Chronic Antibody-mediated Rejection
Review of Literature

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Chronic antibody-mediated rejection among kidney transplant recipients is a major unresolved problem which is covered in this review article which included different lines of its management.

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Review

INTRODUCTION

Late graft loss remains a major obstacle to successful long-term kidney allograft transplantation. Factors contributing to late graft loss include immunological factors (cellular and antibody-mediated injuries) and nonimmunological factors (donor disease, recurrent disease, peritransplant ischemia, viral infection or drug toxicity).[1] Several studies have shown that circulating anti-human leukocyte antigen (HLA) class I or II antibodies, either donor reactive,[2,3] or de novo non-donor reactive,[3,4] are found in a substantial fraction of kidney allograft recipients, and these are associated with later graft loss.

Antibody-mediated rejection (AMR) has become clinically critical because this form of rejection is usually unresponsive to conventional antirejection therapy, and therefore, it has been recognized as a major cause of allograft loss. Although current desensitization protocols have enabled transplantation across donor-specific antibody (DSA) barriers in a growing number of cases,[5,6] these protocols are neither consistently efficacious nor standardized. It reflects an incomplete understanding of the pathogenesis of alloantibody-induced injury as a major cause of allograft loss. Furthermore, patients treated with these modalities persist in having a high risk of multiple AMR episodes.

ANTI-HUMAN LEUKOCYTE ANTIGEN-SPECIFIC ANTIBODIES

In 1968, when kidney transplant patients were first examined for the development of antibodies after graft failure, antibodies were detected in 11 of 29 patients (38%) who had rejected their grafts.[7] The fact that some patients in desensitization protocols developed AMR and others with similar levels of DSA at baseline did not, has remained unexplained due to the lack of detailed studies of these patients posttransplantation. Burns and colleagues[8] aimed to define the natural history of AMR in highly sensitized patients undergoing positive cross-match kidney transplantation. They found that the serum DSA level after transplantation was the major determinant of AMR. Patients who developed high levels of DSA within the first month after transplantation almost invariably developed acute humoral rejection, whereas those who maintained low levels were rejection free. Importantly, more than half of the patients who had high levels of DSA at baseline did not develop high levels of DSA after transplantation. Almost all patients, including those who developed AMR, had a significant decrement or even disappearance of DSA early after transplantation.[9,10] This finding that increases in DSA levels in AMR may be transient and self-limited in many patients presents difficulties in assessing the effectiveness of therapy aimed at treating AMR.

During the 12th International Histocompatibility workshop, a multicenter prospective study was initiated to test patients with functioning kidney transplants once for HLA antibodies posttransplantation. The 806 patients without HLA antibodies had a subsequent 4-year graft survival of

81%, compared with 58% for 158 patients with HLA antibodies (the presence of anti-HLA antibodies led to 5% allograft loss every year; therefore, after 4 years, 20% of the grafts will be lost).[11]

Among 512 patients followed for 1 year after testing in Sao Paulo, 12% of antibody-positive patients lost their grafts, whereas graft failure occurred in only 5.5% of those without HLA antibodies ($P = .03$).[12] These results have been updated, demonstrating that at 3 years posttransplantation, patients without HLA antibodies had a 94% survival rate compared with 79% for those with HLA class II antibodies.[13] Worthington and coworkers[14] showed that among 12 patients who developed enzyme-linked immunosorbent assay-detected HLA antibodies after transplantation, 92% of the grafts failed, whereas among the 64 patients who remained negative, only 11% of the grafts failed ($P < .001$).

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

PATHOGENESIS AND MECHANISM

How allo-antibody and complement activation promote glomerulopathy, arteriopathy, and fibrosis is incompletely clear. Only in the past 7 years, a potential role of allo-antibodies for chronically deteriorating graft function has been postulated. Allo-antibodies preferentially attack a different "location," namely the peritubular and glomerular capillaries.

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.

Antigenic Targets

The main antigenic targets of AMR are major histocompatibility complex (MHC) molecules (both class I and class II)[15] and the ABO blood-group antigens.[16] The 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 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 1600 alleles in humans) aids their main function, which is antigen presentation to T cells.

Production of HLA-specific allo-antibodies depends on exposure to HLA molecules as a consequence of pregnancy, blood transfusion, or transplantation. These antibodies are mainly of the immunoglobulin G 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, B, or AB blood group in response to antigens from the environment, and they are usually of the immunoglobulin M class.[17]

Antibodies to MHC class I antigens can stimulate endothelial and smooth muscle proliferation and expression of fibroblast growth factor receptors.[18] Soluble terminal complement components (C5b-9) trigger the production of fibroblast growth factor and platelet-derived growth factor by endothelial cells.[19] Thus, antibodies and activated complements might induce gene products that promote endothelial activation and injury with consequent basement membrane duplication and arterial smooth muscle proliferation and thickening until finally, the characteristic atherosclerosis lesion of chronic rejection results in obstruction.[21,22]

In addition to MHC molecules and blood group antigens, minor histocompatibility antigens might also be targets of AMR. 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 tumor antigens.[22] In animal studies, non-MHC-specific antibodies can cause endothelial cell apoptosis and graft rejection.[23,24]

The antibody that is specific for MHC-class-I-

polypeptide-related sequence A can be detected in kidney allograft recipients and is associated with later rejection and graft loss,[25,26] that was demonstrated by Zou and coworkers[27] who found that antibodies against minor histocompatibility antigens such as MHC-class-I-polypeptide-related sequence A may be associated with a poorer graft outcome.

Antibodies that recognize self-proteins might also contribute to graft injury. For example, the auto-antibody 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 kidney allografts.[28]

B Lymphocytes

B cells are not just plasma cell precursors, but represent an important population of antigen-presenting cells particularly efficient in the situation of a sensitized recipient, because they have specific immunoglobulin as an antigen-specific receptor on their surface, which leads to efficient uptake and presentation of donor antigens to T cells.[29] Indeed, an increased frequency of alloantigen-specific B cells in sensitized recipients has been reported.[30] Therefore, targeting these B cells will also interfere with activation of indirectly alloreactive T cells, which play an important role in chronic allograft rejection.

In sensitized allograft recipients with DSA, sensitization has always occurred on the level of B and T cells; because B cells need T helpers to produce allo-antibodies of immunoglobulin G isotype as measured by the Luminex technology. Therefore, a combined pathogenesis of rejection must always be postulated, even if not all the pathologic criteria are fulfilled.[31]

However, failure to demonstrate DSA does not rule out a contribution of antibodies to the pathologic process, because absorption of antibodies by the allograft may result in a lack of circulating DSA.[32] Alternatively, DSA against non-HLA antigens or HLA-DP could explain the missing enzyme-linked immunosorbent assay reactivity in the presence of increased cytotoxic anti-B-cell reactivity and ongoing AMR.[27,33]

The combination of allo-antibody, basement membrane multilamination, C4d, and duplication of the glomerular basement membrane has been termed the *ABCD tetrad* by Solez and colleagues.[34]

Plasma Cells

During AMR, it is likely that a portion of the DSA found in the serum is due to ongoing antibody production by pre-existing plasma cells. In addition, the observed increase in DSA during AMR suggests that conversion of allospecific memory B cells to plasma cells also may play a role. Unfortunately, no studies of the activity of memory B cells during AMR exist. Despite this, several groups have developed protocols to treat AMR based on their presumed impact on either B cells or plasma cells.[35]

ACCOMMODATION

Some patients with HLA antibodies have excellent kidney graft function, and it has been documented to be about 20% in studies of 2658 patients with functioning grafts.[10] According to prospective studies, when 158 patients with antibodies were followed up for as long as 4 years, their graft survival was 58% versus 81% for 806 patients without antibodies.[10]

Worthington and colleagues[36] have shown that the mean time from antibody development to failure for class I antibodies was 2.7 years and 3.9 years for class II antibodies. Additionally, antibodies causing humoral rejection may not appear until as many as 13 years,[37] or even after 26 years[38] after transplantation. The reason for this long interval between antibody appearance and graft failure is the time needed for the endothelial walls of the arteries to hypertrophy and close the lumen or for the tubules to disappear because of peritubular capillary damage produced by antibodies.[39]

The phenomenon of accommodation, in which the graft acquires resistance to humoral injury and continues to function well despite the continued presence of antibody against a target antigen expressed on graft endothelium is well documented in ABO-incompatible kidney transplants.[40,41] Alexandre and colleagues[42] initially observed accommodation in recipients of an ABO-incompatible kidney allograft. Transient depletion of the circulating antibodies that are specific for these blood group antigens at the time of transplantation allows immediate graft survival without hyperacute rejection.

A rebound of antibody concentrations (primarily immunoglobulin M) 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 titre.[41,43] Even if the antibody titer returns to pretransplantation levels or higher, the grafts continue to function. It has been proposed that in these cases, complement regulatory proteins and/or other control mechanisms may interrupt the complement cascade distal to the generation of C4d, so the persistence of C4d on graft endothelium represents a marker for the arrest of the complement cascade rather than ongoing complement-mediated graft injury.[44]

At a cellular level, accommodation may occur via multiple mechanisms, including internalization, downregulation, inactivation, and inhibition of the target antigen.[17,45] In HLA-mismatched grafts, allo-antibodies 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 chronic AMR. Accommodation may have different degrees of effectiveness and stability (gradations), ranging from none (hyperacute rejection), to minimal (acute rejection), substantial (chronic rejection), or complete (stable accommodation).[46]

STAGES OF ANTIBODY-MEDIATED REJECTION

At the National Institutes of Health (United States) consensus conference, draft criteria were established for AMR and for 4 theoretical stages in the development of chronic AMR[47] as shown in the Table.[17] 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 acute AMR.

Stages of Antibody-mediated Rejection as Proposed by Colvin and Smith[17]

Stages	Description
Accommodation	
I	De novo antibodies detectable in circulation
II	C4d detectable in graft microvasculature
Rejection	
III	Graft injury (pathologic finding in graft biopsy)
IV	Graft dysfunction (clinical chronic rejection)

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 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. The interval between stages can be long and variable, and it is not known whether progression is inexorable.[17]

PATHOLOGY OF ANTIBODY-MEDIATED REJECTION

As pathologists have become increasingly adept at diagnosing AMR on allograft biopsies, substantial progress has been made in the treatment of AMR and in successful kidney transplantation in recipients with pre-existing antibodies against donor blood group (ABO) and major histocompatibility (HLA) antigens. It has become critical to develop standardized criteria for the pathological diagnosis of AMR.

Chronic AMR is now included in the newest update of the Banff 07 classification of kidney allograft pathology with the following criteria: (1) morphological changes as glomerular double contours compatible with transplant glomerulopathy and severe peritubular capillary basement membrane multilayering, interstitial fibrosis and tubular atrophy with or without peritubular capillary loss, and fibrous intimal thickening in arteries without internal elastica duplication; (2) diffuse C4d deposition in peritubular capillaries; and (3) presence of DSA.[11] Not all these criteria are always fulfilled in an individual patient at every given time point.[31]

Peritubular capillary basement membrane multilayering correlates highly with transplant glomerulopathy, and most of transplant glomerulopathy have evidence of either C4d-positive staining or DSA. However, the proposed criteria do not apply to all situations of chronic active AMR.

Chronic AMR is distinct from acute AMR in that no acute inflammation (neutrophils, edema, necrosis, and thrombosis) is present. However, cellular activity is often reflected by increased mononuclear cells in glomerular capillaries and peritubular capillary.[46] The Banff criteria require peritubular capillary C4d positivity for diagnosis of AMR as well as microcirculation injury. However, C4d is not a sensitive marker of chronic AMR, and in many patients with transplant glomerulopathy, C4d staining is negative in the presence of anti-HLA DSA. Therefore, the recent update of the Banff classification introduced the diagnostic category of "suspicious for AMR." It is defined with the presence of morphologic evidence of antibody-mediated tissue injury and positive anti-HLA antibody with negative C4d, or peritubular capillary C4d positivity in the absence of alloantibody.[48]

C4D AS A MARKER OF ANTIBODY-MEDIATED REJECTION

Feucht and colleagues[49] showed that peritubular capillary C4d deposition in renal transplant biopsies is strongly associated with a poor prognosis and raised the possibility that antibodies were responsible. Currently, C4d has been adopted as a marker of antibody-mediated rejection.[50] The justification for the selection of C4d, a split product of C4, as a marker for AMR comes from its position in the cascade of complement activation.

C4d deposition in renal peritubular capillaries is strongly associated with circulating antibody to donor HLA class I or class II antigens[51,52] and is currently the best single marker of complement-fixing circulating antibodies to the endothelium.

OTHER MARKERS OF ANTIBODY-MEDIATED REJECTION

C4d Pitfalls

C4d is not a magic marker for AMR and it is negative in the presence of anti-HLA DSA in many patients with transplant glomerulopathy.[53] Another issue with chronic active AMR is non-HLA antibody-induced rejection without complement fixation of C4d. Moreover, it was shown in many studies that focal C4d staining was not a reliable indicator of AMR,[54] and it is not a guarantee of AMR. Diffuse C4d staining can occur with no morphologic injury or impaired outcome in ABO-incompatible allografts.[48]

There are significant data to show that C4d positivity is usually long-lasting but is not permanent. C4d staining can change from negative to positive and vice versa within days to weeks. The detection of C4d signifies a humoral alloresponse in a subgroup of kidney transplants, which is often associated with signs of cellular rejection.[55]

NEW DIAGNOSTIC TOOLS

Endothelial-associated Transcripts as a New Marker for Chronic Antibody-mediated Rejection

Recognizing the key role of endothelial changes in AMR, it was postulated by Sis and colleagues[56] that altered expression of endothelial genes in biopsies from patients with allo-antibody would identify kidneys incurring antibody-mediated damage and at risk for graft loss, whether they were C4d positive or negative. They explored whether expression of endothelial genes was increased in biopsies manifesting antibody-mediated graft injury, and whether such changes could be seen in C4d-negative as well as C4d-positive biopsies. They identified 119 endothelial-associated transcripts (ENDATs) from the literature and studied their expression by microarrays in 173 kidney allograft biopsies for cause.

Mean ENDAT expression was increased in all rejection but was higher in AMR than in T-cell-mediated rejection and correlated with histopathologic lesions of AMR and allo-antibody. Many individual ENDATs were increased in AMR and predicted graft loss. Kidneys with high ENDATs and antibody showed increased lesions of AMR and worse prognosis in comparison to controls. Only 40% of kidneys with high ENDAT expression and chronic AMR or graft loss were diagnosed by C4d positivity. High ENDAT expression with antibody predicts graft loss with higher sensitivity (77% versus 31%) and slightly lower specificity (71% versus 94%) than C4d. The results were validated in independent set of 82 kidneys. They concluded that in patients with allo-antibodies, abnormalities in expression of endothelial genes identify not only C4d-positive AMR, but some kidney transplants developing antibody associated graft injury despite negative C4d staining and that ENDAT changes in kidney transplants occur in rejection and in other forms of renal injury, and

their impact on transplant glomerulopathy and graft loss is principally in patients with circulating HLA antibodies.

The elevation of the ENDATs is of value in determining which biopsies for cause in patients with antibody may have antibody-mediated injury, even when they are C4d negative.

TRIB1 as a New Noninvasive Marker for Chronic Antibody-mediated Rejection

Ashton-Chess and colleagues[57] set out to discover novel minimally invasive biomarkers of more precise histologic diagnoses of late graft scarring. Using a literature gene-set comparison approach for late graft injury, they identified TRIB1, a human homolog of Drosophila tribbles,[58] as a potentially informative biomarker. TRIB1 is a scarcely characterized member of the tribbles family that has been shown to be a potent regulator of cell signaling in various cells lines. It was determined that TRIB1 is expressed primarily by antigen-presenting cells and activated endothelial cells. TRIB1 differs from the other minimally invasive biomarkers of transplant rejection described to date that are of T/NK cell origin,[59,60] in that it is expressed primarily by antigen-presenting cells as well as endothelial cells. They explored the potential of TRIB1 as a tissue, peripheral blood, and urine biomarker by measuring its mRNA profiles in graft biopsies, blood, and urine from healthy volunteers and kidney transplant recipients with different histologic and clinical diagnoses.

For testing this, mRNA expression in 76 graft biopsies, 71 blood samples, and 11 urine samples were profiled from independent cohorts of kidney transplant patients with different histologic diagnoses recruited at 2 European centers. TRIB1 but not TRIB2 or TRIB3 was found to be a potential blood and tissue (but not urine) biomarker of chronic AMR. Moreover, TRIB1 mRNA in the blood was more specific and sensitive for diagnosing chronic AMR than TRIB1 mRNA in biopsies. TRIB1 mRNA levels in peripheral blood mononuclear cells discriminated patients with chronic AMR from those with other types of late allograft injury with high sensitivity and specificity, suggests TRIB1 to be a marker of an active immune response. Overall, these data support the potential use of TRIB1 as a biomarker of chronic antibody-mediated allograft failure.

TREATMENT OF CHRONIC ANTIBODY-MEDIATED REJECTION

Unfortunately, no immunosuppressive standard for the prevention or therapy of allo-antibody production has been established yet. Although based on very limited evidence, acute humoral rejections are frequently treated with a switch to tacrolimus, plasmapheresis, or immunoadsorption, as well as T- and B-cell-depleting antibodies. However, the best therapeutic approach for C4d-positive, chronic humoral kidney rejection associated with an unfavorable prognosis remains completely unclear.

Intravenous Immunoglobulins

The immunomodulatory effects of intravenous immunoglobulins (IVIG) are multiple, and the exact mechanisms are not elucidated. However, effective allo-antibody inhibition by IVIG was shown in the context of desensitization protocols only relying on high-dose IVIG treatment.[61] Intravenous immunoglobulins inhibits mixed lymphocyte reactions and induces apoptosis mainly in B cells.[62]

There are numerous proposed mechanisms how IVIG exerts its immunomodulatory action. They include modification of circulating allo-antibody concentration through induction of anti-idiotypic circuits, antigen binding through the Fab part of the immunoglobulin molecule, Fc receptor-mediated interaction with antigen-presenting cells to block T- and B-cell activation, and inhibition of complement activity.[63]

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 signaling.[64] Intravenous immunoglobulins inhibits binding of donor-reactive antibodies to target cells in about 80% of patients, indicating that the presence of blocking antibodies might explain the efficacy of IVIG, although the mechanism is not known.[64]

Billing and colleagues[65] studied 6 pediatric kidney transplant recipients with chronic AMR and gave them 4 weekly doses of IVIG (1 g/kg body weight per dose), followed by a single dose of rituximab (375 mg/m^2 body surface area) 1 week after the last IVIG infusion. Median glomerular filtration rate during the 6 months before intervention dropped by 25 mL/ min/1.73m^2 (range, 11 mL/ min/1.73m^2 to 26 mL/ min/1.73m^2) ($P < .05$) and

increased in response to anti-humoral therapy by 21 mL/ min/1.73m^2 (range, -14 mL/ min/1.73m^2 to +30 mL/ min/1.73m^2) 6 months ($P < .05$) and by 19 mL/ min/1.73m^2 (range, -14 mL/ min/1.73m^2 to +23 mL/ min/1.73m^2) 12 months ($P = .06$) after start of treatment. Glomerular filtration rate improved or stabilized in 4 patients; the two nonresponders had the highest degree of transplant glomerulopathy, the highest degree of C4d deposition in peritubular capillaries, and pronounced interstitial inflammation. The treatment regimen was well tolerated.

Another study was conducted by Fehr and colleagues[31] who reported 4 kidney allograft recipients suffering from chronic AMR 1 to 27 years posttransplant, who were treated with a combination of rituximab and IVIG with improved kidney allograft function in all 4 patients, whereas DSAs were reduced in 2 of 4 patients.

Rituximab

Rituximab, a chimeric monoclonal anti-CD20 antibody directed against B cells, prevents new antibody production by depletion of B cells as precursors of mature plasma cells in the circulation and the lymphoid tissue, although some recent reports demonstrated that depletion in secondary and tertiary lymphoid structures is far less efficient and may not affect an ongoing localized humoral immune response,[66,67] prevention of B-cell proliferation, and induction of apoptosis and lysis of B cells through complement-dependent and complement-independent mechanisms.[68]

Rituximab 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 to 12 months in more than 80% of patients, although the degree of depletion is highly variable and is observed for up to 24 months in some individuals.[69] An additional potential mechanism of action of rituximab is the direct targeting of CD20-positive cells that infiltrate the graft.[70]

Preliminary studies indicate that rituximab decreases the concentration of pre-existing and posttransplantation antibodies.[71,72] 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.

Based on the pathophysiologic condition of this rejection process and efficacy of rituximab in B cells and antibody-mediated autoimmune diseases,[73,74] a combination treatment with rituximab or IVIG represents a logical approach.

Mycophenolic Acid and Sirolimus

In a multicenter study, mycophenolate mofetil in combination with cyclosporine resulted in significantly lower frequencies of HLA antibodies when compared with azathioprine and cyclosporine treatment.[75] Moreover, mycophenolate mofetil was described to be effective in inhibiting primary antigen-specific antibody responses in kidney transplant patients.[76] Heidt and colleagues[77] stimulated purified human B cells devoid of T cells with CD40L expressing L cells, or by anti-CD40mAb with or without Toll-like receptor triggering, all in the presence of B-cell activating cytokines. These three protocols resulted in various degrees of B-cell stimulation. Then, they added 4 commonly used immunosuppressive drugs (tacrolimus, cyclosporin, mycophenolic acid, and rapamycin) to these cultures and tested a variety of parameters of B-cell activity including proliferation, apoptosis induction, and both immunoglobulins M and G production. They found that mycophenolic acid was extremely potent in inhibiting both proliferation and immunoglobulin production. Moreover, these effects persisted when mycophenolic acid was added to already activated B cells, implying that an ongoing B-cell response may be dampened by mycophenolic acid, whereas calcineurin inhibitors are ineffective. Mycophenolic acid levels used are lower than levels that are usually achieved physiologically.

In the same in vitro experiments, rapamycin, like mycophenolate mofetil, was described to be extremely potent in inhibiting humoral responses. Rapamycin was the most effective drug tested, as it inhibited not only B-cell proliferation and immunoglobulin production, but also inhibited the number of immunoglobulin-producing cells. None of the other drugs tested were capable of decreasing the number of immunoglobulin producing cells. By contrast, tacrolimus and cyclosporin marginally inhibited B-cell proliferation and immunoglobulin production, and the extent of inhibition depended on the degree of the B-cell stimulation.

Bortezumib

While the B-cell-depleting anti-CD20 antibody rituximab is increasingly incorporated in treatment protocols of humoral rejection,[78] this reagent is neither effective in eliminating antibody-producing plasma cells–either newly created from memory or naive B cells or from those that existed prior to transplant—nor does it decrease circulating antibody titers.[79] For an effective blockade of alloantibody formation, a specific plasma cells-depleting reagent would be desirable. Bortezomib, a selective inhibitor of the 26S proteasome, has been approved by the Food and Drug Administration for the treatment of relapsed multiple myeloma.

Mechanisms of bortezomib action include inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells and cytokine expression as well as induction of apoptosis as a result of activation of the terminal unfolded protein response.[80] Susceptibility to bortezomib-induced apoptosis is related to the high immunoglobulin synthesis rate of plasma cells associated with accumulation of unfolded proteins/DRiPs inducing endoplasmatic reticulum stress.[80] Moreover, bortezomib not only acted on the humoral response, but also effectively inhibited the influx of major histocompatibility complex class II-positive cells, monocytes or macrophages, CD8+, as well as CD4+ T cells.

In animal models, Vogelbacher and colleagues[81] found that combination of bortezomib and sirolimus inhibited the chronic active AMR in experimental kidney transplantation in the rat. In humans, data are lacking. In one case report, bortezomib failed to treat chronic AMR even after treatment with rituximab and IVIG.

SUMMARY

Immunologic barriers once considered insurmountable are now consistently overcome to enable more patients to undergo organ transplantation. Allo-antibodies are a substantial obstacle to short-term and long-term graft survival. To prevent or reduce allo-antibody titers, more insight is needed to improve our understanding of the regulation of B cells and the developmental and differentiation pathways of memory B cells and plasma cells.

Several important issues regarding AMR remain unclear. First, the immunologic mechanisms responsible for the development of high levels of DSA are still unclear. The contribution of memory B cells versus the role of pre-existing plasma cells has important therapeutic implications since each may have a differential sensitivity to various agents. Whereas several new therapeutic approaches have emerged, more extensive study and follow-up are needed to determine if these apparent advances will improve the outcomes of AMR.

CONFLICT OF INTEREST

None declared.

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