

ANTIBODY-MEDIATED ORGAN-ALLOGRAFT REJECTION

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Abstract | Recent studies show that alloantibodies mediate a substantial proportion of graft-rejection episodes, contributing to both early and late graft loss. Rejection that is caused by antibody is mediated by different mechanisms from rejection that is caused by T cells, thereby requiring other approaches to treatment and prevention. Antibody induces rejection acutely through the fixation of complement, resulting in tissue injury and coagulation. In addition, complement activation recruits macrophages and neutrophils, causing additional endothelial injury. Antibody and complement also induce gene expression by endothelial cells, which is thought to remodel arteries and basement membranes, leading to fixed and irreversible anatomical lesions that permanently compromise graft function.

ACUTE REJECTION

The rapid (within days) clinical deterioration of graft function, which can occur days to years after transplantation. It can be caused by T-cell-dependent reactivity to donor alloantigen (that is, cellular rejection) and/or alloantibody reactivity to donor antigens on the endothelium (that is, antibody-mediated rejection).

For several decades, T cells have been regarded to be the central regulatory and effector cells in graft rejection, because allografts are not rejected in animals that lack T cells¹ or in humans who are depleted of T cells². Consequently, to prevent or treat graft rejection, most current therapies (including T-cell-specific antibodies, calcineurin inhibitors, mycophenolic acid, rapamycin and prednisone) generally target T-cell function. These drugs improve short-term graft survival to 88–95% in the first year for renal and cardiac allografts; however, episodes of ACUTE REJECTION still occur, and long-term allografts often succumb to CHRONIC REJECTION. Recent evidence indicates that antibody is involved in a substantial proportion of these remaining rejection episodes, particularly those that involve total graft failure. This evidence comes from the correlation of graft pathology with the presence of circulating antibody and the deposition of complement component 4d (C4d) on graft endothelium, which is a consequence of antibody-induced complement activation (discussed later). This Review focuses on the mechanisms of antibody-mediated graft injury and graft resistance to injury (known as accommodation) in immunosuppressed recipients, with the goal of stimulating new strategies to prevent and treat antibody-mediated rejection.

Early work by Peter Gorer³ and others showed that mouse skin allografts induced alloantibodies that could

agglutinate donor erythrocytes (which express MHC class I antigens in mice). However, passive transfer of antibody at the time of engraftment generally failed to cause accelerated rejection of skin allografts in naive mice that had grafts from the same donor, whereas the adoptive transfer of sensitized lymphocytes caused prompt rejection of grafts, including transplanted tumours. So, only T cells, and not antibody, were originally thought to mediate allograft rejection. Later studies showed that skin allografts could be rejected by antiserum when it was given during the first week after transplantation⁴. Antibody and complement deposition were shown *in vivo* on the graft endothelium. The underlying basis of resistance to antibody in the earlier experiments was shown to be a consequence of the lack of perfusion of skin-graft vessels in the first 4–6 days after transplantation. Grafts then lost their sensitivity to antibody after several weeks, owing to replacement of graft vessels with recipient endothelium⁵. Transplanted solid tumours are generally resistant to antibody-mediated rejection, in part because the vasculature is of recipient origin.

Evidence later implicated antibody in chronic rejection. In 1970, Paul Russell and colleagues⁶ identified a strong correlation between the presence of circulating antibodies that were specific for donor HLA molecules and the existence of

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CHRONIC REJECTION

The slow (within months to years) clinical deterioration of graft function. It can be caused by T-cell- and/or antibody-mediated reaction to donor alloantigens, which results in a slow, progressive decline in graft function and is typically associated with stenotic intimal hyperplasia of the arteries.

PRE-SENSITIZATION

Immunological reactivity to a donor that is present before transplantation: for example, as a result of a previous transplant, pregnancy or blood transfusion. It is typically detected by the measurement of serum antibody that is specific for donor cells or surrogate targets.

SERUM CREATININE

Creatinine is a component of urine and the final product in the metabolism of creatine. An increase in serum concentration is used as a marker of kidney dysfunction.

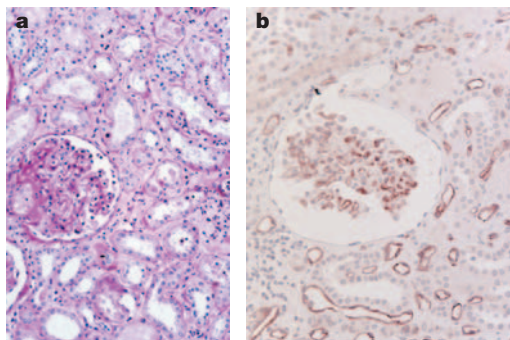


Figure 1 | Acute antibody-mediated rejection. a | Acute humoral rejection in a human renal allograft. Periodic-acid-Schiff staining shows the presence of a sparse interstitial infiltrate of neutrophils, together with oedema. **b** | Complement component 4d (C4d) deposition in peritubular and glomerular capillaries in a human renal allograft. An immunohistochemical stain using polyclonal rabbit antibodies that are specific for C4d shows C4d deposition.

stenotic arterial lesions of renal allografts (known as chronic allograft arteriopathy). Experimental studies using mouse cardiac allografts later showed that the passive transfer of alloantibodies promoted the development of chronic allograft arteriopathy in normal or immunologically deficient mice and that B-cell-deficient mice did not develop fibrotic arterial lesions⁷. Other research groups have confirmed this correlation and have shown that more-severe acute rejection of renal allografts occurs in patients with antibodies that are specific for donor HLA molecules⁸.

Despite these findings, scepticism about the importance of antibody-mediated rejection persisted, because a mechanistic connection and direct link to allografted tissue was lacking: antibody and complement (deposition of C3) were not reliably detectable in grafts. In this respect, a diagnostic breakthrough came in the 1990s, with the use of C4d deposition in graft microvasculature as a marker of complement fixation in tissues⁹ (discussed later), followed by the finding that C4d deposition is usually associated with circulating donor-specific antibodies¹⁰ (FIG. 1). This improvement in diagnosis, coupled with newer methods of detecting donor-reactive antibodies¹¹, has led to a renaissance in the study of antibody-mediated rejection. This Review focuses on the effects of antibody on the endothelium, because endothelial cells are thought to be the main

target of antibody. In a less well-understood process, which is beyond the scope of this Review, antibody might also affect parenchymal cells, as has been postulated to explain the epithelial damage that occurs in lung transplants¹².

Rejection in organ grafts

Rejection is classified as hyperacute, acute or chronic — a classification that is based on time course and not on mechanisms. Hyperacute rejection develops within minutes to hours after transplantation, is caused by PRE-SENSITIZATION to donor tissue, and is usually mediated by the consequences of alloantibody and complement fixation. Acute rejection develops quickly over a few days, occurs days to years after transplantation and is a consequence of the development of antigen-specific T-cell and/or antibody alloreactivity to the graft. Chronic rejection develops over months to years and also involves alloimmunity to the graft by T cells and/or antibody. Grafts can also be injured chronically by non-immune mechanisms, such as drugs, ischaemia, infection, ageing, and *de novo* or recurrent parenchymal disease. However, the strict definition of chronic rejection excludes non-immune processes, and it is the alloimmune processes that we discuss here. Another category of rejection, subclinical rejection, has recently been recognized, and this refers to pathological injury in the graft that has been caused by antibody and/or T cells but has not yet manifested as graft dysfunction.

Acute antibody-mediated rejection. At present, acute antibody-mediated rejection (AAMR) is defined by four criteria^{13,14} (BOX 1). AAMR occurs in 6.7% of kidney-transplant recipients^{13,14} and is present in 32% of renal biopsies from patients who have been diagnosed with acute rejection^{15–17}. AAMR can occur at any time after transplantation (from days to years). It is manifested by a rapid rise in SERUM CREATININE and is resistant to therapy with steroids or T-cell-specific reagents. AAMR has been observed in conjunction with all of the immunosuppressive therapies that are currently used to promote graft acceptance, and it has a poorer prognosis than pure T-cell-mediated acute rejection¹⁸. The pathological features of hyperacute rejection and AAMR are similar, and they include capillaries congested with neutrophils and macrophages, thrombi consisting of platelets and fibrin, parenchymal injury and fibrinoid arterial necrosis^{15,19–21} (FIG. 1). However, some renal biopsies lack histological evidence of rejection and show only acute tubular injury. Detection of C4d deposition in capillaries, which was pioneered by Feucht⁹, has proved to be the most reliable marker of AAMR. We have found C4d deposition to be a highly specific (96%) and sensitive (95%) marker for AAMR compared with circulating donor-HLA-specific antibodies as the gold standard¹⁵. Others using similar techniques have found sensitivities and specificities of 50–67% and 78–88%, respectively^{20,22}. Ischaemia alone rarely, if ever, causes C4d deposition in the kidney²³. A practical guide to C4d staining techniques and interpretation is available²⁴.

Box 1 | Diagnostic criteria for acute antibody-mediated rejection

- Clinical evidence of acute graft dysfunction
- Histological evidence of acute tissue injury: that is, neutrophils, macrophages or thrombi in capillaries, fibrinoid necrosis, or acute tubular injury
- Immunopathological evidence for the action of antibodies: that is, complement component 4d (C4d) deposited in peritubular capillaries, or antibodies or C3 in arteries
- Serological evidence of HLA-specific antibodies or other donor-specific antibodies at the time of biopsy

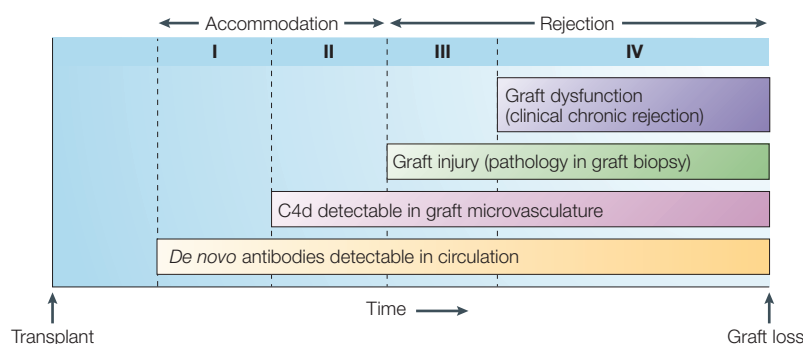


Figure 2 | Proposed sequence of stages of antibody-mediated rejection. Antibodies that are specific for graft antigens (typically HLA molecules) might be produced at any time after engraftment. Pre-sensitized patients have circulating specific antibodies before transplantation (usually causing immediate hyperacute rejection) (not shown). The time between the stages might range from days (acute) to months or years (chronic). The first two stages meet the criteria for accommodation, because the graft is not pathologically injured despite the presence of circulating antibodies. C4d, complement component 4d.

In addition to the kidney, AAMR is recognized frequently in other organs. Fifteen years after it was first described to occur in the heart²⁵, AAMR is now accepted to be a form of rejection of cardiac allografts¹³. The risk factors are similar to those for AAMR of renal allografts, with the notable addition of exposure to cardiopulmonary-assist devices, which increases the risk of alloantibody induction²⁶. C4d deposition in myocardial capillaries strongly correlates with the presence of circulating antibodies that are specific for donor HLA molecules²⁷. Patients who develop HLA-specific antibodies or AAMR early after heart transplantation have poorer graft survival^{25,28–30}. There are also occasional reports of apparent AAMR of lung, pancreas and liver allografts, but diagnostic criteria have not been established^{13,28,31,32}. Further analysis of C4d deposition and correlation with donor-HLA-specific antibodies should clarify the features and significance of AAMR in these other organs.

Chronic antibody-mediated rejection. Recent evidence supports the hypothesis that a subset of cases of chronic rejection might be mediated by alloantibodies. Circulating HLA-specific antibodies are common in patients with long-term organ allografts. In a large multicentre trial, HLA-specific antibodies were detected in 21% of patients with renal allografts and 14–23% of patients with heart, liver or lung allografts³³. Of 2,278 renal-allograft recipients who were followed prospectively, graft failure at 1 year occurred more frequently in patients who developed alloantibodies than in those who did not (8.6% versus 3.0%). Several studies have reported that *DE NOVO* ANTIBODIES that are specific for graft HLA class I and class II molecules are a risk factor for premature graft loss as a consequence of renal and cardiac chronic arteriopathy^{28,34,35}. For example, during a 5-year follow-up period, donor-reactive antibodies were present in 51% of patients with graft failure compared with 2% of stable control individuals. The presence of antibodies preceded graft failure in 60% of cases³⁶.

DE NOVO ANTIBODIES
Antibodies that first appear after transplantation (that is, the patient is not pre-sensitized).

So, circulating HLA-specific antibodies are typically present months to years before graft dysfunction, indicating that antibody-mediated graft injury might be slow to develop.

The features of chronic antibody-mediated rejection (CAMR) in renal allografts include the following: duplication of the glomerular basement membrane (known as chronic allograft glomerulopathy); proliferation in the intima of arteries and infiltration with mononuclear cells (known as chronic allograft arteriopathy); and lamination of the peritubular capillary basement membrane, which occurs together with deposition of C4d in peritubular capillaries^{37–39} and/or glomeruli⁴⁰. At 1 year after transplantation, about one-third of kidney-graft biopsies show evidence of CAMR in the form of C4d deposition³⁸. Most patients with C4d deposition (88%) have circulating antibodies that are specific for donor HLA molecules³⁷. Most importantly, C4d deposition has been shown to precede, and allow the prediction of, the later development of chronic allograft glomerulopathy, which is consistent with the hypothesis that C4d deposition identifies an early stage in the pathogenesis of this glomerulopathy³⁸. A contribution of T-cell-mediated injury to chronic rejection, in particular to the vascular disease and tubular destruction, is also likely.

Stages of antibody-mediated rejection. At a recent National Institutes of Health (United States) consensus conference, draft criteria were established for antibody-mediated rejection and for four theoretical stages in the development of CAMR¹³ (FIG. 2). According to this model, the first evidence of an antibody-mediated response is the *de novo* generation of donor-reactive antibodies (stage I). In many circumstances and for unknown reasons, donor-reactive antibodies do not elicit AAMR. The next stage (stage II) shows evidence of antibody reactivity and complement activation in the graft, with C4d deposition in peritubular or glomerular capillary endothelium. At this stage, there is no evidence of pathological or clinical injury in the graft. Both stage I and stage II fit the criteria for accommodation (that is, graft resistance to antibody; discussed later) and are therefore not necessarily predestined to lead to graft injury. In stage III, in addition to positive staining for C4d, there are identifiable pathological changes, but graft function is still normal (that is, there is subclinical rejection). Finally, in stage IV, in addition to positive staining for C4d and pathological changes, graft dysfunction occurs (which, for renal grafts, results in azotaemia and proteinuria). In cynomolgus monkeys (*Macaca fascicularis*), we have documented the four stages of CAMR in a subset of allografted animals from mixed-chimerism protocols (R.N.S., T. Kawai, S. Boskovic, F. Cardarelli, S. Saidman, D. J. Dorer, O. Nadazdin, D. H. Sachs, A. B. Cosimi and R.B.C., unpublished observations). The interval between stages can be long and variable, and it is not known whether progression is inexorable.

Antigens and antigen presentation

Antigenic targets. The main antigenic targets of antibody-mediated rejection are MHC molecules (both class I and class II)⁴¹ and the ABO blood-group antigens⁴². MHC class I molecules are found at the surface of all nucleated cells, including endothelial cells. By contrast, the distribution of MHC class II molecules is more limited. These molecules are constitutively expressed at the surface of B cells, dendritic cells (DCs) and microvascular endothelial cells (the last applies to humans but not mice) and are expressed by other cells depending on the stimuli that they have been exposed to and their transcriptional activation. The extreme polymorphism of MHC class I and class II polypeptides (more than 1,600 alleles in humans) aids their main function, which is antigen presentation to T cells. Production of HLA-specific alloantibodies depends on exposure to HLA molecules as a consequence of pregnancy, blood transfusion or transplantation. These antibodies are mainly of the IgG class. Blood-group antigens, most importantly the A and B antigens, are carbohydrate epitopes on glycolipids and glycoproteins that are present at the surface of most tissues, including erythrocytes and endothelial cells. Antibodies that are specific for A or B antigens arise 'naturally' in normal individuals who are not of the A and/or B blood group in response to antigens from the environment, and they are usually of the IgM class.

In addition to MHC molecules and blood-group antigens, minor histocompatibility antigens might also be targets of antibody-mediated rejection. Minor histocompatibility antigens, which were originally defined in mice by their ability to cause prompt skin-graft rejection, are also thought to be relevant as targets of graft-versus-host disease and as tumour antigens⁴³. In animal studies, non-MHC-specific antibodies can cause endothelial-cell apoptosis and graft rejection^{44,45}. However, in humans, the molecular characterization of these antigens is limited. MICA (MHC-class-I-polypeptide-related sequence A), one of the few potential endothelial-cell surface alloantigens, has been defined at the molecular level⁴⁶. MICA is a polymorphic non-classical MHC molecule. Antibody that is specific for MICA can be detected in renal-allograft recipients and is associated with later rejection and graft loss^{47,48}.

Antibodies that recognize self-proteins might also contribute to graft injury. For example, autoantibody that is specific for the angiotensin II type 1 receptor, which is expressed by vascular smooth muscle, has been associated with severe hypertension, graft dysfunction and fibrinoid arterial necrosis of human renal allografts⁴⁹. The presence of antibody that is specific for vimentin or myosin correlates with chronic cardiac-allograft arteriopathy⁵⁰ and decreased graft survival⁵¹. Also, in some experimental models, endothelial damage in ischaemic organs is mediated by 'natural' autoantibodies and complement fixation⁵². Natural antibodies are typically of the IgM class and presumably recognize cryptic self-antigens that are exposed

or presented only under certain circumstances. One such autoantigen is non-muscle myosin heavy chain II, which is present in endothelial cells (M. Carroll, personal communication).

Antigen presentation and B cells. Here, we give only an outline of antigen presentation, owing to space constraints, but this topic has recently been reviewed⁵³. The alloantibody response generally requires T-cell help. MHC molecules are presented to recipient T cells by DCs in the graft (the direct pathway) or, after antigen reprocessing, by DCs of the recipient (the indirect pathway). Recent studies indicate that T cells are first triggered by the direct pathway in skin grafts and by the indirect (and later, the direct) pathway in lymph nodes⁵⁴. The activated T cells can then provide help for B-cell memory, and antibody class switching and affinity maturation, through various T-cell-derived cytokines and co-stimulatory factors that recognize receptors at the surface of B cells (such as ICOS (inducible T-cell co-stimulator), CD40 ligand, and CD80 and CD86). Experiments in mice indicate that, for B-cell help, the antigen that is recognized by the T cell needs to be presented by recipient, not donor, DCs, perhaps because the T cell needs to react with, and signal through, self-MHC class II molecules at the surface of the B cell⁵⁵. For example, an IgG alloantibody response requires CD4⁺ T-cell reactivity through the indirect pathway in mice^{55,56} and monkeys⁵⁷. By contrast, production of IgM alloantibodies that are specific for MHC molecules⁵⁵ and carbohydrate antigens (the ABO blood-group antigens)⁵⁵ might not require T-cell help. B cells might also promote rejection by presenting antigen to T cells, although B-cell-deficient mice do not manifest an obvious T-cell defect⁵⁸. However, for some protein antigens, B cells are required for an active T-cell response⁵⁹.

The B-cell response leads to the production of long-lived plasma cells, which migrate to the bone marrow and continue to produce antibody indefinitely, without requiring T-cell help⁶⁰. It is not known whether the presence of antibodies that are specific for graft antigens is maintained as a consequence of the longevity of plasma cells or as a consequence of the continuous generation of new memory B cells. Recent evidence indicates that long-term grafts might become NEO-LYMPHOID ORGANS with organized lymphoid tissue. Stimulation through the indirect pathway could occur in such grafts through the infiltration of recipient DCs and the activation of the endothelium to present antigens. For example, chronically rejecting human renal allografts have a 50-fold increase in lymphatic-vessel density compared with normal kidneys and have organizing nodular infiltrates of T and B cells, DCs and plasma cells^{61,62}. In these studies, the cellular infiltrate expressed CC-chemokine receptor 7 (CCR7) and the lymphatic endothelium expressed the CCR7 ligand CC-chemokine ligand 21 (CCL21; also known as SLC). Lymphatic neo-angiogenesis might be involved in the maintenance of a potentially detrimental, local alloreactive immune response. The graft itself might

NEO-LYMPHOID ORGAN

The organization within a tissue of high endothelial venules, lymphoid follicles and dendritic cells. This can be mediated by persistent inflammation.

therefore be a site for T-cell–B-cell interaction and a niche for long-lived plasma cells⁶³. Indeed, examples of plasma-cell-rich rejection have been described in humans, in some cases associated with C4d deposition and high levels of mRNA encoding interferon- γ (IFN- γ) in the graft⁶⁴. Increased IFN- γ secretion might shorten

graft survival acutely by increasing MHC molecule expression, thereby providing more antigenic targets for alloantibodies⁶⁵.

Mechanisms of antibody-mediated rejection

Complement fixation by antibody is essential for the pathogenesis of acute and hyperacute rejection⁶⁶. COMPLEMENT-FIXING ANTIBODIES are required for passive transfer of AAMR in mouse cardiac allografts^{67,68}. Furthermore, complement antagonists (that is, soluble complement receptors or the expression of complement-regulatory proteins by the endothelium; discussed later) inhibit AAMR and hyperacute antibody-mediated rejection^{69,70}.

Complement activation. Antibody-mediated complement activation is reviewed briefly here (FIG. 3), and it is covered further in REF. 71. Activation of C1 (which is composed of C1q, C1r and C1s) can be initiated by interaction of the globular domains of C1q with IgG or IgM bound to antigen epitopes on the graft endothelium. The C1q-binding potential of human IgG subclasses in order of decreasing capacity is IgG3, IgG1, IgG2 and IgG4. Conformational changes in C1q then allow cleavage of C1r, and activated C1r cleaves and activates C1s, which is the enzyme that activates C2 and C4.

C4 is cleaved by C1s into the small fragment C4a and the large fragment C4b, exposing a sulphhydryl group. The reactive sulphhydryl group of C4b rapidly forms an ester or amide bond with nearby molecules that contain hydroxyl or amino groups. After inactivation of C4b to C4d by factor I, C4d remains covalently bound to the tissue and is thereby a durable *in situ* marker of complement activation⁷², in contrast to deposition of C1q or antibody. For example, in AAMR, C4d is found at the surface of most peritubular capillaries, using immunofluorescence or immunohistochemistry^{10,20} (FIG. 1). C4d co-distributes with type IV collagen along the capillary basement membrane and along endothelial-cell surfaces¹⁰. Eventually, C4d is cleared from the tissue, after the antibody response has ended: loss of C4d has been documented as early as 8 days after treatment¹⁰. So far, no reports indicate that C4d has any functional activity; nevertheless, C4d is a useful marker of antibody deposition and complement activation in the graft. Activation and regulation of the steps of the complement cascade after C4 are crucial, because the pathophysiology of the effects of complement requires activation of the final common pathway. If activation stops at C4 (that is, C4d in the graft) without activation of C3 (that is, no C3d in the graft), then graft injury might be prevented or reduced^{73,74}.

C4b combines with the enzymatically active fragment C2a to form C4b–C2a, which is known as the classical-pathway C3 convertase. After C3-convertase formation, the classical pathway leads to the common step of C3 activation and cleavage to C3a and C3b. When a C3b molecule is covalently deposited in the immediate vicinity of the C3 convertase, the C5 convertase — C4b–C2a–C3b — forms. Cleavage of

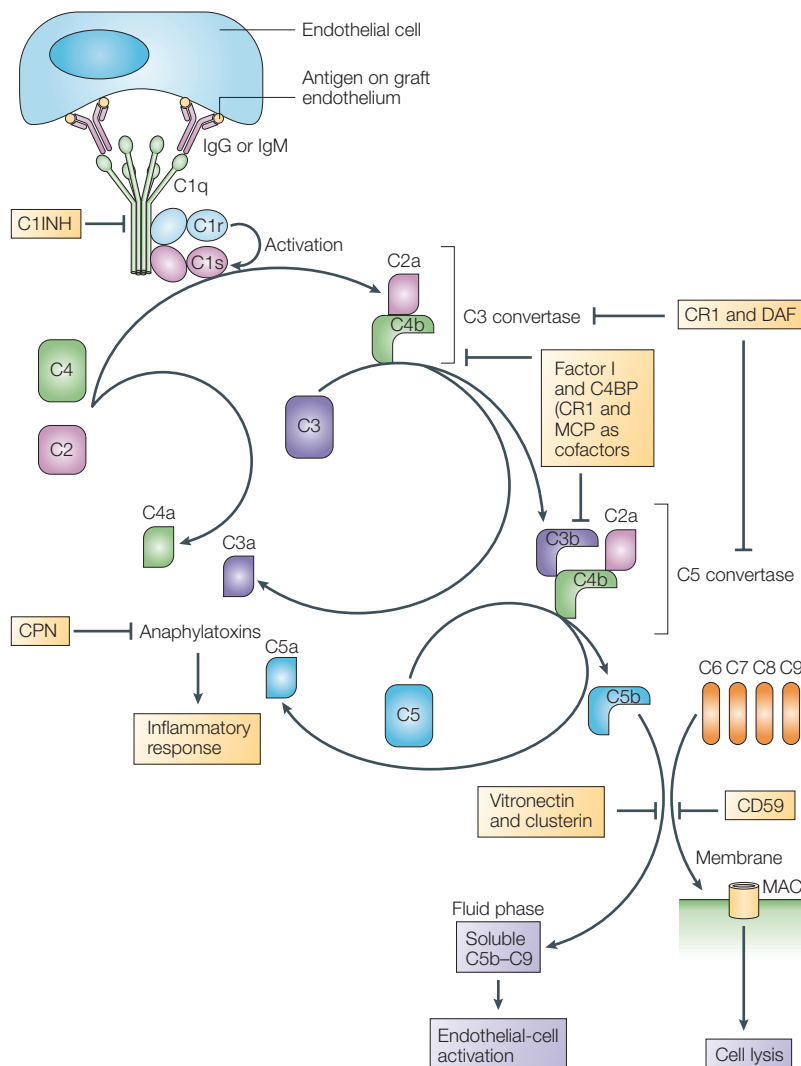


Figure 3 | Classical pathway of complement activation by antigen–antibody complexes. Antibody interacts with complement component 1q (C1q), and this begins the sequence that generates soluble peptides (including C3a and C5a) and bound molecules (including C4b and C3b), and culminates in the formation of the terminal complement components. These components form the membrane attack complex (MAC), which consists of C5b–C6–C7–C8–C9 (denoted C5b–C9), and this results in cell leakage or lysis (for further details, see the main text). Fluid-phase activation causes C5b–C6–C7 complexes to bind vitronectin and clusterin, which are fluid-phase regulators of the terminal pathway. Inhibitor proteins regulate complement activation, including C1 inhibitor (C1INH), carboxypeptidase N (CPN; which inactivates the anaphylatoxins C3a, C4a and C5a) and factor I (which inactivates C3b and C4b, using C4b-binding protein, C4BP). The membrane regulators complement receptor 1 (CR1), membrane cofactor protein (MCP) and decay-accelerating factor (DAF) regulate complement activation by functioning as cofactors for factor-I-mediated cleavage of C3b and C4b (in the case of CR1 and MCP) or by accelerating the decay of C3 convertase and C5 convertase (in the case of CR1 and DAF). CD59, also a membrane regulator, prevents the binding of C9 to C5b–C6–C7–C8 complexes in the terminal pathway. Many of the biological effects resulting from complement activation are mediated by cell-surface receptors, such as the receptors for C1q, C3a, C5a and iC3b (the inactivated form of C3b). Antibody-independent complement activation might also occur by the lectin and/or alternative pathways (not shown).

C5 then releases the bioactive peptide C5a, and C5b. C5b initiates formation of the membrane attack complex (MAC; membrane-bound C5b–C6–C7–C8–C9, denoted as C5b–C9), which causes cell lysis (FIG. 3). In addition, soluble C5b–C9 can also have stimulatory effects on the endothelium (discussed later).

Complement activation mediates acute graft injury by attracting inflammatory cells to the chemoattractants C3a and C5a. Neutrophils and macrophages express the cell-surface receptor for C3a and the receptor for C5a (CD88). C3a is spasmogenic because it elicits the release of prostaglandin E₂ from macrophages, and C5a causes oedema by inducing the release of histamine from mast cells. The MAC can cause lysis of endothelial cells and graft rejection, a process that depends on C6 (REF. 75).

The other effects of complement that are of particular interest are those that activate endothelial cells. ENDOTHELIAL-CELL ACTIVATION in this context refers to increased production of pro-inflammatory molecules (that is, cytokines, chemokines, adhesion molecules and/or growth factors)⁷⁶. Activation of the C3a receptor or the C5a receptor at the surface of endothelial cells causes cytoskeletal changes and cytokine release. The cleavage products C3a and C5a induce endothelial cells to increase the expression of adhesion molecules, such as endothelial-cell selectin (**E-selectin**), vascular cell-adhesion molecule 1 (**VCAM1**) and intercellular adhesion molecule 1 (**ICAM1**), and cytokines and chemokines, such as interleukin-1 α (**IL-1 α**), **IL-6**, **CCL5** (also known as RANTES) and CXC-chemokine ligand 8 (**CXCL8**; also known as IL-8). C3a and C5a also promote signalling through the mitogen-activated protein kinase pathway^{77,78}.

Exposure to soluble C5b–C9 at sub-lytic concentrations also increases the expression of adhesion molecules (such as E-selectin, ICAM1 and VCAM1) by cultured endothelial cells, through the autocrine actions of IL-1 α ⁷⁹. The formation of a pore in the endothelial-cell surface seems to be the essential triggering event⁷⁹. The MAC also elicits signals for the proliferation of endothelial cells, as shown by the release of growth factors, including platelet-derived growth factor (**PDGF**) and basic fibroblast growth factor (bFGF; also known as FGF2)⁸⁰, and for the production of the chemokines **CCL2** (also known as MCP1), **CCL5** and **CXCL8**, which occurs through stimulation of IL-1 α production⁸¹. Soluble C5b–C9 promotes the synthesis and secretion of pro-inflammatory molecules, such as CCL2 and CXCL8, through nuclear translocation of nuclear factor- κ B (NF- κ B)⁸². Finally, C5a and soluble C5b–C9 trigger endothelial-cell synthesis of **tissue factor** (which is part of the extrinsic clotting system)^{83,84}. In severe cases of antibody-mediated rejection, thrombotic injury can dominate so that the rejection resembles thrombotic microangiopathy, with diffuse vascular injury and thrombosis.

Complement also promotes the normal immune response that is elicited by graft antigens. B cells and DCs express complement receptor 1 (CR1; also known as CD35 or the C3b–C4b receptor) and CR2 (also known

as CD21 or the C3d receptor) and can therefore retain antigen covalently linked to C3 or C4. Engagement of CR2 lowers the threshold for B-cell activation and thereby functions as a natural adjuvant⁸⁵. Complement deficiencies prolong graft survival and reduce chronic allograft arteriopathy in mice^{86,87}, and the production of C6 by host macrophages promotes acute rejection⁸⁶. Surprisingly, the *in situ* production of C3 promotes graft rejection, because renal allografts from C3-deficient mice have prolonged survival⁸⁸. (Renal tubular epithelium is the main source of local C3.) So, either host-derived or donor-derived complement can affect acute cellular rejection through stimulating T-cell⁸⁸ or B-cell responses or through inducing endothelial-cell production of potent effector cytokines, which damage the graft.

Complement-independent, antibody-mediated mechanisms. Antibody also has effects on endothelial cells that are independent of complement, and these might contribute to the pathogenesis of graft rejection. Current evidence implicates complement fixation as a mechanism that is required for AAMR, but this might not be the case for CAMR. In the absence of complement or inflammatory cells, antibody can induce endothelial-cell activation. For example, antibodies that are specific for MHC class I molecules increase tyrosine phosphorylation and NF- κ B levels in both human umbilical vein and heart microvascular endothelial cells, and they promote proliferation of these cells *in vitro*⁸⁹. HLA-class-I-specific antibodies stimulate endothelial cells to express FGF receptors, to phosphorylate SRC and to proliferate⁹⁰. Non-complement-fixing, MHC-class-I-specific antibodies activate cultured mouse endothelial cells to produce CCL2 and CXCL1 (also known as KC), an effect that is increased in the presence of tumour-necrosis factor (TNF)⁶⁸. This transcriptional activation might be relevant to the arterial intimal proliferation that is characteristic of CAMR. Other cell types such as bronchial epithelial cells can be triggered to proliferate by MHC-class-I-specific antibodies¹².

Antibody can also lyse target cells by complement-independent pathways, through the low-affinity Fc receptor for IgG Fc γ RIII (CD16) at the surface of natural killer cells and macrophages (a process known as ANTIBODY-DEPENDENT CELL-MEDIATED CYTOTOXICITY, ADCC). The contribution of this mechanism to graft rejection remains uncertain, because there have been few experimental studies. It is intriguing, however, that Fc γ RIIa (CD32) polymorphisms in recipients of renal allografts correlate with the risk of acute rejection⁹¹.

Accommodation: concept and evidence

Accommodation is defined as the resistance of a graft to the acute pathological effects of graft-specific antibodies and complement fixation⁹². Alexandre⁹³ initially observed accommodation in recipients of an ABO-incompatible renal allograft. Transient depletion of the circulating antibodies that are specific for these blood-group antigens at the time of transplantation allows

COMPLEMENT-FIXING ANTIBODIES

Not all antibodies fix or activate complement. In humans, IgM and the IgG subclasses IgG1 and IgG3 readily fix complement, whereas IgG2 is less effective. The IgG subclass IgG4 and other classes of immunoglobulin do not fix complement or activate the classical complement pathway.

ENDOTHELIAL-CELL ACTIVATION

Marked by phenotypic changes that usually include MHC class II expression, tissue-factor activity and increased leukocyte adhesion to the endothelium, all of which are induced by interferon. This often occurs together with morphological changes, including hypertrophy (indicated by a cuboidal appearance), increased biosynthesis and increased permeability.

ANTIBODY-DEPENDENT CELL-MEDIATED CYTOTOXICITY (ADCC)

A mechanism by which natural killer (NK) cells kill other cells: for example, virus-infected target cells that are coated with antibodies. The Fc portions of the coating antibodies interact with the Fc receptor that is expressed by NK cells (Fc γ RIII; CD16), thereby initiating a signalling cascade that results in the release of cytotoxic granules (containing perforin and granzyme B), which induce apoptosis of the antibody-coated cell.

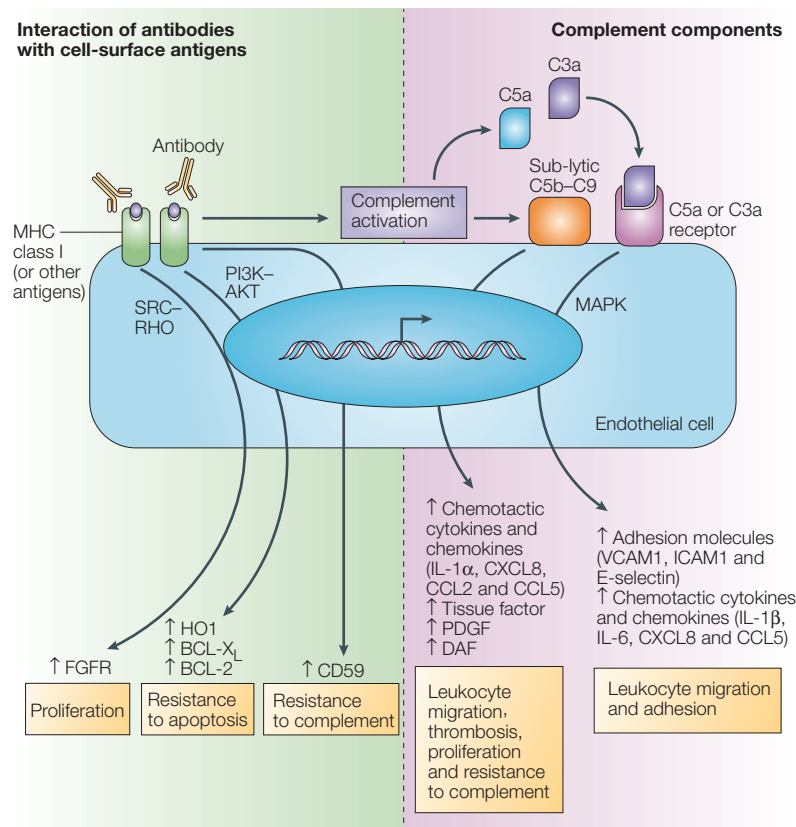


Figure 4 | Summary of reported effects of antibody and complement components on human endothelial cells. Effects that are mediated by the interaction of antibody with antigen at the surface of endothelial cells are listed on the left; those caused by complement components — complement component 3a (C3a), C5a and the membrane attack complex (C5b–C6–C7–C8–C9; denoted C5b–C9) — are listed on the right. The known target antigens are the MHC class I and class II molecules and, in some cases, the ABO blood-group antigens. In fact, any molecule at the surface of the endothelium that elicits an antibody response in a recipient might be a target (for further details, see the main text). BCL, B-cell lymphoma; CCL, CC-chemokine ligand; CXCL8, CXC-chemokine ligand 8; DAF, decay-accelerating factor; E-selectin, endothelial-cell selectin; FGFR, fibroblast-growth-factor receptor; ICAM1, intercellular adhesion molecule 1; IL, interleukin; MAPK, mitogen-activated protein kinase; PDGF, platelet-derived growth factor; PI3K, phosphatidylinositol 3-kinase; RHO, RAS homologue; VCAM1, vascular cell-adhesion molecule 1.

immediate graft survival without hyperacute rejection. A rebound of antibody concentrations within the first 10 days occurs together with rejection in 90% of cases. However, after 21 days, for the remaining grafts, there is no correlation between the occurrence of rejection and the antibody titre⁹⁴. Even if the antibody titre returns to pre-transplantation levels or higher, the grafts continue to function. Studies in mice show that, in the absence of T-cell help, B cells that are exposed to incompatible carbohydrate antigens on allografts differentiate into cells that can produce non-complement-fixing antibody, and these B cells gradually become tolerant after prolonged exposure⁹⁵.

In HLA-mismatched grafts, alloantibodies can be found in the absence of clinical graft dysfunction, thereby fitting the definition of accommodation. However, patients with circulating HLA-specific antibody have a greater likelihood of later graft loss, indicating that, if accommodation occurs, then it is either

transient or insufficient to prevent CAMR. Long-term, complete accommodation has not been documented for MHC molecules, and the phenomenon might therefore be partly determined by the nature of the antigen.

Anti-apoptotic proteins. The resistance of the graft to the acute effects of antibody and complement is usually thought of in terms of resistance developed by the graft endothelium through the expression of cytoprotective proteins⁹⁶. Accommodated rodent xenografts have increased expression of the anti-apoptotic proteins B-cell lymphoma 2 (BCL-2), BCL-X_L and haem oxygenase 1 (HO1)⁹⁷ (FIG. 4). Increased expression of BCL-X_L was found in the endothelium of renal grafts from patients with circulating donor-HLA-specific antibodies, and BCL-X_L expression could be induced in cultured human endothelial cells by HLA-specific antibodies⁹⁸. Further studies showed that low concentrations of HLA-class-I-specific antibodies increased endothelial-cell expression of BCL-2, BCL-X_L and HO1, and increased the activity of phosphatidylinositol 3-kinase and AKT, which phosphorylate and inactivate the pro-apoptotic protein BAD (BCL-2-antagonist of cell death)⁹⁹. Differential gene expression occurs in accommodated human grafts. DNA-microarray analysis of gene expression in accommodated, ABO-incompatible renal allografts in humans has detected higher levels of mRNA encoding signalling molecules (including SMADs (mothers against decapentaplegic homologues) and protein tyrosine kinases), TNF and mucin-1, but not BCL-2, BCL-X_L or HO1, than in ABO-compatible grafts¹⁰⁰.

Complement-regulatory factors. Complement regulation is thought to be involved in accommodation (FIG. 3). Four cell-surface inhibitors of classical and alternative complement activation have been identified in humans. Three of these inhibitors — CRI1, decay-accelerating factor (DAF; CD55) and membrane cofactor protein (MCP; CD46) — inhibit both the classical and the alternative complement pathways at the level of C3 convertase and C5 convertase; the fourth inhibitor — CD59 (also known as protectin) — inhibits the MAC. In humans, normal renal capillary endothelium expresses low or undetectable levels of these inhibitory molecules, except for CD59, which is expressed at variable levels¹⁰. So, at the peritubular capillary surface, classical-pathway activation of C4 is relatively unopposed. Mice and rats do not express MCP, but they do express another protein, complement-receptor-related protein (CRRY), that has the functions of MCP and DAF¹⁰¹ and is strongly expressed by endothelium¹⁰¹.

The expression of DAF by cultured endothelial cells is increased in the presence of thrombin or vascular endothelial growth factor, through protein kinase C (PKC)-dependent pathways. DAF expression is also promoted by exposure to TNF, IFN- γ , C5b–C9 or bFGF, through PKC-independent pathways¹⁰². These mediators do not affect endothelial-cell expression

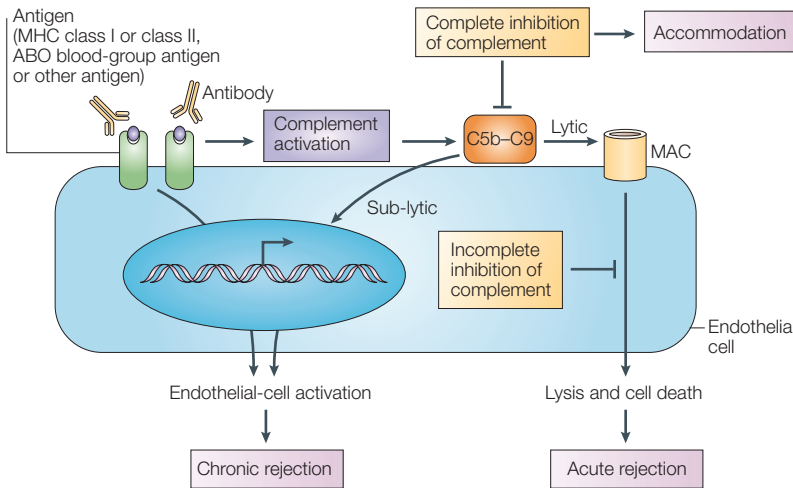


Figure 5 | Schematic diagram of the three postulated outcomes of the binding of complement-fixing alloantibody to endothelial cells. Full activation of complement leads to lysis of endothelial cells and acute rejection. Endothelial-cell activation might also be important in acute rejection (for example, by promoting coagulation). If complement activation is completely inhibited, then no injury results (and this state is known as accommodation). Incomplete inhibition of complement might be sufficient to prevent cell lysis but not complement activation, leading to endothelial-cell activation and chronic antibody-mediated rejection. Antibody alone, independent of complement, can activate endothelial cells and might cause pathogenesis. C, complement component; C5b–C9, C5b–C6–C7–C8–C9; MAC, membrane attack complex.

of CD59 or MCP. The upregulation of DAF expression by bFGF inhibits complement-mediated cytotoxicity of endothelial cells and reduces deposition of C3 *in vitro*. We have shown that DAF is required to prevent AAMR induced by low titres of α -gal (galactose- α -1,3-galactose)-specific antibodies¹⁰³. Exposure to C-reactive protein increases the expression of CD59, DAF and MCP by cultured endothelial cells¹⁰⁴. Antibody alone can also promote the expression of complement-regulatory proteins. For example, cultured pig endothelial cells respond to the presence of human natural IgM that is specific for pig α -Gal by synthesizing more cell-surface CD59 (REF. 104). Expression of CD59 was identified in allografts that could recover function after rejection⁶². We have reported that increased CD59 expression by endothelial cells is a common event in acute and chronic renal-allograft rejection and that increased endothelial-cell expression of DAF is found occasionally in CAMR (L. Cornell, P. Della Pelle, N. Brousaides and R.B.C., unpublished observations). Although this response might function to attenuate and contribute to recovery from AAMR, the accommodation was inadequate to prevent the onset of CAMR. A short-term study of three pig-to-baboon cardiac xenografts showing accommodation supported the concept that accommodation is a consequence of the inhibition of full complement activation¹⁰⁵. Grafts showed deposition of early (C4d and C3d), but not late (C5b and MAC), complement components on the microvasculature. The block in the complement pathway did not seem to result from the increased expression of complement-regulatory proteins but rather from

the persistence of heparan sulphate, which can aid in complement control. Preservation of syndecan-4 and HO1 expression by the endothelium was also observed. Further studies are required to understand how complement activation is controlled in accommodated grafts.

Mechanisms that mimic accommodation. Other mechanisms that could mimic accommodation have also been reported. Circulating endothelial progenitor cells of recipient origin might repopulate donor vessels, replacing donor endothelium¹⁰⁶. Complete loss of expression of donor ABO blood-group antigens from graft endothelium has been described to occur after 5 years¹⁰⁷. However, other evidence does not support a substantial replacement of donor endothelium with recipient endothelium, because most replacement does not occur in accommodated grafts but in grafts with evidence of past acute rejection¹⁰⁸. Another potential mechanism that could mimic accommodation is the production of non-complement-fixing antibody, as has been described for mice^{95,109}, which potentially competes with complement-fixing antibody and induces or mimics accommodation.

Accommodation is assumed to be all-or-none, but we propose that there are gradations. A graft that is transplanted into a pre-sensitized recipient has no opportunity for accommodation, and hyperacute rejection and graft loss occur rapidly (within minutes to hours). After transplantation, partial accommodation might occur, which would function to attenuate and slow the process of antibody-mediated rejection. In some cases, the accommodation might be sufficient to prevent only acute endothelial injury but insufficient to prevent smouldering, subclinical endothelial-cell injury or activation that might lead to chronic rejection. So, we propose that CAMR might be partially accommodated AAMR (FIG. 5). Drugs that promote more effective accommodation would potentially be useful clinically.

Therapy today

At present, treatment of AAMR is still evolving and cannot be adequately summarized in this Review. The most common strategies are based on the quick reduction of antibody titres using plasmapheresis, plus immunosuppression using drugs such as tacrolimus (Prograf; Astellas Pharma US, Inc.) or mycophenolic acid³³. INTRAVENOUS IMMUNOGLOBULIN (IVIG) is often used because of its immunomodulatory effects, especially on B cells and antibody. IVIG inhibits MIXED LYMPHOCYTE REACTIONS and induces apoptosis mainly in B cells¹¹⁰. *In vivo*, IVIG reduces the number of B cells and monocytes, and it reduces CD19, CD20 and CD40 expression by B cells, thereby modulating B-cell signalling¹¹¹. IVIG inhibits binding of donor-reactive antibodies to target cells in ~80% of patients, indicating that the presence of blocking antibodies might explain the efficacy of IVIG, although the mechanism is not known¹¹¹. IVIG might also function, in part, by inhibition of complement activation¹¹².

INTRAVENOUS IMMUNOGLOBULIN
Immunoglobulin that is pooled from a large number of individuals. It is used as a replacement for patients who have been depleted of immunoglobulins and for the immunomodulatory treatment of patients with some immune disorders.

MIXED LYMPHOCYTE REACTION
A tissue-culture technique for testing T-cell reactivity. The proliferation of responder T cells (in this case, recipient T cells) that is induced by exposure to inactivated stimulator cells (in this case, donor T cells) is determined by measuring the incorporation of ³H-thymidine into the DNA of dividing cells.

New therapies

B cells. There are no therapeutic small molecules available that interfere selectively with B-cell function. The most specific drug, rituximab (Rituxan; Genentech, Inc. and Biogen Idec Inc.), binds CD20 at the surface of precursor and mature B cells and leads to transient B-cell depletion, with typical B-cell recovery after 6–9 months. Preliminary studies indicate that rituximab decreases the concentration of pre-existing and post-transplantation antibodies^{108,113–115}. Conclusions and extrapolations from these studies are limited, because rituximab is usually combined with other therapies in these small and uncontrolled trials. The risk of bacterial infection as a result of immunoglobulin deficiency is also an important consideration. It is therefore necessary to search for other potential therapeutic targets. Memory B cells are heterogeneous but have cell-surface markers (CD24, CD27, CD43 and CD79b) that are potential therapeutic targets¹¹⁶. B cells also express TACI (transmembrane activator and calcium-modulating cyclophilin-ligand interactor), BCMA (B-cell maturation antigen) and BAFF receptor (B-cell-activating factor receptor), all of which are members of the TNF-receptor family that are triggered by the ligands BAFF and APRIL (a proliferation inducing ligand), which are expressed at the cell surface of DCs¹¹⁷. A soluble TACI-immunoglobulin fusion protein blocks B-cell development by inhibiting the interaction between B cells and DCs¹¹⁸. These cell-surface markers might be useful targets to prevent the development of B cells into plasma cells.

Plasma cells. Normal plasma cells express little or no CD20 and are therefore resistant to rituximab-mediated depletion. Several cell-surface molecules that are expressed by plasma cells might be considered as drug targets — syndecan-1 (CD138), CD38, $\alpha_4\beta_1$ -integrin (CD49d-CD29) and CXC-chemokine receptor 4 (CXCR4) — although none of these is entirely plasma-cell specific. Plasma-cell longevity is thought to be an extrinsic phenomenon that is mediated by survival signals delivered by bone-marrow stromal cells. Recent findings in rodents show that BAFF delivers survival signals through ligation of the receptor BCMA at the surface of plasma cells. The TACI-immunoglobulin fusion protein blocks these interactions by binding BAFF¹¹⁹. Indeed, treatment of mice with soluble TACI-immunoglobulin depleted long-lived plasma cells¹¹⁸, and TACI-immunoglobulin is already in Phase I clinical trials (which are being carried out by ZymoGenetics and Serono S.A.) for the treatment of systemic lupus erythematosus and B-cell malignancies. Because the transcription factors BLIMP1 (B-lymphocyte-induced maturation protein 1) and XBP1 (X-box-binding protein 1) (as well as the repression of *PAX5*, paired box gene 5) are required to maintain plasma-cell function, their inhibition might result in the loss of plasma-cell function⁶⁰.

Complement antagonists. Complement antagonists could prevent the acute pathological effects of complement activation. For example, soluble CR1 delays antibody-mediated rejection in xenograft models but is insufficient to prevent graft rejection completely⁷⁰. Other complement antagonists, such as C5-specific antibody, which blocks activation of C5 and formation of both C5a and the MAC, are in preliminary stages of evaluation (by Alexion Pharmaceuticals Inc.). Transgenic expression of human complement-regulatory proteins (DAF and CD59) in pigs has shown potency for preventing xenograft rejection⁶⁹, but the relevance of these studies to allografts needs to be extended and tested.

Tolerance protocols. Infants have been successfully transplanted with hearts from ABO-incompatible donors. Newborn infants lack antibodies that are specific for the ABO blood-group antigens, because these antibodies are mainly IgM and are therefore not transported across the placenta¹²⁰. Infants with ABO-incompatible grafts develop B-cell unresponsiveness (that is, tolerance) to donor A and/or B antigens in the graft. Tolerance seems to occur by elimination of donor-reactive B cells and might depend on the persistence of low levels of antigen expression¹²¹. Curiously, in mixed-chimerism protocols in adults, antibody-mediated rejection of HLA-mismatched kidneys has been observed (T. Kawai, D. Sachs, T. Spitzer, N. Tolckoff-Rubin, F. Delmonico, F. Saidman, J. Shaffer, W. Williams, B. Dey, S. McAfee, D. Ko, M. Hertl, N. Goes, W. Wong, J. Fishman, R.B.C., M. Sykes and A. Cosimi, unpublished observations). Therapy with the antibody Campath-1H (alemtuzumab; Campath; Genzyme Corporation) and rapamycin, which together cause marked T- and B-cell depletion, was also shown to lead to a higher risk of AAMR¹²². Why AAMR should emerge during the conditioning period, when robust T-cell depletion occurs, is unclear. It could be that T cells that normally inhibit B-cell function are depleted or that sensitized memory cells escape depletion.

Conclusion

Alloantibodies are a substantial obstacle to short- and long-term graft survival. Antibodies that are specific for alloantigens at the surface of donor endothelial cells might have various pathological consequences, including endothelial-cell activation or lysis. Alternatively, under some circumstances, antibodies or antibodies together with complement activation might cause graft accommodation. To prevent or reduce alloantibody titres, more insights are needed to improve our understanding of the regulation of B cells and the developmental and differentiation pathways of memory B cells and plasma cells. Methods to promote accommodation and to prevent or mitigate acute complement-mediated graft injury are desperately needed. Such methods and insights will create a scientific basis for the development of innovative approaches to prevent and treat antibody-mediated rejection.

1. Manning, D. D., Reed, N. D. & Shaffer, C. F. Maintenance of skin xenografts of widely divergent phylogenetic origin of congenitally athymic (nude) mice. *J. Exp. Med.* **138**, 488–494 (1973).
2. Pascual, M. *et al.* in *Clinical Transplants 2001* 123–130 (UCLA Immunogenetics Center, Los Angeles, 2002).
3. Gorer, P. The detection of antigenic differences in mouse erythrocytes by the employment of immune sera. *Br. J. Exp. Pathol.* **17**, 42–50 (1936).
4. Gerlag, P. G., Koene, R. A., Hagemann, J. F. & Wijdeveld, P. G. Hyperacute rejection of skin allografts in the mouse. Sensitivity of ingrowing skin grafts to the action of alloantibody and rabbit complement. *Transplantation* **20**, 308–313 (1975).
5. Jooste, S. V., Colvin, R. B., Soper, W. D. & Winn, H. J. The vascular bed as the primary target in the destruction of skin grafts by antiserum. I. Resistance of freshly placed xenografts of skin to antiserum. *J. Exp. Med.* **154**, 1319–1331 (1981).
6. Jeannot, M., Pinn, V. W., Flax, M. H., Winn, H. J. & Russell, P. S. Humoral antibodies in renal allotransplantation in man. *N. Engl. J. Med.* **282**, 111–117 (1970).
7. Russell, P. S., Chase, C. M. & Colvin, R. B. Alloantibody- and T cell-mediated immunity in the pathogenesis of transplant arteriosclerosis: lack of progression to sclerotic lesions in B cell-deficient mice. *Transplantation* **64**, 1531–1536 (1997).
8. Halloran, P. F., Schlaut, J., Solez, K. & Srinivasa, N. S. The significance of the anti-class I antibody response. II. Clinical and pathologic features of renal transplants with anti-class I-like antibody. *Transplantation* **53**, 550–555 (1992).
9. Feucht, H. E. *et al.* Vascular deposition of complement-split products in kidney allografts with cell-mediated rejection. *Clin. Exp. Immunol.* **86**, 464–470 (1991).
10. Collins, A. B. *et al.* Complement activation in acute humoral renal allograft rejection: diagnostic significance of C4d deposits in peritubular capillaries. *J. Am. Soc. Nephrol.* **10**, 2208–2214 (1999).
This was the first paper to show that the presence of C4d in the peritubular capillaries of renal allografts is associated with circulating donor-HLA-specific antibodies and distinctive histological features.
11. Pei, R., Lee, J. H., Shih, N. J., Chen, M. & Terasaki, P. I. Single human leukocyte antigen flow cytometry beads for accurate identification of human leukocyte antigen antibody specificities. *Transplantation* **75**, 43–49 (2003).
12. Jaramillo, A. *et al.* Anti-HLA class I antibody binding to airway epithelial cells induces production of fibrogenic growth factors and apoptotic cell death: a possible mechanism for bronchiolitis obliterans syndrome. *Hum. Immunol.* **64**, 521–529 (2003).
13. Takemoto, S. K. *et al.* National conference to assess antibody-mediated rejection in solid organ transplantation. *Am. J. Transplant.* **4**, 1033–1041 (2004).
14. Racusen, L. C. *et al.* Antibody-mediated rejection criteria — an addition to the Banff 97 classification of renal allograft rejection. *Am. J. Transplant.* **3**, 708–714 (2003).
15. Mauiyyedi, S. *et al.* Acute humoral rejection in kidney transplantation: II. Morphology, immunopathology, and pathologic classification. *J. Am. Soc. Nephrol.* **13**, 779–787 (2002).
16. Nickleit, V., Zeiler, M., Gudat, F., Thiel, G. & Mihatsch, M. J. Detection of the complement degradation product C4d in renal allografts: diagnostic and therapeutic implications. *J. Am. Soc. Nephrol.* **13**, 242–251 (2002).
17. Herzenberg, A. M., Gill, J. S., Djurdjev, O. & Magil, A. B. C4d deposition in acute rejection: an independent long-term prognostic factor. *J. Am. Soc. Nephrol.* **13**, 234–241 (2002).
18. Lorenz, M. *et al.* Risk factors for capillary C4d deposition in kidney allografts: evaluation of a large study cohort. *Transplantation* **78**, 447–452 (2004).
19. Trpkov, K. *et al.* Pathologic features of acute renal allograft rejection associated with donor-specific antibody: analysis using the Banff grading schema. *Transplantation* **61**, 1586–1592 (1996).
20. Bohmig, G. A. *et al.* Capillary C4d deposition in kidney allografts: a specific marker of alloantibody-dependent graft injury. *J. Am. Soc. Nephrol.* **13**, 1091–1099 (2002).
21. Magil, A. B. & Tinckam, K. Monocytes and peritubular capillary C4d deposition in acute renal allograft rejection. *Kidney Int.* **63**, 1888–1893 (2003).
22. Lederer, S. R. *et al.* Impact of humoral alloreactivity early after transplantation on the long-term survival of renal allografts. *Kidney Int.* **59**, 334–341 (2001).
This is a recent paper by the research group of Helmut Feucht, who was the first to stain renal allografts for C4d and show that it predicted a poor prognosis (reported in reference 9).
23. Haas, M., Ratner, L. E. & Montgomery, R. A. C4d staining of perioperative renal transplant biopsies. *Transplantation* **74**, 711–717 (2002).
24. Rotman, S., Collins, A. B. & Colvin, R. B. C4d deposition in allografts: current concepts and interpretation. *Transplant. Rev.* (in the press).
25. Hammond, E. H. *et al.* Vascular (humoral) rejection in heart transplantation: pathologic observations and clinical implications. *J. Heart Transplant.* **8**, 430–443 (1989).
26. John, R. *et al.* Immunologic sensitization in recipients of left ventricular assist devices. *J. Thorac. Cardiovasc. Surg.* **125**, 578–591 (2003).
27. Smith, R. N. *et al.* C4d deposition in cardiac allografts correlates with alloantibody. *J. Heart Lung Transplant.* **24**, 1202–1210 (2005).
This paper shows that C4d deposition in myocardial capillaries is associated with circulating HLA-specific antibodies rather than ischaemia.
28. Michaels, P. J. *et al.* Humoral rejection in cardiac transplantation: risk factors, hemodynamic consequences and relationship to transplant coronary artery disease. *J. Heart Lung Transplant.* **22**, 58–69 (2003).
29. Reed, E. F. *et al.* Monitoring of soluble HLA alloantigens and anti-HLA antibodies identifies heart allograft recipients at risk of transplant-associated coronary artery disease. *Transplantation* **61**, 566–572 (1996).
30. Behr, T. M. *et al.* Detection of humoral rejection in human cardiac allografts by assessing the capillary deposition of complement fragment C4d in endomyocardial biopsies. *J. Heart Lung Transplant.* **18**, 904–912 (1999).
31. Magro, C. M. *et al.* Use of C4d as a diagnostic adjunct in lung allograft biopsies. *Am. J. Transplant.* **3**, 1143–1154 (2003).
32. Krukemeyer, M. G. *et al.* Description of B lymphocytes and plasma cells, complement, and chemokines/receptors in acute liver allograft rejection. *Transplantation* **78**, 65–70 (2004).
33. Terasaki, P. I. & Ozawa, M. Predicting kidney graft failure by HLA antibodies: a prospective trial. *Am. J. Transplant.* **4**, 438–443 (2004).
34. Piazza, A. *et al.* Impact of donor-specific antibodies on chronic rejection occurrence and graft loss in renal transplantation: posttransplant analysis using flow cytometric techniques. *Transplantation* **71**, 1106–1112 (2001).
35. Pelletier, R. P. *et al.* Clinical significance of MHC-reactive alloantibodies that develop after kidney or kidney-pancreas transplantation. *Am. J. Transplant.* **2**, 134–141 (2002).
36. Worthington, J. E., Martin, S., Al-Husseini, D. M., Dyer, P. A. & Johnson, R. W. Posttransplantation production of donor HLA-specific antibodies as a predictor of renal transplant outcome. *Transplantation* **75**, 1034–1040 (2003).
This report shows that circulating antibodies that are specific for donor HLA class I or class II antigens predict the subsequent development of chronic rejection of a renal allograft.
37. Mauiyyedi, S. *et al.* Chronic humoral rejection: identification of antibody-mediated chronic renal allograft rejection by C4d deposits in peritubular capillaries. *J. Am. Soc. Nephrol.* **12**, 574–582 (2001).
The paper identifies and establishes the immunopathological criteria for a new form of chronic renal-allograft rejection that is mediated by antibodies.
38. Regele, H. *et al.* Capillary deposition of complement split product C4d in renal allografts is associated with basement membrane injury in peritubular and glomerular capillaries: a contribution of humoral immunity to chronic allograft rejection. *J. Am. Soc. Nephrol.* **13**, 2371–2380 (2002).
This was the first paper to show that the presence of C4d in the peritubular capillaries of renal allografts precedes the development of chronic allograft glomerulopathy. It also shows that C4d deposition correlates with lamination of the peritubular capillary basement membrane.
39. Vongwiwatana, A., Gourishankar, S., Campbell, P. M., Solez, K. & Halloran, P. F. Peritubular capillary changes and C4d deposits are associated with transplant glomerulopathy but not IgA nephropathy. *Am. J. Transplant.* **4**, 124–129 (2004).
40. Sijpkens, Y. W. *et al.* Immunologic risk factors and glomerular C4d deposits in chronic transplant glomerulopathy. *Kidney Int.* **65**, 2409–2418 (2004).
41. Erlich, H. A., Opelz, G. & Hansen, J. HLA DNA typing and transplantation. *Immunity* **14**, 347–356 (2001).
42. Race, R. R. & Sanger, R. *Blood Groups in Man* (Blackwell Scientific, Oxford, 1958).
43. Chao, N. J. Minors come of age: minor histocompatibility antigens and graft-versus-host disease. *Biol. Blood Marrow Transplant.* **10**, 215–223 (2004).
44. Derhaag, J. G., Duijvestijn, A. M., Damoiseaux, J. G. & van Breda Vriesman, P. J. Effects of antibody reactivity to major histocompatibility complex (MHC) and non-MHC alloantigens on graft endothelial cells in heart allograft rejection. *Transplantation* **69**, 1899–1906 (2000).
45. Wu, G. D. *et al.* Vascular endothelial cell apoptosis induced by anti-donor non-MHC antibodies: a possible injury pathway contributing to chronic allograft rejection. *J. Heart Lung Transplant.* **21**, 1174–1187 (2002).
46. Kooijmans-Coutinho, M. F. *et al.* Interstitial rejection, vascular rejection, and diffuse thrombosis of renal allografts. Predisposing factors, histology, immunohistochemistry, and relation to outcome. *Transplantation* **61**, 1338–1344 (1996).
47. Sumitran-Holgersson, S., Wilczek, H. E., Holgersson, J. & Soderstrom, K. Identification of the nonclassical HLA molecules, MICA, as targets for humoral immunity associated with irreversible rejection of kidney allografts. *Transplantation* **74**, 268–277 (2002).
48. Mizutani, K. *et al.* Serial ten-year follow-up of HLA and MICA antibody production prior to kidney graft failure. *Am. J. Transplant.* **5**, 1–8 (2005).
49. Dragun, D. *et al.* Angiotensin II type 1-receptor activating antibodies in renal-allograft rejection. *N. Engl. J. Med.* **352**, 558–569 (2005).
50. Jurcevic, S. *et al.* Antivimentin antibodies are an independent predictor of transplant-associated coronary artery disease after cardiac transplantation. *Transplantation* **71**, 886–892 (2001).
51. Morgun, A. *et al.* Pre- and post-transplant anti-myosin and anti-heat shock protein antibodies and cardiac transplant outcome. *J. Heart Lung Transplant.* **23**, 204–209 (2004).
52. Austen, W. G. Jr *et al.* Murine hindlimb reperfusion injury can be initiated by a self-reactive monoclonal IgM. *Surgery* **136**, 401–406 (2004).
53. Mitchison, N. A. T-cell–B-cell cooperation. *Nature Rev. Immunol.* **4**, 308–312 (2004).
54. Baratin, M., Bonin, K. & Daniel, C. Peripheral priming of alloreactive T cells by the direct pathway of allorecognition. *Eur. J. Immunol.* **34**, 3305–3314 (2004).
55. Steele, D. J. *et al.* Two levels of help for B cell alloantibody production. *J. Exp. Med.* **183**, 699–703 (1996).
56. Edwards, J. C. *et al.* Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. *N. Engl. J. Med.* **350**, 2572–2581 (2004).
57. Asiedu, C. K., Dong, S. S., Lobashevsky, A., Jenkins, S. M. & Thomas, J. M. Tolerance induced by anti-CD3 immunotoxin plus 15-deoxyspergualin associates with donor-specific indirect pathway unresponsiveness. *Cell. Immunol.* **223**, 103–112 (2003).
58. Epstein, M. M., Di Rosa, F., Jankovic, D., Sher, A. & Matzinger, P. Successful T cell priming in B cell-deficient mice. *J. Exp. Med.* **182**, 915–922 (1995).
59. Williams, G. S., Oxenius, A., Hengartner, H., Benoist, C. & Mathis, D. CD4⁺ T cell responses in mice lacking MHC class II molecules specifically on B cells. *Eur. J. Immunol.* **28**, 3763–3772 (1998).
60. Shapiro-Shelef, M. & Calame, K. Regulation of plasma-cell development. *Nature Rev. Immunol.* **5**, 230–242 (2005).
61. Kerjaschki, D. *et al.* Lymphatic neoangiogenesis in human kidney transplants is associated with immunologically active lymphocytic infiltrates. *J. Am. Soc. Nephrol.* **15**, 603–612 (2004).
62. Sanwal, M. *et al.* Molecular heterogeneity in acute renal allograft rejection identified by DNA microarray profiling. *N. Engl. J. Med.* **349**, 125–138 (2003).
63. Cassese, G. *et al.* Inflamed kidneys of NZB/W mice are a major site for the homeostasis of plasma cells. *Eur. J. Immunol.* **31**, 2726–2732 (2001).
64. Desvaux, D. *et al.* γ -Interferon expression and poor clinical outcome. *Nephrol. Dial. Transplant.* **19**, 933–939 (2004).
65. Hidalgo, L. G. & Halloran, P. F. Role of IFN- γ in allograft rejection. *Crit. Rev. Immunol.* **22**, 317–349 (2002).
66. Auchincloss, H. Jr & Sachs, D. H. Xenogeneic transplantation. *Annu. Rev. Immunol.* **16**, 433–470 (1998).
67. Wasowska, B. A. *et al.* Passive transfer of alloantibodies restores acute cardiac rejection in IgKO mice. *Transplantation* **71**, 727–736 (2001).

68. Rahimi, S. *et al.* Non-complement- and complement-activating antibodies synergize to cause rejection of cardiac allografts. *Am. J. Transplant.* **4**, 326–334 (2004).
69. Menoret, S. *et al.* Characterization of human CD55 and CD59 transgenic pigs and kidney xenotransplantation in the pig-to-baboon combination. *Transplantation* **77**, 1468–1471 (2004).
70. Azimzadeh, A. *et al.* Hyperacute lung rejection in the pig-to-human model. 2. Synergy between soluble and membrane complement inhibition. *Xenotransplantation* **10**, 120–131 (2003).
71. Proding, W. M., Wurzn, R., Stoiber, H. & Dierich, M. P. in *Fundamentals of Immunology* (ed. Paul, W. E.) 1077–1103 (Lippincott Williams & Wilkins, Philadelphia, 2003).
72. van den Elsen, J. M. *et al.* X-ray crystal structure of the C4d fragment of human complement component C4. *J. Mol. Biol.* **322**, 1103–1115 (2002).
73. Baldwin, W. M. 3rd, Kasper, E. K., Zachary, A. A., Wasowska, B. A. & Rodriguez, E. R. Beyond C4d: other complement-related diagnostic approaches to antibody-mediated rejection. *Am. J. Transplant.* **4**, 311–318 (2004).
74. Sund, S. *et al.* Complement activation in early protocol kidney graft biopsies after living-donor transplantation. *Transplantation* **75**, 1204–1213 (2003).
75. Nakashima, S., Qian, Z., Rahimi, S., Wasowska, B. A. & Baldwin, W. M. 3rd. Membrane attack complex contributes to destruction of vascular integrity in acute lung allograft rejection. *J. Immunol.* **169**, 4620–4627 (2002).
76. Saadi, S. & Platt, J. L. Humoral rejection and endothelial cell activation, 2001. *Xenotransplantation* **9**, 239–241 (2002).
77. Albrecht, E. A. *et al.* C5a-induced gene expression in human umbilical vein endothelial cells. *Am. J. Pathol.* **164**, 849–859 (2004).
78. Monsinjon, T. *et al.* Regulation by complement C3a and C5a anaphylatoxins of cytokine production in human umbilical vein endothelial cells. *FASEB J.* **17**, 1003–1014 (2003).
79. Saadi, S., Holzknicht, R. A., Patte, C. P. & Platt, J. L. Endothelial cell activation by pore-forming structures: pivotal role for interleukin-1 α . *Circulation* **101**, 1867–1873 (2000).
80. Benzaquen, L. R., Nicholson-Weller, A. & Halperin, J. A. Terminal complement proteins C5b–9 release basic fibroblast growth factor and platelet-derived growth factor from endothelial cells. *J. Exp. Med.* **179**, 985–992 (1994).
81. Selvan, R. S., Kapadia, H. B. & Platt, J. L. Complement-induced expression of chemokine genes in endothelium: regulation by IL-1-dependent and -independent mechanisms. *J. Immunol.* **161**, 4388–4395 (1998).
82. Kilgore, K. S. *et al.* Sublytic concentrations of the membrane attack complex of complement induce endothelial interleukin-8 and monocyte chemoattractant protein-1 through nuclear factor- κ B activation. *Am. J. Pathol.* **150**, 2019–2031 (1997).
83. Ikeda, K. *et al.* C5a induces tissue factor activity on endothelial cells. *Thromb. Haemost.* **77**, 394–398 (1997).
84. Saadi, S., Holzknicht, R. A., Patte, C. P., Stern, D. M. & Platt, J. L. Complement-mediated regulation of tissue factor activity in endothelium. *J. Exp. Med.* **182**, 1807–1814 (1995).
85. Dempsey, P. W., Allison, M. E., Akkaraju, S., Goodnow, C. C. & Fearon, D. T. C3d of complement as a molecular adjuvant: bridging innate and acquired immunity. *Science* **271**, 348–350 (1996).
86. Qian, Z. *et al.* C6 produced by macrophages contributes to cardiac allograft rejection. *Am. J. Pathol.* **155**, 1293–1302 (1999).
87. Ota, H. *et al.* Terminal complement components mediate release of von Willebrand factor and adhesion of platelets in arteries of allografts. *Transplantation* **79**, 276–281 (2005).
88. Pratt, J. R., Basheer, S. A. & Sacks, S. H. Local synthesis of complement component C3 regulates acute renal transplant rejection. *Nature Med.* **8**, 582–587 (2002).
89. Smith, J. D., Lawson, C., Yacoub, M. H. & Rose, M. L. Activation of NF- κ B in human endothelial cells induced by monoclonal and allospecific HLA antibodies. *Int. Immunol.* **12**, 563–571 (2000).
90. Jin, Y. P. *et al.* Ligation of HLA class I molecules on endothelial cells induces phosphorylation of Src, paxillin, and focal adhesion kinase in an actin-dependent manner. *J. Immunol.* **168**, 5415–5423 (2002).
- This is the most recent in a series of papers from this research group. It identifies the activation pathways in endothelial cells that are triggered by HLA-specific antibodies.**
91. Yuan, F. F. *et al.* Association of Fc γ receptor IIA polymorphisms with acute renal-allograft rejection. *Transplantation* **78**, 766–769 (2004).
92. Koch, C. A., Khalpey, Z. I. & Platt, J. L. Accommodation: preventing injury in transplantation and disease. *J. Immunol.* **172**, 5143–5148 (2004).
93. Alexandre, G. P. J. *et al.* Present experience in a series of 26 ABO-incompatible living donor renal allografts. *Transplant. Proc.* **19**, 4538–4544 (1987).
94. Shishido, S. *et al.* ABO-incompatible living-donor kidney transplantation in children. *Transplantation* **72**, 1037–1042 (2001).
95. Ogawa, H. *et al.* Mouse-heart grafts expressing an incompatible carbohydrate antigen. II. Transition from accommodation to tolerance. *Transplantation* **77**, 366–373 (2004).
96. Soares, M. P., Brouard, S., Smith, R. N. & Bach, F. H. Heme oxygenase-1, a protective gene that prevents the rejection of transplanted organs. *Immunol. Rev.* **184**, 275–285 (2001).
97. Tabata, T. *et al.* Accommodation after lung xenografting from hamster to rat. *Transplantation* **75**, 607–612 (2003).
98. Salama, A. D. *et al.* Transplant accommodation in highly sensitized patients: a potential role for Bcl-X $_L$ and alloantibody. *Am. J. Transplant.* **1**, 260–269 (2001).
- This paper shows antibody-induced increases in endothelial-cell expression of BCL-X $_L$, a potential molecular mechanism of accommodation in human renal transplants.**
99. Narayanan, K., Jaramillo, A., Phelan, D. L. & Mohanakumar, T. Pre-exposure to sub-saturating concentrations of HLA class I antibodies confers resistance to endothelial cells against antibody complement-mediated lysis by regulating Bad through the phosphatidylinositol 3-kinase/Akt pathway. *Eur. J. Immunol.* **34**, 2303–2312 (2004).
100. Park, W. D. *et al.* Accommodation in ABO-incompatible kidney allografts, a novel mechanism of self-protection against antibody-mediated injury. *Am. J. Transplant.* **3**, 952–960 (2003).
101. Lee, P. C. *et al.* All chronic rejection failures of kidney transplants were preceded by the development of HLA antibodies. *Transplantation* **74**, 1192–1194 (2002).
102. Mason, J. C. *et al.* Decay-accelerating factor induction on vascular endothelium by vascular endothelial growth factor (VEGF) is mediated via a VEGF receptor-2 (VEGF-R2)- and protein kinase C- α (PKC α)-dependent cytoprotective signaling pathway and is inhibited by cyclosporin A. *J. Biol. Chem.* **279**, 41611–41618 (2004).
103. Shimizu, I. *et al.* DAF prevents acute humoral rejection induced by low titres of anti- α Gal antibodies in GAL KO mice. *Transplantation* (in the press).
104. Grubbs, B. C., Benson, B. A. & Dalmasso, A. P. Characteristics of CD59 up-regulation induced in porcine endothelial cells by α Gal ligation and its association with protection from complement. *Xenotransplantation* **10**, 387–397 (2003).
105. Williams, J. M. *et al.* Acute vascular rejection and accommodation: divergent outcomes of the humoral response to organ transplantation. *Transplantation* **78**, 1471–1478 (2004).
106. Hillebrands, J. L. *et al.* Origin of neointimal endothelium and α -actin-positive smooth muscle cells in transplant arteriosclerosis. *J. Clin. Invest.* **107**, 1411–1422 (2001).
107. Koestner, S. C. *et al.* Histo-blood group type change of the graft from B to O after ABO mismatched heart transplantation. *Lancet* **363**, 1523–1525 (2004).
108. Lagaaij, E. L. *et al.* Endothelial cell chimerism after renal transplantation and vascular rejection. *Lancet* **357**, 33–37 (2001).
109. Mohiuddin, M. M., Ogawa, H., Yin, D. P., Shen, J. & Gallili, U. Antibody-mediated accommodation of heart grafts expressing an incompatible carbohydrate antigen. *Transplantation* **75**, 258–262 (2003).
- This report describes a pilot study of gene expression in ABO-incompatible human cardiac allografts with accommodation.**
110. Toyoda, M., Petrosyan, A., Pao, A. & Jordan, S. C. Immunomodulatory effects of combination of pooled human gammaglobulin and rapamycin on cell proliferation and apoptosis in the mixed lymphocyte reaction. *Transplantation* **78**, 1134–1138 (2004).
111. Jordan, S. C. *et al.* Intravenous immune globulin treatment inhibits crossmatch positivity and allows for successful transplantation of incompatible organs in living-donor and cadaver recipients. *Transplantation* **76**, 631–636 (2003).
112. Frank, M. M., Miletic, V. D. & Jiang, H. Immunoglobulin in the control of complement action. *Immunol. Res.* **22**, 137–146 (2000).
113. Vieira, C. A. *et al.* Rituximab for reduction of anti-HLA antibodies in patients awaiting renal transplantation: 1. Safety, pharmacodynamics, and pharmacokinetics. *Transplantation* **77**, 542–548 (2004).
- This paper describes encouraging results from a Phase I clinical trial of rituximab in pre-sensitized patients who are awaiting a renal transplant. It shows some efficacy at the inhibition of presumed pre-existing memory B cells and long-lived plasma cells.**
114. Gloor, J. M. *et al.* Overcoming a positive crossmatch in living-donor kidney transplantation. *Am. J. Transplant.* **3**, 1017–1023 (2003).
115. Tyden, G., Kumlien, G. & Fehrman, I. Successful ABO-incompatible kidney transplantations without splenectomy using antigen-specific immunoabsorption and rituximab. *Transplantation* **76**, 730–731 (2003).
116. McHeyzer-Williams, L. J. & McHeyzer-Williams, M. G. Antigen-specific memory B cell development. *Annu. Rev. Immunol.* **23**, 487–513 (2005).
117. Craxton, A., Magaletti, D., Ryan, E. J. & Clark, E. A. Macrophage- and dendritic cell-dependent regulation of human B-cell proliferation requires the TNF family ligand BAFF. *Blood* **101**, 4464–4471 (2003).
118. Gross, J. A. *et al.* TACH-Ig neutralizes molecules critical for B cell development and autoimmune disease: impaired B cell maturation in mice lacking BLYS. *Immunity* **15**, 289–302 (2001).
119. O'Connor, B. P. *et al.* BCMA is essential for the survival of long-lived bone marrow plasma cells. *J. Exp. Med.* **199**, 91–98 (2004).
120. West, L. J. *et al.* ABO-incompatible heart transplantation in infants. *N. Engl. J. Med.* **344**, 793–800 (2001).
121. Fan, X. *et al.* Donor-specific B-cell tolerance after ABO-incompatible infant heart transplantation. *Nature Med.* **10**, 1227–1233 (2004).
122. Knechtle, S. J. *et al.* Campath-1H induction plus rapamycin monotherapy for renal transplantation: results of a pilot study. *Am. J. Transplant.* **3**, 722–730 (2003).

Competing interests statement

The authors declare no competing financial interests.

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