease up to 15 months.	Nelsestuen et al. 2005
	CCL2	Kidney	Urine	Early detection of CCL2 in urine is a marker of a development of IF/TA at 24 months.	Ho et al. 2010
	CXCL9 CXCL10	Kidney	Urine	Increased expression of CXCL9 and CXCL10 in urine of patients with chronic rejection.	Schaub et al. 2009
	CCR1 RANTES	Kidney	Biopsies	CCR1 and RANTES promote ongoing IF/TA and are linked to worst outcome.	Dikow et al. 2010
	Immunoproteasome β subunit 10	Kidney	Blood and biopsies	Immunoproteasome β subunit 10 mRNA expression is specifically up-regulated during chronic active antibody-mediated rejection.	Ashton-Chess et al. 2010
	Tribbles-1, -2, and -3	Kidney	Blood, biopsies and urine	Only mRNA expression of Tribbles-1 is significantly increased in blood and graft biopsies of kidney transplant recipients with chronic antibody-mediated rejection compared to patients with other histological and clinical diagnoses. In peripheral blood, mononuclear cell mRNA Tribbles-1 expression discriminates patients with chronic antibody-mediated rejection from those with other types of late allograft injury with high sensitivity and specificity. Tribbles-1 was found to be a potential blood and tissue biomarker of chronic antibody-mediated rejection.	Ashton-Chess et al. 2008

Acute and chronic	CXCL10	Kidney	Blood, serum	High serum levels of CXCL10 is associated with both acute rejection and chronic allograft nephropathy.	Lazzeri et al. 2005
	CXCL10 MIG MIP-1 OPG	Kidney	Urine	Chemokines screening using Luminex multiplex assay. CXCL10 and MIG: markers of acute rejection, but not chronic rejection and stable graft function. MIP-1 (Δ) and OPG: markers of both acute and chronic rejection, but not stable graft function.	Hu et al. 2009
	FoxP3 T-bet Granzyme B	Kidney	Blood, PBMC and biopsies	mRNA expression of FoxP3 and T-bet increase in biopsies, but not in PBMC samples of patients with acute rejection. mRNA expression of granzyme B increased in biopsy samples of patients with acute (cellular) and chronic (antibody-mediated) rejection. mRNA expression of granzyme B in PBMC is significantly reduced for patients with chronic rejection. Granzyme B, but not FoxP3 and T-bet, should be considered as a biomarker of chronic rejection.	Ashton-Chess et al. 2009

IF/TA, interstitial fibrosis and tubular atrophy.

urine of a kidney transplant recipient was shown to correlate with incidence of acute rejection episodes (Matz et al. 2006). Moreover, high levels of CXCL3 (Tatapudi et al. 2004) and CD103 mRNA (Ding et al. 2003) were present in a recipient undergoing acute rejection. Nevertheless, chemokines are not the only markers of rejection. In fact, other proteins, such as granzyme B and FoxP3, miRNAs, and cells could also be used as predictive biomarkers of rejection (Table 1).

Nonspecific Effector Cells (NK Cells, Macrophages, Monocytes, DCs, and Neutrophils)

Despite the finding that NK cell detection in the graft infiltrate was a negative prognostic factor (Sorrentino et al. 2006), this cell type does not appear to be necessary or sufficient to mediate allograft rejection. Some recent studies have even highlighted the involvement of NK cells in the phenomenon of tolerance (van der Touw et al. 2012). NK cells seem to act mainly as a bridge between innate and adaptive immune responses. This has been underlined by experiments using CD28-deficient mice. For example, by depleting NK1.1$^+$ cells using antibodies in CD28-deficient mice, some groups showed that absence of NK cells reduce cardiac allograft rejection in mice (Maier et al. 2001; McNerney et al. 2006). In this model, NK cells, but not NKT cells, were required for rejection by promoting T-cell expansion and effector function (McNerney et al. 2006). This effect was independent of the NK-cell activator NKG2D receptor.

In contrast, several studies have highlighted the role of the NKG2D receptor in rejection. In a model of cardiac transplantation in CD28-deficient mice, blocking NKG2D prevented graft rejection (Kim et al. 2007). Zhang and coworkers confirmed the involvement of NKG2D in graft rejection as they showed that the NKG2D receptor present on NK cells recognizes renal tubular epithelial cells, leading to their killing by a perforin-dependent pathway during ischemia reperfusion injury (Zhang et al. 2008). Promotion of CD4$^+$ T-cell activation by the indirect allorecognition pathway is another mechanism of action of NK cells investigated by NK-cell depletion and NKG2D blockade in a murine skin graft model (Ito et al. 2008). Two ligands of NKG2D, namely RAEL1 and RRLT, were found to be expressed in rat liver allograft and to be linked to acute rejection (Zhuo et al. 2010). Moreover, in human kidney transplant biopsies, an elevated level of NKG2D mRNA was also associated with acute rejection (Seiler et al. 2007). NK cells produce a variety of cytokines, including IFN-γ, TNF-α, and TGF-β, as well as colony-stimulating factors (Cuturi et al. 1987, 1989; Anegon et al. 1988). IFN-γ produced by NK cells is known to induce an up-regulation of MHC class I and class II molecules by endothelial cells leading to their killing by CD8$^+$ T cells (McDouall et al. 1997; Ayalon et al. 1998). More recently, a role for IL-15 in NK-cell-mediated mechanisms was shown, as in vivo stimulation of NK cells with IL-15 was found to induce skin graft rejection in RAG$^{-/-}$ mice (Kroemer et al. 2008). Last, an interesting effect of NK cells on donor DCs has been described. Coudert et al. (2002) showed that interaction of NK cells with donor DCs in the absence of CD8$^+$ T cells promoted CD4$^+$ T-cell priming and regulated the Th balance. In a more recent study performed in a model of CD4$^+$ T-cell-mediated allogeneic skin graft rejection, NK cells killed donor DCs in draining LNs by a perforin-dependent pathway that avoided T-cell activation (Laffont et al. 2008) thereby confirming that NK cells favor indirect allorecognition (Garrod et al. 2010).

Macrophages constitute another innate cell population that contributes to the tissue damage observed in rejected organs. In humans, macrophages were found to represent 40%–60% of the cellular infiltrate in renal allotransplants (Hancock et al. 1983). A large number of monocytes were also detected in infiltrates from the graft biopsies of kidney transplant recipients under T-cell-depleting therapy (anti-CD52 and FK506 monotherapy) (Salama et al. 2007). These cells were recruited to inflammatory sites by monocyte chemoattractant protein-1 (MCP-1). Blockade of MCP-1 was shown to increase pancreatic islet transplant survival in rodents (Lee et al. 2003). In addition, blockade of macrophage colony-stimulating factor-

reduced macrophage proliferation and accumulation in the graft leading to a decrease in the severity of kidney transplant rejection in mice (Jose et al. 2003). Other studies have confirmed in macrophages a decreased infiltration and an increase in graft survival in murine models of both kidney (Qi et al. 2008) and heart (Takeiri et al. 2011) transplantation. Furthermore, in the context of human kidney transplantation, CX3CR1 expression by macrophages was associated with acute rejection, and was a negative prognostic factor in human kidney graft (Hoffmann et al. 2010).

There are several macrophage subsets, and specialized tissue-resident macrophages are widely distributed across the body including the liver (Kupffer cells) and bone (osteoclasts). Macrophages can also display heterogeneity in terms of their functional phenotype as well as their activation state. In the same terms, activated macrophages are classified as classically or alternatively activated macrophages, named M1 and M2 macrophages, respectively, referring to T-cell nomenclature (Mantovani et al. 2004).

M1 macrophages are activated by a combination of IFN-γ and TNF released by Th1 cells. M1 macrophages have the capacity to present antigen to T cells and express high levels of costimulatory molecules (CD86 and CD80) (Mantovani et al. 2004). These cells also express large amounts of the proinflammatory cytokine IL-12 and promote the development of the Th17 immune response by secreting IL-1, IL-6, and IL-23. M1 macrophages are thought to play a role in the defense against bacteria or viruses as well as tumor resistance. However, in several conditions, this macrophage subset can take part in chronic inflammation and autoimmune diseases (Mosser and Edwards 2008).

M2 macrophages, on the other hand, are associated with immunoregulation. Macrophage phenotype and function are thought to differ according to the chemokine environment at the time of their activation (Mantovani et al. 2004). In fact, macrophages develop a regulatory profile when activated by IL-10 or immune complexes associated with a second stimulus. These cells act as regulatory cells by producing high levels of IL-10 and other anti-inflammatory cytokines such as TGF-β, for example (Mosser and Edwards 2008). Alternatively, if resident macrophages are activated in the presence of IL-4 and IL-13, they are considered as M2a, or wound-healing macrophages, as they promote tissue repair. The latter cells are characterized by an upregulation of mannose receptor and production of polyamine (Mantovani et al. 2004; Mosser and Edwards 2008).

However, depending on the context, activated macrophages are able to switch their phenotype and change their function too (Mosser and Edwards 2008). One example of this is the differentiation of M1 to hybrid macrophages that share the characteristics of both regulatory and wound-healing macrophages. Unfortunately, these cells are tumor-associated macrophages and inhibit antitumor immunity (Duluc et al. 2007). Another example is in the context of the autoimmune disease diabetes, in which adipose-tissue-associated macrophages with a wound-healing phenotype polarize toward a phenotype similar to classically activated macrophages (Lumeng et al. 2007).

A recent study performed in a rat model of lung transplantation showed that $CD68^+$ macrophages were the most abundant cell type observed during acute rejection, whereas $CD163^+$ cells were distributed around vessels and bronchioles (Jungraithmayr et al. 2010). Regarding their tissue localization, macrophage infiltration was significant in the interstitium, but also in the arterial intima of vascularized grafts (Sun et al. 2011). Infiltration was also observed in the glomeruli of kidney transplants.

In contrast to the arterial intima in which no correlation between antibody-mediated rejection (AMR) and macrophage infiltration was shown (Kozakowski et al. 2009), macrophages/monocytes were associated with AMR when present in the glomeruli. Moreover, presence of focal or diffuse C4d deposits was associated with macrophage/monocyte infiltrates, whereas T cells predominated when no C4d deposits were detected (Magil 2005; Magil and Tinckam 2006). These results were confirmed by Fahim et al. (2007), who showed an abundant recruitment of monocytes to peritubular and glomerular capillaries during humoral rejection. More-

over, in a murine model of hepatocellular allotransplantation in which rejection is mediated by a CD4-dependent alloantibody response, depletion of macrophages delayed hepatocyte rejection and inhibited in vivo allocytotoxicity (Horne et al. 2008).

Polymorphonuclear cells are also efficient producers of chemokines (Molesworth-Kenyon et al. 2005). As described in the previous section, chemokines act on T-cell activation, proliferation, and function. One type of polymorphonuclear cell, the neutrophil, was detected in large numbers among the cells infiltrating murine skin allografts during acute rejection (Celli et al. 2011). Neutrophils have also been shown to contribute to allograft rejection in models of costimulation blockade (El-Sawy et al. 2005), or in the absence of IFN-γ (Miura et al. 2003) and IL-4 (Surquin et al. 2005). There is also evidence of eosinophil involvement in models of rejection and their activation was shown to be dependent on a Th2 $CD4^+$ T-cell response (IL-4-dependent) (Braun et al. 2000; Surquin et al. 2005). Eosinophils were also abundant in intestinal graft infiltrates on T-cell elimination (Wu et al. 2006).

Recently, the role of platelets has been shown in allograft rejection, as these cells are able to induce immune stimulation. In this context, platelets are believed to act by recruiting mononuclear cells by secreting cytokines/chemokines and by stimulating monocytes, macrophages, and T cells by interaction with them via P-selectin/PSGL-1 or CD40/CD40L pathways (Xu et al. 2006; Kirk et al. 2009).

Last, endothelial cells are also able to induce allogeneic $CD4^+$ and $CD8^+$ T-cell activation and proliferation, favoring IFN-γ and IL-2 secretion and leading to graft rejection (Epperson and Pober 1994). In a recent review, Taflin et al. (2011, 2012) described how in the context of inflammation, such as observed in transplantation, endothelial cells are activated by proinflammatory cytokines and TLR ligands. This activation leads to memory $CD4^+$ T-cell proliferation and Th1/Th17 expansion by endothelial cell secretion of proinflammatory cytokines and chemokines (such as CXCL10, IL-6, and IL-1α) and overexpression of costimulatory molecules (such as LFA-3 or OX-40L) or adhesion molecules (such as ICAM-1) (Griffin et al. 2012). In humans, three nonclassical MHC class I antigens exist: HLA-E, HLA-F, and HLA-G. Coupel et al. (2007) showed that HLA-E is expressed only by leukocytes, such as NK cells, B and T lymphocytes, macrophages, and by endothelial cells. Moreover, they show that endothelial cells up-regulate HLA-E in inflammatory conditions, and endothelial cells produce a soluble form of HLA-E. Coupel's group additionally showed that increased expression of the membrane-bound form of HLA-E protected IFN-γ-activated endothelial cells from CD94/NKG2A-mediated cytolysis, whereas the soluble form of HLA-E protected other cell types. Endothelial cells expressed the MHC class-I related chain A, MICA, which is a ligand for NKG2D. NK cells and cytotoxic T cells express this activating receptor. Under inflammatory stimulation by TNF-α, human endothelial cells up-regulate the expression of MICA (Lin et al. 2012). These results are consistent with previous data suggesting that MICA is expressed by kidney macrovascular endothelial cells in kidney transplant recipients (Sumitran-Holgersson et al. 2002). Moreover, this study showed that MICA acts as an antibody target, which confirmed anti-MICA antibodies are associated with the reduction of graft survival (Zou et al. 2007).

Acute Cellular Response

T-Cell Activation and Effector T Cells

Effector T cells can mediate cell lysis either via cytokine or chemokine secretion, which induces necrosis of transplant tissue, or via direct contact with epithelial or endothelial cells and various mechanisms of cytotoxicity. However, T cells can also stimulate B cells to initiate humoral rejection, or other cells such as macrophages or neutrophils, whose role in transplant rejection is described above.

On activation by DCs, naïve alloreactive $CD4^+$ T cells can differentiate into T helper cells, including Th1, Th17, Th2, or into regulatory T cells (Tregs). In a proinflammatory environment, naïve $CD4^+$ T cells differentiate mainly

into Th1 and Th17 cells. Th1 cells produce IFN-γ and IL-2 and are involved in cytotoxic T lymphocyte (CTL) priming, stimulation of the humoral response, and activation of other cell types such as NK cells. IL-17 produced by Th17 cells stimulates the production of inflammatory cytokines and chemokines leading to the recruitment of neutrophils and macrophages to the graft. In a Th1-depleted environment, Th17 cells (Yuan et al. 2008) or Th2 cells (Barbara et al. 2000) can efficiently promote graft rejection. Th2 cells release anti-inflammatory cytokines such as IL-10 and IL-4 T follicular helper cells and Th9 helper cells have not yet been described in detail, but they have been shown to induce B-cell maturation and mast cells recruitment, respectively.

In contrast, $CD4^+$ Tregs have been shown to be involved in the tolerance process and to prevent graft rejection in mice (Kingsley et al. 2002). These cells are able to suppress $CD4^+$ and $CD8^+$ effector T cells and can also target APCs, decreasing their capacity for antigen-presentation and costimulation (Shevach 2009). A recent study performed using humanized mice showed the superiority of donor alloantigen-specific $CD4^+$ Tregs over their polyclonal counterparts to suppress alloimmune responses (Sagoo et al. 2011). In tolerant liver transplant patients, an increase in peripheral blood $CD4^+$ Tregs has been observed by several groups (Li et al. 2004; Martinez-Llordella et al. 2007; Pons et al. 2008; Nafady-Hego et al. 2010). Although this Treg signature does not seem to be present in the peripheral blood of kidney transplant patients, an increase in intragraft Tregs was detected in such patients (Bestard et al. 2007).

$CD8^+$ T cells can differentiate into CTLs, which themselves can be classified as Tc1, Tc2, or the more recently described Tc17 (Yuan et al. 2009). Although, naïve $CD8^+$ cells can only be activated by DCs, $CD4^+$ T cells have been shown to facilitate $CD8^+$ T-cell differentiation either by cell-to-cell contact or by secretion of IL-2/IFN-γ cytokines. In the latter case, both APCs and $CD4^+$ helper T cells have been shown to be involved in CTL differentiation (Ridge et al. 1998). CTLs subsequently migrate to the graft where they recognize target cells via their allogeneic MHC class I molecules. Killing by CTLs is mediated mainly by the secretion of perforin and granzyme B, or by the Fas/FasL pathway. These two pathways induce target cell apoptosis. CTLs are also able to secrete soluble mediators such as TNF-α. In terms of the dynamics of CTL action, in a model of skin graft rejection, an accumulation of CTLs surrounding the graft was detected, followed by an early phase of killing in small areas of the dermis/epidermis junction. The CTLs were shown to disseminate to the whole graft and induce tissue destruction (Celli et al. 2011).

Compared to their naïve counterparts, memory T cells induce a faster and more effective immune response with lower antigen stimulation (Rogers et al. 2000; Veiga-Fernandes et al. 2000). This is supported by data in humans showing that the pretransplant frequency of donor-specific memory cells correlates with the posttransplant risk of developing acute rejection episodes (Heeger et al. 1999). These results were confirmed in liver transplant patients in whom the presence of a high number of $CD8^+$ cells with a memory phenotype before transplantation was associated with reduced graft survival (Tanaka et al. 2006). More recently, in a model of kidney allotransplantation in nonhuman primates, Nadazdin et al. (2011) showed that the presence of memory T cells before transplantation was a barrier to transplantation tolerance.

Despite $CD8^+$ Tregs being of considerable interest in transplantation, their subsets and mechanisms of action are less understood in comparison to their $CD4^+$ Treg counterparts (Guillonneau et al. 2010). Understanding the function and mechanisms of action of the different $CD8^+$ Treg subsets is essential. In rodent models, several studies have shown the role of $CD8^+$ Treg subsets in the suppression of transplant rejection. For example, donor-antigen-specific $CD8^+CD45^{low}$ Tregs were shown to develop in a fully mismatched heart allograft model in rat on treatment with CD40Ig (Guillonneau et al. 2007). The therapeutic potential of these regulatory cells has also been highlighted in human studies; in liver and cardiac transplant recipients, expansion of $CD8^+CD28^-$ Tregs was shown to correlate with low exposure to immu-

nosuppressive drugs and a decrease in acute and chronic allograft rejection (Guillonneau et al. 2010).

Memory T Cells

The presence of memory T cells able to react with alloantigens was shown in healthy individuals by Lombardi's group in 1990 (Lombardi et al. 1990). Memory T cells showed a faster proliferation, in accordance with observations in vivo during secondary-type immune responses (Akbar et al. 1990). Memory $CD8^+$ T cells act as CTLs as they are able to directly kill target cells, mainly via the granzyme/perforin pathway (Barber et al. 2003).

Two distinct subsets of memory T cells have been identified, and are termed central and effector memory cells (Brook et al. 2006). Central and effector memory cells are $CD45RO^+$ $CD45RA^-$, but differ by their expression of the CD62L/CCR7 markers. Contrary to central memory cells, effector memory cells express more β_1 and β_2 integrins. Moreover, the two subsets differ in their localization because central memory T cells express intermediate levels of CCR4 and CCR6, whereas effector memory T cells express high levels of CCR1, CCR3, and CCR5. Although central memory T cells require restimulation, this subset is more efficient in their killing function (Sallusto et al. 1999; Barber et al. 2003; Hengel et al. 2003).

In sensitized patients, the presence of memory T cells specific for alloantigens can be caused by prior blood transfusion, pregnancy, or transplant rejection. The presence of allospecific memory T cells is more difficult to explain in nonsensitized individuals, but may be the result of heterologous immunity or homeostatic proliferation.

Heterologous immunity is defined as the ability of alloreactive memory T cells specific for a microbial antigen to cross-react with allogeneic MHC molecules through direct allorecognition. This affinity of the T-cell receptor for allogeneic MHC molecules can be higher than that for self-antigens. Heterologous immunity has been shown both in mice and in humans (Smith et al. 2012). Homeostatic proliferation occurs in lymphopenic conditions (such as those caused by certain immunotherapies) and in the absence of an antigenic stimulus. In these conditions, peripheral T cells rapidly proliferate and acquire a memory phenotype that spontaneously skews toward a Th1 phenotype (Moxham et al. 2008). Certain cytokines, such as IL-7, have been shown to mediate this homeostatic proliferation (Schluns et al. 2000).

Memory T cells generated in the context of viral infections or by homeostatic proliferation may prevent tolerance induction, as shown using well-established rodent models of tolerance (Valujskikh et al. 2002; Zhai et al. 2002; Wu et al. 2004). These results show that memory T cells can act as barriers to tolerance induction. It is therefore necessary to develop therapies to prevent memory T-cell generation or to eliminate such cells. The effect of immunosuppressive drugs on memory T cells has started to be studied. So far, it is known that $CD4^+$ memory T cells are resistant to steroids, deoxyspergualin, and sirolimus. In contrast, calcineurin inhibitors tacrolimus and cyclosporine A inhibit their proliferation and in vitro activation (Pearl et al. 2005). Optimal activation of memory T cells requires costimulatory molecules, which are different from those acting on naïve cells, such as ICOS/ICOSL, OX40/OX40L, and CD27/CD70 (Croft 2003; Wu et al. 2004). Consequently, blockade of the CD27/CD70 pathway prolongs the survival of heart transplant in mice (Yamada et al. 2005). Moreover, IL-7 inhibition blocks allograft rejection mediated by memory T cells without affecting Tregs (Wang et al. 2006).

Acute Humoral Response

Although T cells have been considered for many years as playing a predominant role in graft rejection, it is now known that the acute humoral response is the main cause of acute graft loss (Terasaki 2003). In fact, acute humoral rejection accounts for 15% to 20% of graft rejection within the first posttransplant year, despite immunosuppressive therapies (Montgomery et al. 2004; Lucas et al. 2011).

The criteria for the diagnosis of acute humoral rejection of kidney transplants were ini-

tially established by the Banff working group in 2003 (Racusen et al. 2003). At least one of the following criteria must be present for a diagnosis of acute humoral rejection to be made: (1) morphological criteria: presence of neutrophils and/or monocytes/macrophages in peritubular capillaries (PTC) and/or glomeruli, fibrinous arterial necrosis, thrombosis in the glomerular capillaries, arterioles, and/or small arteries and acute tubular damage; (2) immunohistological criteria: C4d deposits in PTC, presence of immunoglobulins, or complement in the fibrinous necrosis of the arteries; (3) serological criteria: presence of circulating anti-donor (HLA and non-HLA) antibodies called donor-specific antibodies (DSA).

These criteria were optimized during the 9th Banff conference by including the quantification of C4d deposition (Solez et al. 2008).

Generally, detection of DSA and C4d deposits is associated with an increase in AMR and an inferior graft outcome (Kedainis et al. 2009). Nevertheless, although the presence of C4d deposits in kidney biopsies appears to be a good marker of AMR, during the last Banff meeting it was agreed that AMR can also be diagnosed in the absence of C4d detection (Mengel et al. 2012).

The new objectives of the Banff committee are therefore to characterize AMR by defining: (1) thresholds for microvascular injury and for DSA in AMR, noting that all injuries are specific and all patients with DSA develop AMR, (2) C4d-negative AMR before adding this category to the Banff classification, (3) acute versus chronic AMR, and (4) the significance of intimal arteritis in the absence of necrosis as a histological criteria (Mengel et al. 2012).

B Cells

The importance of B cells in allograft rejection was shown using B-cell-deficient mice. Several groups, including Brandle and coworkers, as well as Wasowska and colleagues showed that acute allograft rejection was delayed in B-cell-deficient mice and could be restored by the administration of hyperimmune sera (Brandle et al. 1998; Wasowska et al. 2001). In humans, the primary role of B cells as amplifiers of the alloresponse is suggested by the positive reports of B-cell depletion therapy (rituximab) as induction for ABO-incompatible renal transplant recipients (Tyden et al. 2009). However, rituximab-induction therapy was reported to lead to acute cellular rejection in some kidney transplant recipients (Clatworthy et al. 2009). It is therefore thought that B-cell depletion may be quicker at depleting the B cells that contribute to tolerance than those that promote rejection, thereby temporarily amplifying the alloresponse.

The ability of B cells to present antigen and to produce antibodies and cytokines means that these cells can take part in allograft rejection by different mechanisms. B cells have the ability to promote T-cell activation/differentiation through costimulatory pathways, or cytokine release (Constant 1999; Zarkhin et al. 2010). B cells have also been shown to promote alloreactive T-cell differentiation into memory T cells (Ng et al. 2010). Moreover, a deficiency in B-cell antigen presentation can prolong graft survival (Noorchashm et al. 2006).

More recently, several groups suggested that B cells might be involved in tolerance. For example, Deng and coworkers showed that anti-CD45RB-induced tolerance to heart transplants requires the presence of B cells (Deng et al. 2007). Furthermore, resting B cells expressing donor antigen indefinitely prolonged heterotopic heart transplant survival (Niimi et al. 1998). In rats, long-term allograft tolerance is characterized by an accumulation of B cells expressing genes such as BANK-1, which are associated with tolerance (Le Texier et al. 2011). In addition, anti-CD20-mediated depletion of B cells in mice was shown to accelerate skin graft rejection (DiLillo et al. 2011). A specific population of B cells expressing TIM-1 was identified as having regulatory properties in mouse models of islet transplantation (Ding et al. 2011). Furthermore, a B-cell signature was described in immunosuppressant-free transplant patients who were spontaneously tolerant to their HLA mismatched kidney transplant (Newell et al. 2010; Pallier et al. 2010; Sagoo et al. 2010; Silva et al. 2012). In contrast, spontaneously tolerant liver trans-

plant recipients were reported to display an NK cell signature (Martinez-Llordella et al. 2008). These results were confirmed by the analysis of PBMC from kidney and liver transplant patients by the same laboratory, showing that the B-cell signature was only detectable in kidney in immunosuppressant-free kidney transplant patients (Lozano et al. 2011).

Antibodies

Halloran's group showed the role of alloantibodies in acute humoral rejection in the 1990s. They described a correlation between the prognosis of acute rejection and the production of antidonor antibodies after transplantation (Halloran et al. 1990). In presensitized patients, the presence of DSA at the time of transplantation was identified as a high risk factor for AMR and patients who develop anti-HLA DSA tend to have inferior long-term graft survival compared to those that do not (Lefaucheur et al. 2008, 2010). In support of these results, a recent study by Cooper et al. (2011) also showed that patients who develop de novo DSA after transplantation have an inferior graft outcome.

Alloantibodies that develop against the donor organ can recognize several types of antigens (for review, see Dragun 2008): HLA antigens class I and II (Terasaki and Ozawa 2004; Terasaki and Cai 2005), MICA and MICB antigens (MHC class I-related molecules A and B) (Zou et al. 2007; Li et al. 2010), minor histocompatibility antigens and non-HLA antigens including the angiotensin II type 1 receptor (Dragun et al. 2005), vimentin (Mahesh et al. 2007), myosin, the ABO blood group antigens (Montgomery et al. 2012), perlecan, type IV and VI collagen, agrin, unknown endothelial antigens (Jackson et al. 2011b), and ICAM-1 (Lawson et al. 2005).

Alloantibodies mainly induce AMR by complement-dependent mechanisms. In fact, the alloantibodies that fix C1q complement are associated with a worse outcome that does not fix complement (Yabu et al. 2011). A greater risk of acute humoral rejection was clearly shown when C4d deposits were associated with DSA and, more specifically, anti-HLA class I but not anti-HLA class II antibodies (Cosio et al. 2010). However, in the absence of C4d staining, association of DSA with altered expression of endothelial genes was also found to be a marker of AMR in kidney patients, as the alloantibodies modified the microcirculation (Sis et al. 2009). NK cells and macrophages were also involved in AMR in patients with DSA. On stimulation by alloantibodies, NK cells cause endothelial injury and act as effector cells by killing target cells through the antibody-dependent cellular cytotoxicity pathway (Hidalgo et al. 2010). The role of NK cells is particularly well-defined as NKG2D is a receptor for stress-inducible MICA and, as described previously, this molecule is also highly efficient at stimulating NK cells and certain T-cell subsets (Bauer et al. 1999).

Several desensitizing protocols have been tested to reduce the DSA that cause AMR, including plasmapheresis or IVIg, as well as treatments targeting complement C5 molecules, proteasome, or $CD20^{+}$ cells (Gueler et al. 2008; Raedler et al. 2011; Yoo et al. 2012). A combination of these treatments was shown to improve graft survival outcome (Lefaucheur et al. 2009; Montgomery et al. 2011).

CHRONIC REJECTION

The Origins and Mechanisms of Chronic Rejection

Since the early 1980s, 1-year graft survival of kidney allografts has increased significantly, reaching more than 90% (Hariharan et al. 2000; Pascual et al. 2002). Nevertheless, the graft survival in the long term has changed minimally, and the percentage of grafts lost annually after the first year has not changed (Meier-Kriesche et al. 2004). Death with a functioning graft and chronic allograft nephropathy (CAN) are the main causes of graft loss (Halloran et al. 1999; Ojo et al. 2000; Matas et al. 2002; Pascual et al. 2002). The predominance of CAN is 60%–70% in the first year posttransplant (Solez et al. 1998; Nankivell et al. 2003). CAN is an entity that encompasses several different mechanisms that include interstitial fibrosis and tubular atrophy. The natural history of CAN suggests that it can result from both immunological and nonimmu-

nological phenomena, including calcineurin inhibitors (CNI) toxicity (Nankivell et al. 2003). Chronic dysfunction is also observed in other organ transplants and is often responsible for premature graft loss.

In recent years, significant efforts have been made to describe and classify CAN. In 1990, analyses based in histological studies were performed in the field of kidney transplantation, resulting in the description of a classification system for chronic allograft dysfunction (Classification "Banff 97") (Racusen et al. 1999). This classification is revisited regularly to incorporate the latest scientific data and to try to differentiate lesions associated with chronic rejection (CR) from those observed in response to cardiovascular risk factors or CNI toxicity. The 2005 Banff meeting differentiated the lesions suggestive of chronic allograft rejection (including antibody-dependent complement activation lesions) and cell arteritis from the less specific lesions of interstitial fibrosis/tubular atrophy (IF/TA) (Solez et al. 2007, 2008). IF/TA lesions can appear very early after transplantation. At 1 year posttransplant, >80% of kidneys have minimal lesions of IF/TA that will deteriorate over time, reaching >50% of kidneys with severe lesions at 5 years. The 2011 Banff meeting focused on refining the diagnostic criteria for AMR. As such, C4d-negative AMR was recognized, in which NK cells and endothelial activation were proposed to play a role (Mengel et al. 2012).

The Histological Lesions

Significant progress has been made in understanding the mechanisms involved in the onset of CR, with the description of arteritis lesions in animal models (Yuan et al. 2002). One predominant observation made during chronic allograft rejection is an increase in the thickness of the intima, resulting in a decrease in vessel caliber with destruction of the internal elastic lamina. This thickening is also because of an accumulation of extracellular matrix and proliferation of myofibroblasts (Pedagogos et al. 1997; Pilmore et al. 2000; Ramirez et al. 2006). An accumulation of macrophages and $CD4^+$ T cells has been observed at the periphery of the vessels (Thaunat and Nicoletti 2008; Thaunat et al. 2005, 2006, 2008), whereas $CD8^+$ T cells are rarely present. The involvement of T cells in the pathogenesis of CR has been investigated through the study of genetically invalidated mice. In a mouse model of cardiac allotransplantation in which the genes encoding CD40 or CD40L were invalidated, CR was not observed, suggesting that activated T lymphocytes are required to initiate the phenomenon of CR. Nevertheless, in a rat model of cardiac allotransplantation in which animals were treated with CD40Ig (a molecule interfering with CD40-CD40L interaction), acute rejection was successfully inhibited, but CR occurred in long-term-surviving animals (Guillot et al. 2002). In this same model, the development of CR was prevented by simultaneous blockade of other costimulation pathways, such as RANK and ICOS (Guillonneau et al. 2004, 2005).

In some cases, the graft infiltrate has been found to adopt an organization resembling that of ectopic lymphoid tissues, with the infiltrating B cells capable of producing antidonor antibodies. These lesions of tertiary lymphoid tissue have been identified in kidney transplants, and the process is referred to as lymphoid neogenesis, which is analogous to the ontogenic program triggered during embryo development, the ectopic germinal centers (eGCs) (Thaunat et al. 2010; Cheng et al. 2011). The eGCs participate in the mechanisms of CR maintaining local memory T and B cells capable of synthesizing cytokines and antibodies. Interestingly, there is minimal overlap between the intragraft humoral response, suggesting a local antibody repertoire. Recently, Thaunat and coworkers showed that a breakdown of B-cell tolerance occurs within the graft during CR (Thaunat et al. 2012). It is thought that the micrograft environment interferes with peripheral deletion of autoreactive immature B cells that, in turn, produce autoantibodies. Recent studies have shown an important role for autoimmunity in the pathogenesis of CR suggesting a cross talk between the alloimmune response and autoimmunity (Sarma et al. 2012). Although all types of Th polarization profiles can lead to terminal CR, Thaunat and coworkers (Deteix et al. 2010) reported on a correlation between shorter graft survival and the

presence of Th17 cells that produce IL-17 and IL-21 in human transplant patients. The latter group hypothesized that IL-21 promotes intragraft lymphoid neogenesis, which in turn supports the development of a local humoral immune response. Moreover, Burlingham et al. (Burlingham et al. 2007; Keller and Burlingham 2011) showed that the alloimmune response is partly responsible for the development of a Th17 autoimmune response. In this context, Fukami et al. (2009) showed that an anti-IL-17 therapy decreases the production of autoantibodies and the development of CR in a murine model.

The Role of Antibodies and Endothelial Cells

The involvement of antibodies directed against the graft in chronic rejection has been suggested by several groups showing a negative correlation between the appearance of antidonor antibodies and graft survival (Mao et al. 2007a,b; Sis et al. 2007). The emergence of new techniques to identify antibodies by fluorescence-activated cell sorting, flow cytometry, ELISA (enzyme-linked immunosorbent assay), and the most sensitive Luminex technique (flow cytometry with microbeads coated with peptides) now enables a better analysis of the humoral component (Pei et al. 2003). Moreover, recent data showing the existence of deposits during CR suggest the involvement of anti-HLA antibodies with the ability to activate complement (Nickeleit et al. 2002; Regele et al. 2002). However, observations from ABO-incompatible transplants, a context in which complement activation occurs, suggest the existence of adaptation mechanisms, allowing endothelial cells to resist complement activation (Gloor et al. 2006).

However, the effects of antibodies are diverse as described in Figure 2, and not limited to complement activation. Antibodies can also bind to the surface molecules of target cells and recruit other cells by interactions via their constant domain (Rebellato et al. 2006; Won et al. 2006). Recently, Hirohashi et al. described a new role for NK cells in chronic allograft rejection in which donor-specific antibodies interact with NK cells in the absence of complement and trigger transplant vasculopathy (Hirohashi et al. 2012; Li and Baldwin 2012). Last, there is some in vitro data showing that the culture of endothelial cells with antidonor antibodies results in

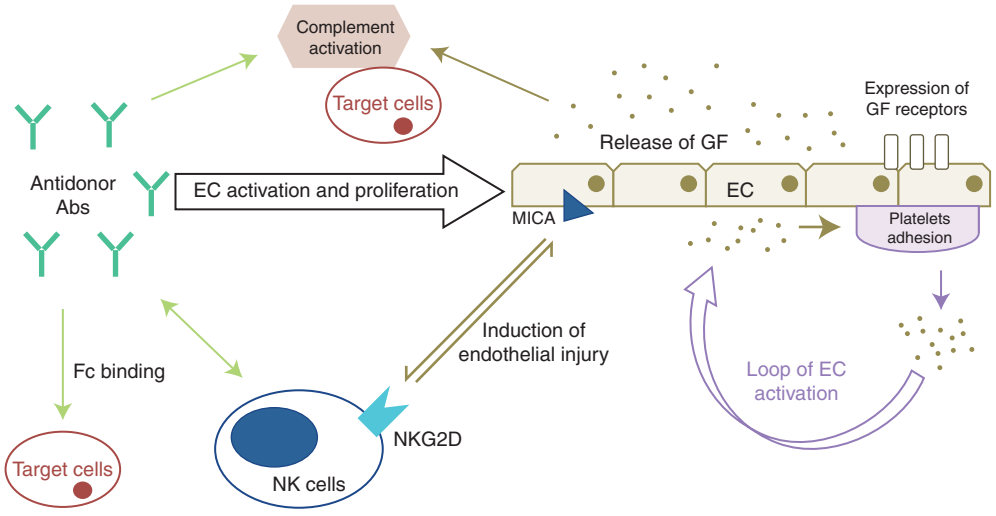

Figure 2. Induction of endothelial cells activation by antidonor antibodies. Antidonor antibodies (Abs) are known to induce chronic allograft rejection by several mechanisms of action involving their constant domain (Fc) or their capacity to induce activation of complement. Antidonor Abs also induce endothelial cell activation. Activated ECs secrete notably growth factors (GF) that induce the recruitment and activation/proliferation of several cells implicated in allograft rejection.

the activation and proliferation of endothelial cells (Bian and Reed 1999).

This activation step of endothelial cells is associated with the expression of various receptors on their cell surface (PDGF-R, EGF-R, FGF-R), as well as the synthesis of numerous growth factors (PDGF, EGF, FGF, VEGF, TGF-β, etc.) and synthesis of endothelin I (Bian and Reed 2001; Chen et al. 2001; Rossini et al. 2005). The local presence of growth factor is increased by the platelet adhesion that occurs during endothelial-cell activation, resulting in the generation of a local amplification loop with the release of many growth factors such as PDGF and TGF-β (MacDermott 1996; Yang et al. 2005). This phase of endothelial-cell activation promotes the stimulation of smooth muscle cells via release of endothelin I, and indirectly via the local synthesis of angiotensin II. It also causes local recruitment of inflammatory cells through the release of chemokines (MCP-1, IP-10), local activation of complement, local coagulation activation, promoting platelet adhesion, and releasing thromboxane A2. Finally, endothelial-cell activation stimulates the differentiation and proliferation of myofibroblast cells that synthesize the extracellular matrix involved in CR (Abbate et al. 2002; Cogan et al. 2002; Dewald et al. 2005; Li et al. 2007; Frangogiannis 2008; Haurani et al. 2008; Kennard et al. 2008; Wynn 2008). Myofibroblastic cells are important constituents of CR as they infiltrate the vessel wall and interstitial space. These cells express different cytoskeletal proteins (vimentin, α smooth muscle actin) as well as the myosin light chain, but do not express markers of lymphoid or epithelial cells (E-cadherin, ZO-1) (Badid et al. 2002). The origin of these cells is diverse. They originate from circulating stem cells capable of differentiating into endothelial cells or myocardiocytes (Direkze et al. 2003; Li et al. 2007). They can also originate from the transdifferentiation of endothelial cells or from renal tubular epithelial cells that transdifferentiate into myofibroblasts (Gressner 1996; Sommer et al. 2005; Hertig et al. 2006). The mechanisms involved remain poorly understood. However, the changes induced by ischemia/reperfusion or during CR appear to promote epithelial-mesenchymal transdifferentiation. TGF-β was also shown to be important for the promotion of epithelial-mesenchymal transdifferentiation (Fan et al. 1999; Mezzano et al. 2003; Lindert et al. 2005; Jiang et al. 2006; Meyer-ter-Vehn et al. 2006). Cell differentiation into myofibroblasts and expansion involves various growth factors including TGF-β, FGF, PDGF, IGF-1, angiotensin II, MCP-1, RANTES, TNF-α, IL-15, and connective tissue growth factor.

The Antigenic Targets of Antigens (Antidonor and Autoantibodies)

One of the independent risk factors for the development of chronic rejection is the presence of anti-HLA class I and especially class II antibodies (Ozawa et al. 2007). More than 80% of patients with transplant glomerulopathy have anti-HLA antibodies, 85% being directed against class I or class II antigens (Gloor et al. 2007; Sis et al. 2007; Issa et al. 2008). Less than half of biopsies (40%) display deposits of C4d, suggesting that mechanisms other than complement activation may be associated with CR (Solez et al. 2008). Apart from antibodies directed against the major histocompatibility complex HLA, other non-HLA antibodies may also contribute to the structural changes observed during chronic rejection. These include anti-MICA, anti-MICB (MHC class I-related molecules A and B), antiendothelial cells, antivimentin, and other autoantibodies directed against angiotensin II receptor (Dragun et al. 2005, 2008; Hilbrands et al. 2005; Zafar et al. 2006; Panigrahi et al. 2007; Zou et al. 2007; Baid-Agrawal and Frei 2008; Kamoun and Grossman 2008; Sumitran-Holgersson 2008).

Tissue Fibrosis

During the various immunological and nonimmunological rejection processes, several cell types, particularly endothelial cells, are responsible for the development of an extracellular matrix through the release of growth factors or cytokines such as endothelin I, angiotensin II, TNF, PDGF, TGF, etc. (Coupes et al. 1994; Lu et al. 2002; el-Agroudy et al. 2003; Baczkowska

et al. 2005; Summers et al. 2005; Roos-van Groningen et al. 2006). Extracellular matrix accumulation is a lesion of fibrosis. In recent years, it has become apparent that tissue fibrosis is a dynamic equilibrium. Activation of different tissue protease, such as metalloproteinases, or the use of blocking molecule should prevent fibrosis and degrade extracellular matrix. These approaches must be validated in clinical models.

In conclusion, although significant progress has been made in developing immunosuppressive therapies that reduce the occurrence of acute rejection and improve allograft survival at 1 year, little progress has been made regarding graft survival in the longer term. Long-term survival is limited by complex and intricate mechanisms including chronic rejection, immunosuppressive drug toxicity, cardiovascular factors, dyslipidemia, diabetes, and so on. Understanding the mechanisms involved in the onset of chronic rejection should be promoted by approaches such as DNA chips or protein chips to better understand the different stages of development of chronic rejection and the different factors involved. This understanding should help develop new preventive and/or therapeutic approaches in organ transplantation.

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Effector Mechanisms of Rejection

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