The Biology and Molecular Basis of Organ Transplant Rejection

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Contents

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Abstract

Allograft rejection is defined as tissue injury in a transplanted allogeneic organ produced by the effector mechanisms of the adaptive alloimmune response. Effector T lymphocytes and IgG alloantibodies cause two different types of rejection that can occur either individually or simultaneously: T cell-mediated rejection (TCMR) and antibody-mediated rejection (ABMR). In TCMR, cognate effector T cells infiltrate the graft and orchestrate an interstitial inflammatory response in the kidney interstitium in which effector T cells engage antigen-presenting myeloid cells, activating the T cells, antigen-presenting cells, and macrophages. The result is intense expression of IFNG and IFNG-induced molecules, expression of effector T cell molecules and macrophage molecules and checkpoints, and deterioration of parenchymal function. The diagnostic lesions of TCMR follow, i.e. interstitial inflammation, parenchymal deterioration, and intimal arteritis. In ABMR, HLA IgG alloantibodies produced by plasma cells bind to the donor antigens on graft microcirculation, leading to complement activation, margination, and activation of NK cells and neutrophils and monocytes, and endothelial injury, sometimes with intimal arteritis. TCMR becomes infrequent after 5–10 years post-transplant, probably reflecting adaptive mechanisms such as checkpoints, but ABMR can present even decades post-transplant. Some rejection is triggered by inadequate immunosuppression and non-adherence, challenging the clinician to target effective immunosuppression even decades post-transplant.

Keywords

Alloimmune response · Antibody-mediated rejection · Donor-specific antibody · Organ transplantation · Rejection · T cell-mediated rejection

1 Introduction and Overview

This chapter focuses on organ transplant (allograft) rejection, with a particular focus on kidney and heart transplants, but we will also consider the effects of parenchymal injury. T cells with alpha-beta receptors (TCRs) recognizing major histocompatibility complex (MHC – human, HLA) proteins are essential for all graft rejection: animals with no thymus and no T cells cannot reject organ allografts. For reviews, see Halloran (2004); Halloran et al. (2016a). Aspects of the rejection process have been covered in previous reviews (Einecke and Halloran 2007).

Much new information about rejection included here has been generated in the development of the Molecular Microscope Diagnostic System (MMDx) (Halloran

et al. 2018) for organ transplants biopsies, including kidney (Reeve et al. 2017), heart (Parkes et al. 2019), lung transbronchial biopsies (Halloran et al. 2019), lung mucosal biopsies (Halloran et al. 2020), and liver biopsies (Madill-Thomsen et al. 2020).

Much of what we know about rejection comes from kidney transplant studies because the core biopsies are abundant and more easily read than the more challenging heart and lung biopsies. This chapter will often refer to kidney studies but most lessons are generalizable to other organ transplants.

It is necessary to understand tissue injury, which is universal in donation and implantation of organ transplants and may help activate antigen presentation and adaptive immune responses. Nonimmune and immune injury is additive. However, injury is probably not necessary for activating the immune response: some "ticking over" of the antigen presentation system may always be occurring.

1.1 Structure and Function of the Immune System and Some Molecules to Know

We cannot cover the molecular biology of all elements of adaptive and innate immunity (inflammation) in this chapter, and the reader is encouraged to have some familiarity with the development of the adaptive immune system; the lymphoid organs; the key antigen recognition molecules – T cell receptors, B cell receptors and immunoglobulins; the major histocompatibility complex proteins (in humans, the HLA complex), the cytokines and the chemokines; and the mechanisms of inflammation.

T cells are generated in the thymus from marrow precursors, rearranging their TCR genes, expressing TCRs, and undergoing positive and negative selection. B cells arise from marrow precursors in the bone marrow, rearranging their immunoglobulin light and heavy chain genes, and undergo negative selection. Mature T and B cells then populate the secondary lymphoid organs.

Organ transplantation between genetically non-identical humans leads to the activation of a large number of alloreactive clones of T and B lymphocytes that specifically recognize the mismatched donor alloantigens and can generate antigen-specific effector functions leading to the destruction of the transplant. Alloantigens are antigenic differences between individuals controlled by polymorphic gene differences. MHC alloantigens are of two classes – class I (specialized to engage CD8 T cells) and class II – specialized to engage CD4 T cells. All contain peptides in their groove between alpha-helices. Each can be "seen" by T cells in three ways: as intact donor molecules (direct) recognition); as peptides in host MHC molecules (indirect); or as intact donor molecules on islands on the membrane from donor cells that have been acquired by host cells (semi-direct"). Thus MHC alloantigens are peptide-MHC complexes that present non-self features, due to either non-self (donor) amino acid sequences in the MHC protein itself, non-self donor MHC peptides in MHC grooves, or both. B cell alloantigens (that will generate alloantibodies) are intact non-self MHC proteins; the peptide is usually not relevant.

The two cognate (i.e., antigen-specific) effector systems of the adaptive immune response generated during the alloimmune response are the effector T lymphocytes, which cause T cell-mediated rejection (TCMR) and IgG alloantibodies, which cause antibody-mediated rejection (ABMR). Under usual immunosuppressive regimens (Halloran 2004), clinical rejection episodes take characteristic TCMR and ABMR and mixed forms and are diagnosed by biopsies read by histology and molecular platforms. The possibility of NK cell recognition of "missing self" must also be considered within the syndrome of ABMR phenotypes (Callemeyn et al. 2021).

2 Three-Signal Model of the Alloimmune T Cell Response in the SLO

Alloimmune responses are initiated by activation of antigen-presenting cells (APCs) through innate immune recognition systems (Fig. 1). In the graft and surrounding tissues, dendritic cells of donor and host origin become activated and move to T cell areas of secondary lymphoid organs (SLO).

In the SLO, antigen-bearing dendritic cells engage alloantigen-reactive naive T cells and memory T cells (Fig. 1). While naive T cells are optimally triggered by dendritic cells in SLO (Lakkis et al. 2000; Zhou et al. 2003), previously stimulated or "antigen-experienced" memory cells may be activated by other cell types, such as graft endothelium (Biedermann and Pober 1998). This is an issue in clinical transplantation since human adults have large numbers of memory T cells activated previously by viral antigens that cross-react with alloantigens (Adams et al. 2003a) (heterologous memory (Adams et al. 2003b)). Some estimates indicate that many of the antigen-specific T cells reacting with donor antigens are memory T cells, not naïve T cells (Lombardi et al. 1990).

An antigen on the surface of dendritic cells that triggers T cells with cognate T cell receptors constitutes "signal 1," transduced through the CD3 complex. Dendritic cells provide costimulation, or "signal 2," delivered when CD80 and CD86 on the surface of dendritic cells engage CD28 on T cells. Memory T cells have less requirement for costimulation.

Signals 1 and 2 activate three signal-transduction pathways: the calcium–calcineurin pathway, the RAS–mitogen-activated protein (MAP) kinase pathway, and the nuclear factor-B pathway (Wang et al. 2004). These pathways activate transcription factors that trigger the expression of many new molecules, including interleukin-2, CD154, and CD25. Interleukin-2 and other cytokines (e.g., interleukin-15) activate the "target of rapamycin" pathway to provide "signal 3," the trigger for cell proliferation. Proliferation and differentiation lead to a large number of effector T cells.

B cells are activated when antigen engages their antigen receptors, usually in lymphoid follicles or in extrafollicular sites, such as red pulp of spleen (MacLennan et al. 2003), or possibly in the transplant (Sarwal et al. 2003), producing alloantibody against donor HLA antigens. However, follicular helper T cells are essential to the

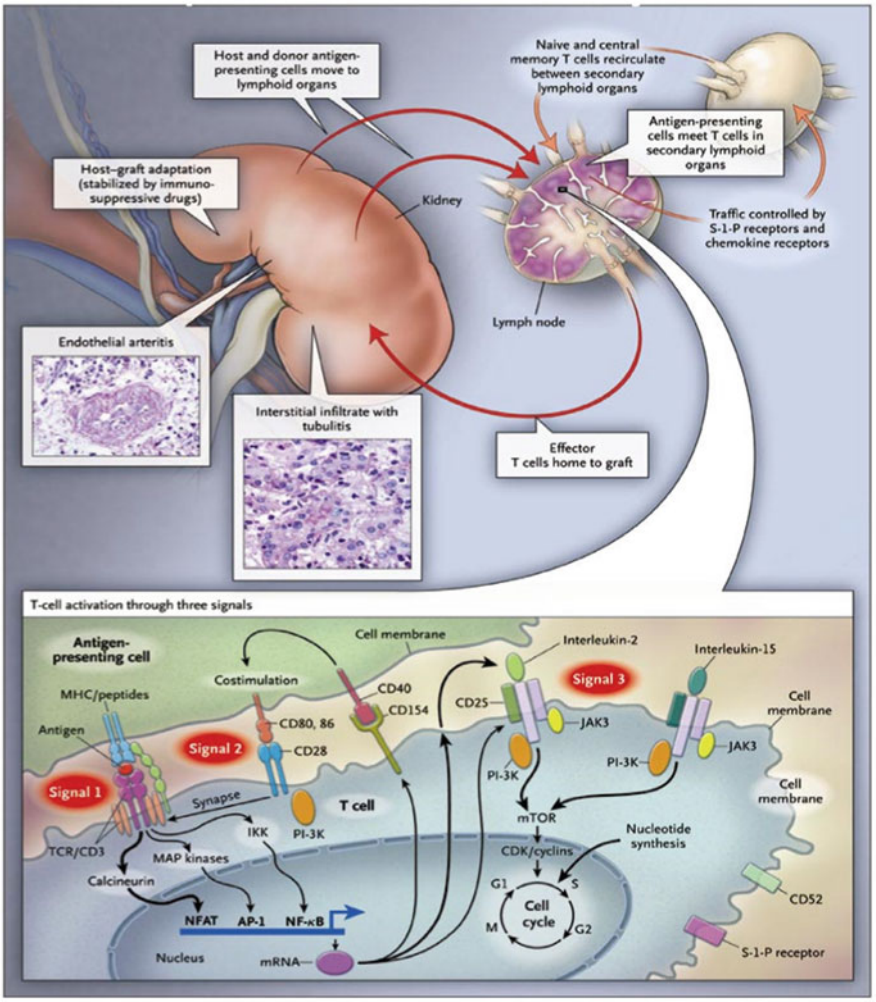

Fig. 1 Steps in T cell-mediated rejection. Antigen-presenting cells (APCs) of host or donor origin migrate to secondary lymphoid organs. APCs present donor antigen to naive and central memory T cells. These T cells ordinarily circulate between lymphoid tissues, regulated by chemokine and sphingosine-1-phosphate (S-1-P) receptors (Mandala et al. 2002). T cells are activated and undergo clonal expansion and differentiation to express effector functions. Antigen triggers T-cell receptors (TCRs) (signal 1) and synapse formation. CD80 (B7-1) and CD86 (B7-2) on the APC engage CD28 on the T cell to provide signal 2. These signals activate three signal-transduction pathways – the calcium–calcineurin pathway, the mitogen-activated protein (MAP) kinase pathway, and the protein kinase C–nuclear factor-B (NF-B) pathway – which activate transcription factors nuclear factor of activated T cells (NFAT), activating protein 1 (AP-1), and NF-B, respectively. The result is expression of CD154 (which further activates APCs), interleukin-2 receptor chain (CD25), and interleukin-2. Receptors for a number of cytokines (interleukin-2, 4, 7, 15, and 21) share the common chain, which binds Janus kinase 3 (JAK3). Interleukin-2 and interleukin-15 deliver growth signals (signal 3) through the phosphoinositide-3-kinase (PI-3 K) pathway and the molecular-target-of-rapamycin (mTOR) pathway, which initiates the cell cycle. Antigen-experienced T cells home to and infiltrate the graft and engage the parenchyma to create typical rejection lesions such as tubulitis and, in more advanced rejection, endothelial arteritis. However, if the rejection does not destroy the

generation of effective B cell transformation into mature plasma cells producing high-affinity IgG that can engage donor endothelium and produce ABMR.

Thus, within days the immune response generates the effector mechanisms that can damage the organ and mediate allograft rejection, effector T cells, and alloantibody. In naïve recipients the first rejection to appear is TCMR. New TCMR and ABMR responses can be initiated later, especially during periods of under-immunosuppression.

3 Effectors, Lesions, and Molecular Phenotype of Rejection

Rejection is defined as tissue injury produced by the effector mechanisms of the adaptive alloimmune response, leading to deterioration in organ function. Rejection has many dimensions: clinical, immunologic, molecular, and histologic.

There are two types of rejection: T cell-mediated rejection (TCMR) and antibody-mediated rejection (ABMR) (Fig. 2). TCMR, ABMR, and mixed rejection can be early or late, fulminant and rapid, or relatively indolent and slow. Increasingly new dimensions such as microarray or RNA sequencing analysis of genome-wide gene expression are being added.

T cells serve as the main effectors and regulators of the alloimmune response. Macrophages are possible effectors and aid in the removal of apoptotic cells. Theoretically, B cells and plasma cells could contribute to the production of alloantibodies within the graft but they are seen more often in TCMR and are not per se part of the criteria for ABMR. In ABMR, the high-affinity damaging IgG antibodies are probably made in SLO or the marrow.

3.1 T Cell-Mediated Rejection (TCMR)

Most rejection in clinical organ transplantation was previously TCMR, but effective ISDs have made TCMR later and less common, yet still important, e.g. in non-adherence. Cognate effector T cells that emerge from SLO infiltrate the graft and orchestrate an inflammatory response including recruitment of activated macrophages. Cognate effector T cells home to the graft by recognizing alloantigen on dendritic cell processes that emerge through the endothelium and guide the T cells through the capillary endothelium. In the interstitium they are then activated by dendritic cells to create the inflammatory environment that is the fundamental feature of TCMR (Halloran et al. 2016a), recruiting many other inflammatory cells:

Fig. 1 (continued) graft, adaptation occurs and is stabilized by immunosuppressive drugs. The photomicrographs of tubulitis and endothelial arteritis are taken from a mouse model in which these lesions are T cell-dependent but independent of perforin, granzymes, and antibody. IKK denotes inhibitor of nuclear factor-B kinase, CDK cyclin-dependent kinase, and MHC major histocompatibility complex. (Halloran, P. F. N Engl J Med 2004;351:2715–2729)

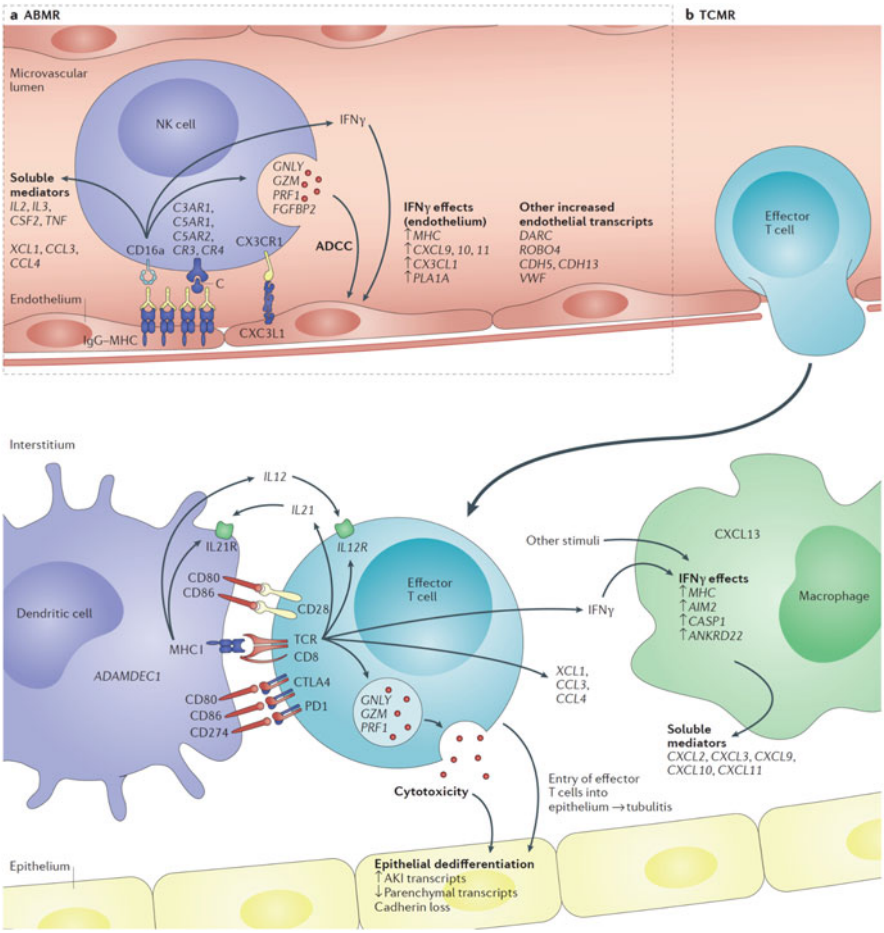

Fig. 2 (**a**) Tubulitis in T cell-mediated rejection (PAS, 40×) (**b**) Endothelialitis in T cell-mediated rejection (PAS, 40×)

non-cognate effector and memory T cells, macrophages, B cells, and plasma cells – the cellular infiltrate observed in TCMR biopsies. (The plasma cells seen in TCMR and damaged tissue generally are probably not fully mature and are not an important source of high-affinity alloantibody, which generally comes from mature bone marrow plasma cells.)

The diagnostic lesions of T cell-mediated rejection reflect mononuclear cells accumulating in the interstitium and in the cases of epithelial organs invading the epithelium, e.g. kidney tubules (tubulitis) and the intima of small arteries (arteritis) (Racusen et al. 1999). In the heart, the parenchyma manifests myocyte injury and necrosis. The molecular hallmark of all rejection is that the graft displays intense IFNG expression and IFNG-induced molecules such as CXCL9, CXCL10, and

CXCL11, accompanied in TCMR by many TCMR-selective transcripts expressed in effector T cells (e.g., IFNG), APCs, and macrophages (e.g., CXCL13 and ADAMDEC1), and checkpoint transcripts such as CTLA4. The parenchyma deteriorates with loss of the transcripts associated with normal function and expression of acute injury transcripts (Halloran et al. 2018; Venner et al. 2014; Venner et al. 2015; Loupy et al. 2017).

The recruitment of the other inflammatory cells into the graft across the microcirculation endothelium – diapedesis – is a result of the expression of chemokines and adhesion molecules by the endothelium of the graft. The steps are: rolling on selectins, engagement of chemokines, tight binding to adhesion molecules, then transendothelial migration. The endothelium of postcapillary venules serves as the entry point of recipient leukocytes from the bloodstream into the allograft. Endothelial cells are activated by proinflammatory cytokines and injury to express adhesion molecules and chemokines necessary for transendothelial migration. The recruitment of leukocytes is initiated by the release of chemokines by tubular cells, interstitial cells, endothelial cells, and infiltrating recipient cells within the allograft. T cells expressing the respective chemokine receptors extravasate through the endothelium and are guided by a chemokine gradient within the graft. The binding of chemokines to their receptors induces a conformational change in integrins, which are normally present on circulating leukocytes in an inactive state. Tight adhesion occurs when activated integrins bind their ligands on graft cells. The most common integrins present on lymphocytes are LFA-1 that binds ICAM-1 and -2, and VLA-4 that binds VCAM-1. Unfortunately, treating or preventing rejection by blocking adhesion has not been successful, likely due to redundancy among the multiple adhesion molecules and their ligands, and involvement of these mechanisms in many other types of inflammation.

An interesting but unexplained feature of rejection is that antigen-triggered effector T cells cross the donor endothelium without killing the endothelial cells. TCMR can smolder for days or weeks as an interstitial process, yet the graft remains viable. This could be related to T cell exhaustion.

3.1.1 Tissue Injury in TCMR

The main lesion for diagnosing kidney rejection in the Banff schema is tubulitis. E-cadherin on the basolateral membrane of the tubular epithelium of rejecting grafts may play a role in the development of tubulitis. Invasive lesions correlate with functional deterioration (Solez et al. 1993a, b) and may be relevant to the tubular atrophy that often follows rejection. TCMR can develop in mouse hosts lacking B cells and alloantibody (Jabs et al. 2003), and intense tubulitis is not a characteristic of human ABMR, although tubulitis is non-specific and can occur in ABMR and injury (Trpkov et al. 1996). In hearts, the corresponding lesion is myocyte injury and necrosis.

Alloimmune T cells may mediate parenchymal injury either through direct contact (cytotoxicity) or through contact-independent inflammatory mechanisms analogous to delayed-type hypersensitivity (DTH). Infiltrating effector T cells display many cytotoxic molecules: enzymes in their granules – perforin (Prf1),

granzymes A and B (GzmA/B), and granulysin (GNLY) – as well as Fas ligand on their membranes (Robertson et al. 1996; Einecke et al. 2005). CTL could engage or even synapse with epithelial cells via specialized molecules to damage individual epithelial cells via cytotoxic mechanisms. The enzymes from stored granules released into the cytosol of target cells could initiate a cascade of events that leads to apoptosis, and engagement of Fas on target cells by FasL can cause apoptotic death of the target cell. However, TCMR is not dependent on granule-associated CTL mechanisms: it can develop in allografts rejecting in hosts lacking Prf1 or granzyme A (GzmA) and granzyme B (GzmB) (Halloran et al. 2004) or Fas ligand.

It has been suggested that the integrin CD103, by binding its ligand E-cadherin on epithelial cells, may permit CD8 T cells to engage the renal epithelium (Hadley et al. 1999; Robertson et al. 2001) and mediate invasion into tubular cells. However, mice deficient in CD103 develop tubulitis and deterioration similar to wild-type hosts, indicating that CD103 is not critical for TCMR.

The independence of the epithelial deterioration from cytotoxic mechanisms suggests that the interstitial inflammatory cells such as macrophages and effector T cells produce epithelial dedifferentiation by synergy among inflammatory molecules such as IFNG and TNF in a general inflammatory process called "delayed-type hypersensitivity" (DTH). Parenchymal deterioration in DTH is mediated by contact-independent mechanisms. APCs and macrophages are activated by effector T cells participate in TCMR through DTH mechanisms (Bogman et al. 1989), but the injury remains antigen-specific (Rosenberg and Singer 1992). Mechanisms directly altering the epithelium could include the release of soluble effector T cell or macrophage products (cytokines, superfamily members, reactive oxygen species, nitric oxide, eicosanoids, and enzymes). Additional effects may operate by changing the extracellular matrix (e.g., synthesis of hyaluronic acid) or the microcirculation. Tubulitis in kidney transplants may be a relatively late change in the epithelium, reflecting loss of epithelial integrity that permits entry of lymphocytes, which would explain the lack of requirement for cytotoxic mechanisms and the occurrence of tubulitis in atrophic tubules independent of rejection. The conditions for tubulitis may simply be interstitial infiltration and compromised epithelial integrity, and the diagnostic value of tubulitis may be as an indicator of this loss of epithelial integrity.

While our current belief is that TCMR is at least in part an interstitial inflammatory process mediated by effector T cells with cytotoxic activity but via delayed-type hypersensitivity (DTH) mechanism, it is also possible that the T cells sometimes augment this via their cytotoxic mechanisms. The epithelium deteriorates and loses its ability to exclude inflammatory cells, permitting T cells to enter to create tubulitis. PRF1, GZMA, GZMB, GNLY, and FAS-FAS ligand may be supplementary but are not essential in this model.

The intensity of TCMR correlates with the expression of checkpoint molecules (see below), indicating that T cell mechanisms are never activated without activation of inhibitory processes. This is because of the vast power of T cell effector mechanisms to do harm. TCMR also becomes rare as the years go by, suggesting that the clonal T cells eventually become exhausted (Halloran et al. 2015).

TCMR is "treatable" but is still a serious event for an organ transplant because of its ability to directly damage the parenchyma (nephrons, myocytes, etc.). TCMR on current immunosuppressive protocols usually occurs in one of four situations:

1. In the first 3–12 months, often due to failure to sustain target ISD levels or ill-advised attempts to "minimize" ISDs below recommended levels;
2. Following ISD reduction associated with virus infections and other complications, where a TCMR-like process is common. Note that TCMR-like inflammation develops in some virus infections (e.g., polyomavirus infection in kidney transplants) and may be virus-immune or alloimmune (Halloran et al. 2021a);
3. In non-adherent patients;
4. During treatment of cancer with checkpoint inhibitors.

3.2 Antibody-Mediated Rejection

ABMR is now recognized as a major cause of loss of kidney and heart transplants (and possibly lung and liver transplants, although less is known about ABMR in these organs). ABMR is a major target for efforts to reduce transplant failure (Djamali et al. 2014; Einecke et al. 2009; Sellares et al. 2012).

Mechanism of ABMR. ABMR represents the effect of alloantibodies against donor antigens (donor-specific antibodies, DSA) binding to the graft microcirculation, leading to complement activation and margination of neutrophils, monocytes, and NK cells in the glomeruli and peritubular capillaries – glomerulitis and peritubular capillaritis – and endothelial injury (Halloran et al. 1990). The main antigenic targets of ABMR are MHC molecules, both class I and class II.

Alloantibodies against non-MHC proteins could potentially mediate ABMR but this has not been proven. Antibodies directed against self-proteins such as AT1R can be associated with graft injury (Dragun et al. 2005), but their role in ABMR is a matter of debate.

There is increasing recognition that ABMR often occurs in the absence of DSA recognized by current measurement platforms – see below.

3.3 Triggering of Host B Cell Clones with Cognate Receptors for Native Donor HLA Molecules

The B cell response to antigens generates germinal centers in lymph nodes as the B cells, helped by follicular helper T cells, undergo clonal expansion, class switching (IgM to IgG) and affinity maturation, leading to the production of plasma cells, which migrate to the bone marrow where they continue to produce antibody.

De novo IgG anti-HLA production requires triggering of host B cell clones with IgM receptors for donor HLA antigens to develop mature IgG-producing plasma cells producing anti-HLA IgG (Fig. 3). B cells in SLO engage the donor HLA

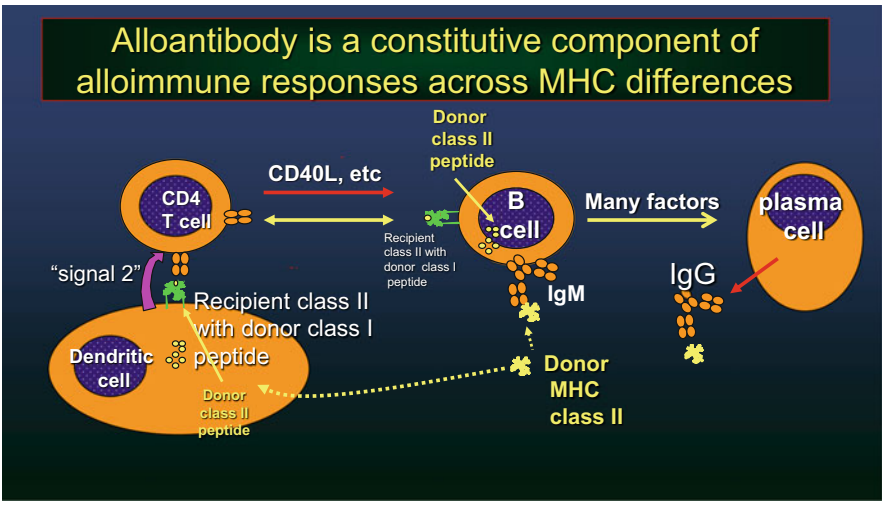

Fig. 3 Triggering of host B cell clones with antigen-specific receptors for donor HLA antigens to develop mature plasma cells producing anti-HLA IgG. B cells in SLO engage the donor HLA molecules and begin their triggering process, and express class II with donor HLA peptides. However, they require "help" from follicular helper T cells (TFH) that which recognize the donor HLA proteins as peptides in host class II molecules. The TFH are primed by host APC that have incorporated donor HLA antigen and expressed host class II with donor HLA peptides. The TFH then engage the B cells, which express the same host class II with donor HLA peptides, and provide help, permitting clonal expansion, affinity maturation, and generation of plasma cell precursors that eventually home to the bone marrow as mature plasma cells. Plasma cell maturation requires support from many molecules, including IL6

molecules and begin the triggering process, and express class II with donor HLA peptides. However, they require "help" from follicular helper T cells (TFH) that recognize the host class II molecules with donor HLA peptides. The TFH is primed by host APC that have non-specifically ingested donor HLA antigen and expressed host class II with donor HLA peptides. The TFH then engages B cells that express the same host class II with donor HLA peptides, and provides help, permitting clonal expansion, affinity maturation, and generation of plasma cell precursors that eventually home to the bone marrow as mature plasma cells. Plasma cell maturation requires support from many molecules, including IL6.

It is not known whether the long-term production of antibodies specific for donor antigens is maintained by long-lived plasma cells or by continuous generation of new memory B cells or both. Some late failing grafts near end-stage become "tertiary lymphoid organs" with organized lymphoid follicles (Colvin and Smith 2005). However, these late changes are agonal in near end-stage tissues and are never seen in early ABMR. High-affinity damaging IgG antibodies are probably produced by fully mature affinity matured bone marrow plasma cells.

3.4 Effector Mechanisms in ABMR

The potential effector functions of DSA against donor endothelium include direct effects (although this has not been demonstrated in vivo), complement activation, and recruitment of effector cells through engagement of Fc receptors (Colvin and Smith 2005; Lee et al. 2007). In general, IgG probably requires hexamer formation to activate complement (Lee et al. 2011) and possibly Fc receptors. Many IgG antibodies may be unable to form such hexamers, explaining why some apparent DSA may not produce injury.

Complement activation is often observed in ABMR but its actual role in most cases is questionable because blocking complement does not prevent the progression of ABMR (Bohmig et al. 2019). Complement-fixing DSA is more diagnostic for ABMR than non-complement fixing antibodies in kidney transplants (Loupy et al. 2013), but C4d deposition is not evident in many ABMR (Einecke et al. 2009; Sis et al. 2009; Haas 2011). Complement activation mediates injury by lysis or attracting inflammatory cells via chemoattractants C3a and C5a.

In severe cases of ABMR, glomerular capillary thrombosis can occur in ABMR, but thrombotic microangiopathy is very rare, and often due to other causes.

Leukocytes in the microcirculation in biopsies with ABMR (in kidneys, peritubular capillaritis or ptc-lesions and glomerulitis or g-lesions) are the main feature of ABMR, suggesting an effector role for these cells, but whether such cells are mediators of injury or are recruited by injury or both is difficult to establish. The strongest molecular associations point to NK cells. NK cells are a hallmark of ABMR, whether DSA-positive or DSA-negative. Activated NK cells produce IFNG and probably account for the IFNG effects in ABMR. NK cell transcripts such as KLRD1 are prominent of kidney and heart ABMR, and NK cells may be critical effectors of ABMR (Halloran et al. 2016a; Venner et al. 2015).

A possible mechanism of the microcirculation injury in ABMR is antibody-dependent cellular cytotoxicity (ADCC) through CD16a Fc gamma receptors on NK cells (Venner et al. 2015; Hidalgo et al. 2010). The principal Fc gamma receptor on human NK cells is CD16a (FcγRIIIa or FCGR3A), an activating receptor with signal-transducing mechanisms like the T cell receptor. Like effector T cell activation, CD16a triggers calcineurin and releases cytokines and cytotoxic molecules that induce injury and target cell apoptosis (Halloran et al. 2016a; Venner et al. 2015).

NK cells also have other activating and inhibitory receptors (Long et al. 2013), many with the ability to engage MHC class I, which may help them recognize "missing self" (Callemeyn et al. 2021). This raises the possibility of considering donor-recipient matching for NK receptors to avoid triggering NK recognition.

Studies of kidney and heart transplant biopsies provide strong support for the role of NK cells in ABMR syndromes (Parkes et al. 2017). These data support a model of ABMR inducing injury in the microcirculation endothelium, induced by donor-specific antibody or missing self mechanisms (e.g., CD16a activation of NK cells, triggering IFNG release and NK cell-mediated ADCC (Venner et al. 2015)).

Clinical presentations of ABMR. The dynamic range of ABMR is highly variable, from fulminant failure within hours (Patel and Terasaki 1969) to a relatively indolent course progressing over years (Sis et al. 2007) or even stable or burned out. ABMR is diagnosed by clinical (Halloran et al. 1990), immunologic (Terasaki 2003), and histologic criteria. The key kidney histology lesions in ABMR are microcirculation inflammation (peritubular capillaritis and/or glomerulitis lesions) and glomerular double contours (cg lesions). Hearts with ABMR have microcirculation inflammation but lack chronicity lesions for staging. ABMR can produce arteritis, like TCMR, although this is relatively uncommon. The Banff and ISHLT guidelines are empirically derived to achieve a reasonable trade-off between over- and underdiagnosis. Both recognize that DSA may not be demonstrable (Halloran et al. 2017).

New insights on the phenotypes of ABMR come from molecular assessment of ABMR phenotypes (Reeve et al. 2017; Venner et al. 2015). By molecular analysis, ABMR occupies a continuum of molecular space from early-stage to fully developed to late-stage (and even burned out) in the natural history of ABMR (Reeve et al. 2017). At least 25% of MMDx ABMR is DSA negative (Einecke et al. 2021).

3.5 Classification of ABMR

It is useful to characterize ABMR as

1. Hyperacute, mediated by very high levels of circulating DSA at transplantation;
2. Type 1 ABMR, mediated by re-emergence of a previously sensitized DSA due to memory, producing large amounts of DSA in the early post-transplant period;
3. Type 2 ABMR, mediated by the later appearance of ABMR independent of previous sensitization, often due to a documented de novo DSA. Of interest, type 2 has a poorer prognosis than type 1 (Aubert et al. 2017). Most ABMR now is type 2 with de novo DSA. Type 1 ABMR may do better because for unknown reasons the presensitized DSA response may eventually attenuate and disappear on immunosuppression, unlike most de novo DSA.

3.5.1 Hyperacute ABMR

This condition is prevented by crossmatching and is virtually never encountered unless a serious error is made in selecting donors. If recipients have been sensitized by previous transfusions, pregnancies, or transplants bearing donor MHC molecules, they may have high levels of pre-formed alloantibody against the donor. This can lead to disastrous hyperacute rejection, even on the operating table (Kissmeyer-Nielsen et al. 1966). Similar changes occur with incompatibility between donor and recipient at the ABO blood group locus if A- or B-antibodies are high-titer, analogous to incompatible blood transfusion. In these cases, the entire endothelium of the graft is injured, and the large vessels usually fail, leading to immediate complete failure of the graft.

The existence of pre-formed alloantibodies against HLA or AB antigens can be detected by crossmatching and ABO matching prior to transplantation to prevent

hyperacute rejection. Effective crossmatching effectively eliminated such cases, except for catastrophic failures of the safety checks.

3.5.2 Early Acute ABMR in Sensitized Patients (Type 1)

Early ABMR in the days post-transplant reflects an anamnestic burst of donor-specific HLA antibody, classically associated with the triad of decreased renal function, the presence of circulating DSA, and histological evidence of active antibody-mediated tissue injury (microvascular inflammation) (Trpkov et al. 1996), and often with deposition of complement component 4d (C4d) in peritubular capillaries (Feucht 2003). (ABO incompatibility can cause very early ABMR if the levels of antibody are high but is usually well tolerated after the initial period, despite C4d staining.) This ABMR phenotype can also emerge in the next few weeks. Type 1 kidney ABMR presents as early-stage molecular features with ptc- and g-lesions, and progresses to fully developed molecular features (glomerular double contours) over the next year.

3.5.3 ABMR Apparently Independent of Pre-Transplant Sensitization (Type 2)

Type 2 ABMR, by far the commonest type of ABMR, presents as early-stage ABMR (EABMR) in its molecular features and ptc- and g-lesions. Like type 1 ABMR, type 2 progresses to fully developed ABMR (FABMR) with histologic glomerular double contours, usually after at least 12 months. However, EABMR often escapes detection and presents as FABMR.

Type 1 EABMR usually is observed in high-risk transplants. Type 2 EABMR starts to become common to become common at the end of the first year post-transplant, and new cases continue to appear indefinitely, with molecular findings: NK transcripts and IFNG-inducible transcripts associated with histologic ABMR-related lesions peritubular capillaritis and glomerulitis.

The features of type 1 and type 2 are identical at the FABMR stage, include NK transcripts, IFNG-inducible transcripts, and certain endothelial transcripts such as ROBO4, as well as the triad of histologic ABMR-related lesions: peritubular capillaritis, glomerulitis, glomerular double contours). Double contours (duplication of the glomerular basement membrane or transplant glomerulopathy) are accompanied by lamination of the peritubular capillary basement membrane (Mauiyyedi et al. 2001; Regele et al. 2002; Vongwiwatana et al. 2004). These changes represent stages of progression of microcirculation changes after many months of ABMR (Lefaucheur et al. 2013; Cosio et al. 2008; Loupy et al. 2014). It would be useful to find such ABMR staging lesions in heart ABMR.

3.6 Late-Stage ABMR (LABMR)

FABMR often progresses to LABMR after several years, with atrophy-fibrosis and glomerular sclerosis. DSA may become negative, perhaps reflecting immune adaptations and long-term immunosuppression, or perhaps the natural history of

the antibody response. Moreover, new-onset EABMR becomes uncommon after 10 years, perhaps reflecting the adaptations in TFH.

3.7 Mixed Rejection

This phenotype is frequently seen in severe TCMR, often associated with non-adherence (Halloran et al. 2016b) and with intimal arteritis (v-lesions). A characteristic is a lack of afferent arteriolar hyalinosis due to inadequate exposure to calcineurin inhibitor ISDs (Einecke et al. 2017). A common presentation is severe TCMR followed by emergence of early-stage ABMR. With treatment of TCMR, and given the difficulty of treating ABMR, ABMR may then become the dominant long-term phenotype.

3.8 Sub-Threshold ABMR-Like Changes

We have recently found that mild ABMR-like changes exist in many biopsies that are currently diagnosed as no rejection, often but not always associated with DSA (Madill-Thomsen et al. 2021; Halloran et al. 2021b). At least in kidney transplants, the grafts with these changes are at risk of future deterioration.

3.9 DSA-Negative ABMR

ABMR molecular and histologic features can be found in kidneys and hearts in patients with no demonstrable DSA. In kidneys, the mean time of onset is somewhat earlier than DSA-positive ABMR, but the same genes are induced, e.g., NK transcripts and IFNG-induced transcripts although with moderately lower expression; the same histology microcirculation lesions are present; and both impair graft survival. Thus at least in kidneys, DSA-negative ABMR presents as an earlier and milder form of the same disease as DSA-positive ABMR.

The possible mechanisms operating in DSA-negative ABMR include:

1. Anti-HLA that is not detected by the usual platforms such as Luminex.
2. NK cell recognition of missing self HLA proteins such as HLAC.
3. Antibody against non HLA alloantigens.
4. Autoantibody.

3.10 Unsolved Issues in ABMR

It is clear that there are fundamental issues that need to be addressed in ABMR. What is the natural history of DSA and of ABMR? Can DSA and ABMR spontaneously disappear? What determines the pathogenicity of DSA? How can silent ABMR

phenotypes be detected in the clinic, and if they can, how should they be managed? What is the mechanism of DSA-negative ABMR, and if it is DSA that is not detectable by current tests, what is the target antigen, and how can we detect this antibody? Above all, there is a need for a safe and effective way of suppressing ABMR without putting patients at risk since current treatments are far from satisfactory.

4 Donor-Derived Cell-Free DNA (dd-cfDNA)

We do not have time for a detailed cover of dd-cfDNA; this subject has recently been reviewed (Kataria et al. 2021), and this is an active area for investigation as a blood screening test for rejection. ABMR and to a lesser extent TCMR and tissue injury release donor cfDNA, which has a short half-life and represents a potentially useful signal for monitoring the organ. The utility and cost-effectiveness of dd-cfDNA for monitoring organ transplant patients are under review (Puttarajappa et al. 2021).

5 Host-Graft Adaptation

Over many years, transplant patients on current ISD protocols develop reduced T cell responsiveness to donor antigens, although they still require immunosuppression. Clonal T cell responses to donor alloantigens, which are required both for generating effector T cells for TCMR and helper T cells for new DSA production to initiate ABMR, have constitutive controls that are activated from the first steps in the response. These controls are needed to avoid uncontrolled proliferation and to avoid destruction of host tissues if viruses cannot be cleared. Some are intrinsic to the cognate T cells clones, e.g., exhaustion. Others reflect those actions on these clones of regulatory cells such as Tregs. The term "host–graft adaptation" describes the decrease in both donor-specific responsiveness and the risk of rejection in the years after a successful transplantation maintained with immunosuppression (Starzl et al. 1963). Changes in the organ – a loss of donor dendritic cells ("passenger leukocytes") and resolution of injury – probably play little if any role. The crucial element is the change in the cognate clones: anergy or clonal exhaustion.

5.1 Immune Checkpoint Molecules

Exhaustion is a general characteristic of T cell responses in vivo when antigen persists (Schwartz 2003) and is mediated by immune checkpoints, which was the basis of the 2018 Nobel prize in medicine for J. P. Allison and T. Honjo. Immune checkpoint molecules represent surface molecules on T cells that engage ligands and act as brakes on the T cell system that are essential for induction of exhaustion and the maintenance of immune homeostasis. The suppressive functions of immune checkpoints usually depend on ligand-induced signaling. These receptors often use

mono-tyrosine signaling motifs, such as immunoreceptor tyrosine-based inhibitory motif (ITIM) and immunoreceptor tyrosine-based switch motif (ITSM), to deliver inhibitory signals. Inhibitory immunoreceptor-ligand combinations include PD1, CTLA4, and their ligands (He and Xu 2020).

CTLA4 is structurally related to CD28 and binds CD80/B7.1 and CD86/B7.2 with greater affinity and avidity than CD28, thus enabling it to outcompete CD28 for its ligands. CTLA4 transmits an inhibitory signal to T cells, thereby limiting T cell immune responses (Sansom 2000). PD-1 is expressed on antigen-activated T cells and upregulated in T cell exhaustion (Mumprecht et al. 2009). In the presence of its ligands (PD-L1/PD-L2) on the surface of APCs and peripheral tissue, PD-1 signaling results in decreased proliferation, IL-2 production, protein synthesis, and survival of T cells (Francisco et al. 2010), thereby suppressing T cell inflammatory activity. PD1 ligands are inducible by IFNG, helping them to control TCMR.

In addition to the CTLA4-B7 and PD1-PD1L mechanisms, other checkpoints could be relevant to transplantation, including BTLA, CD160, LAG3, TIGIT, CD244/2B4, and HAVCR2/TIM3. All of these genes show increased expression in TCMR.

As surface molecules, the activity of these checkpoints can be inhibited by blocking antibodies that prevent ligand-receptor engagement, and this forms the basis of successful anti-cancer therapy. Like organ allografts, cancer represents persistence of antigen and induces adaptations that limit effector T cell generation. The most successful immune checkpoint blockade therapy for cancers targets PD-1/PD-L1 and has been approved to treat a wide variety of cancers (Ribas and Wolchok 2018).

In transplant patients, immune checkpoint inhibition is a considerable risk for TCMR (Manohar et al. 2020; Abdel-Wahab et al. 2019). Although patients with long-term transplantation are less prone to acute rejection, there was no correlation between the rate and timing of checkpoint-inhibitor-induced allograft rejection and the time since transplantation in those patients treated with checkpoint inhibitors. Although spontaneous TCMR occurs only rarely beyond 10 years after transplantation, catastrophic TCMR can occur after checkpoint inhibition (Lipson et al. 2016). Transplant biopsies demonstrated an acute TCMR process in half of the patients who received checkpoint inhibitor therapy, even at 25 years after transplantation. ABMR is usually not induced by checkpoint inhibitors. These observations suggest that the PD-1 pathway (as well as other checkpoints) stabilizes the T cell system in transplant patients and contributes to long-term graft stability.

5.2 Regulatory T Cells

Regulatory T cells (Tregs) emerged as a mechanism in the control of autoimmunity (Sakaguchi et al. 2001; Kim et al. 2007; Lahl et al. 2007), and considerable interest has focused on their role in organ transplantation and their potential for cell-based therapy (Wood and Sakaguchi 2003; Fehervari and Sakaguchi 2005). Such studies often incorporate the transcript factor forkhead box P3 (FOXP3), a forkhead-winged

helix transcription factor important in the development and function of Tregs (Ziegler 2006; Walker et al. 2003; Yagi et al. 2004). Foxp3 knockout mice exhibit severe systemic autoimmune-like syndrome (Chikuma and Bluestone 2007; Sharma et al. 2007). Humans with mutations of FOXP3 manifest X-linked IPEX syndrome: immune dysregulation, polyendocrinopathy, and enteropathy (Wildin and Freitas 2005). Thus FOXP3 is important in cells that regulate self-tolerance.

In human organ transplantation, the significance of FOXP3+ cells remains unclear. In transplant biopsies for cause, FOXP3 mRNA expression is not a feature of pristine transplants but transplants with rejection, inflammation, and injury (Bunnag et al. 2008). FOXP3 expression in kidney tissue is a feature of renal inflammation, which is never beneficial compared to the absence of inflammation, but within such inflammation FOXP3 positive Tregs may help stabilize the inflamed site. In addition, FOXP3 positive Treg cells may be stabilizing T cell responses in SLO, preventing effector T cell generation. FOXP3 positive Tregs probably contribute to the control of all immune responses, including alloimmune responses, by analogy with their ability to suppress autoimmunity (Sakaguchi 2004). But the importance of Tregs in the events in individual patients has not been demonstrated.

Note that some researchers propose to inject regulatory cells as "drugs" to help immunosuppress transplant patients (Miller et al. 2004), but their short half-life makes this very challenging and no benefits have been shown.

5.3 Transplant Tolerance

Tolerance is a state of non-responsiveness to specific antigens induced by previous exposure to those antigens in an immunocompetent host. Transplant tolerance would allow organ transplantation without ISDs and risks of infection and cancer if it could be induced safely and last indefinitely. Unfortunately, there is no current strategy that has been proven to induce durable safe long-term transplant tolerance for HLA antigen mismatched organ transplants.

6 Late Slow Deterioration of Organ Transplants

Much of late kidney and heart loss occurs after late slow deterioration of graft function, characterized by characteristic histologic and molecular changes and loss of GFR, usually with proteinuria. (The term chronic rejection is not useful and should be avoided. If TCMR or ABMR are present use those terms.) Kidney histology shows parenchymal atrophy, interstitial fibrosis, fibrous intimal thickening of arteries, and hyalinosis of afferent arterioles. MMDx shows transcripts associated with atrophy-fibrosis: transcripts for plasma cells and B cells (Einecke et al. 2008) and mast cells (Mengel et al. 2009), AKI molecules (Einecke et al. 2010), and loss of normal parenchymal transcripts (Venner et al. 2016). These are the features of irreversible parenchymal loss, the final common pathway of many diseases (Risdon

and Sloper 1968), and reflect the cumulative burden of injuries, perhaps superimposed on advancing organ aging.

Some late slow graft deterioration is due to late uncontrolled antibody responses, but recurrent primary disease can produce similar results.

Many late losses remind us of the need for life-long immunosuppression and surveillance of renal transplant recipients, and the risks of graft loss if we "minimize" immunosuppression. The contribution of non-adherence to graft loss is considerable (Sellares et al. 2012), often presenting as TCMR but evolving to ABMR if the TCMR is treated. Understanding, preventing, and managing under-immunosuppression and non-adherence remains a major unsolved problem in organ transplantation.

The problem of parenchymal loss and deterioration of function must be seen in the context of the natural history of the organ with aging, beyond transplantation. Some parenchymal loss (atrophy-fibrosis) often manifests in the first year due to the effects of donation-implantation injury after the early injury response has resolved, and is not progressive (Venner et al. 2016). But progression often reflects some new injury process such as rejection, infection, or primary diseases, or in kidney CNI toxicity (although CNI toxicity has been over-estimated in the past by reliance on hyalinosis, which can be due to donor age, glomerular global sclerosis (Einecke et al. 2017), or hypertension (Trpkov et al. 1996; Schneeberger et al. 1999; Racusen et al. 2002; Halloran 2002; Halloran et al. 1999; Bonsib et al. 2000; Solez et al. 1998). Parenchymal atrophy-fibrosis is not believed to be inherently progressive if the stress is terminated, e.g. withdrawal of CNIs if the cause is CNI toxicity, but more information is needed about how the parenchymal elements "remember" previous injuries, and whether there is a point of no return where progression becomes inevitable with no further insults.

7 Effects of Injury

Late allograft failure as a composite phenotype reflects the total burden of injury, including pretransplant factors, aging, and post-transplant immune and nonimmune injuries, plus limitations on organ homeostasis. Nonimmune stresses, such as brain death-related organ injury and warm and cold ischemia, and the stresses of preservation and implantation, have a direct effect on parenchyma and the circulation, acting as a challenge to homeostatic mechanisms.

In renal transplant populations, the probability of late graft loss is determined by five major groups of risk factors:

1. Organ "quality" (age, size, quality, and previous disease stresses, such as hypertension, cardiovascular disease, and diabetes, donor age);
2. Brain death;
3. Preservation and implantation injury (cold preservation plus rewarming, reperfusion);

4. Alloimmune injury (rejection): in human population data this is represented by the degree of HLA mismatch, sensitization, immunosuppressive drugs, and rejection episodes;
5. New stresses in the recipient environment (infection, hypertension, recurrent disease, drug toxicity, and advancing aging).

7.1 Does Injury Evoke Rejection?

While nonimmune injury and rejection injury can be additive, the basic science behind the relationships between injury and rejection is incomplete, and cannot be modeled in rodents or young primates because these models lack donor aging. We previously postulated that tissue injury, by evoking inflammation (innate immunity activation), increases the probability of rejection (Halloran et al. 1997), but this remains unproven. Living donor kidneys have some advantage in graft survival compared to deceased donor kidneys despite extensive HLA mismatching because they lack the injury associated with brain death and cold storage (Terasaki et al. 1995), but they are still at risk of rejection. Two kidneys from one deceased donor show similar function at all times post-transplant (Gourishankar et al. 2003), but they are not paired for rejection, which is driven mainly by non-donor factors.

Injury evokes response-to-injury effects on the organ as complex as the immune response itself (Halloran et al. 2021c). Inflammation – macrophage infiltration – is the normal response to wounding and should not be considered undesirable. However, the effects of injury on the parenchyma itself are often overlooked because the study of inflammatory and adaptive immune response is a natural priority of transplantation scientists. Inflammation is our hammer, and all the world looks like a nail. But the transplant patient wants high-quality parenchyma, and the reduction of parenchymal stress – peri-transplant stress, surgical and cold stress, ischemia, drugs, and infections – should be a priority, as well as understanding how to help injured parenchyma recover from these wounding effects.

8 Summary

The course of an organ transplant reflects the previous history of the organ (e.g., age, hypertension), its burden of injuries and stresses in the peri-transplant and post-transplant period, its post-transplant experiences, its intrinsic limitations on repair, and homeostasis imposed by aging and previous injuries, and rejection. The pathologic changes of rejection can explain how rejection can be associated with permanent loss of the limiting elements in an organ transplant. This puts the course of an organ transplant into the same context as the general problem of repair and homeostasis of that organ in the original host. The most preventable stress is rejection and identifying and treating all uncontrolled alloimmune injury remains the key to long-term graft stability. But in the long-term, the focus must include parenchymal health and homeostasis, and promotion of recovery from injury without atrophy-fibrosis.

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