ABSTRACT

This paper reviews the most important studies on chronic antibody-mediated rejection (cABMR), which is an important cause of late graft dysfunction after renal transplantation. Several antibodies seem to be responsible for chronic rejection; new techniques have allowed us to identify these antibodies in circulation. The pathogenetic role of the antibodies generally includes the complement pathway, but may also be complement-independent. This paper also examines the pathogenesis of chronic endothelial lesions, as well as the histopathological aspects. Antibodies responsible for chronic rejection may preexist before transplantation or may develop after transplantation. The possible therapeutic approaches are poor and principally based on early identification and desensitisation techniques. New B cell targeting drugs are aimed at an improved control of the relevant condition.

Keywords: Chronic rejection, antibodies, complement, donor-specific antibodies, renal transplantation.

INTRODUCTION

Despite improvements in outcomes of renal transplantation, kidney allograft loss remains substantial and is associated with increased morbidity, mortality, and costs. Clearly, the identification of critical pathologic pathways responsible for the allograft loss and the development of therapeutic intervention to improve the duration and the quality of allograft function are among the most important targets of transplant medicine. One of the most important advances in the past decade has been the realisation that the insufficient control of the humoral arm of a recipient's immune system by the current immunosuppressive regimens is the factor primarily responsible for allograft dysfunction and loss. This notion is now superseding the historical dogma that allograft losses were caused by the calcineurin inhibitors' (CNIs) toxicity and by the chronic allograft nephropathy (CAN). Indeed nephrotoxicity and CAN as causes of late graft failure are being challenged by the findings of the Long-Term Deterioration of Kidney Allograft Function (DeKAF) and by other studies.

The emergence of sensitive techniques to detect donor-specific anti-human leukocyte antigen antibodies (HLA-DSAs) and other HLA and non-HLA antibodies, together with the advances in assessment of graft pathology, have expanded the spectrum of what constitutes as antibody-mediated rejection (ABMR). The different technologies used by researches and the significance of alloantibody found by such technologies recently led to a consensus conference with the elaboration of consensus guidelines on the testing and clinical management issues associated with HLA and non-HLA antibodies in transplant recipients.

As a consequence of such knowledge increment, since the Banff 2005 meeting report, the term CAN has been deleted, and in Banff 2007 and Banff 2009 Conferences, the concept of chronic ABMR (cABMR) had been further evaluated and cABMR was definitively included in the Banff classification. The Banff 2011 meeting report further confirmed these data.
Epidemiology

Due to the continuous evolution of techniques, it is difficult to evaluate incidence and prevalence of cABMR as a cause of graft failure. In a prospective study by Einecke, 63% of late kidney failures were attributable to cABMR, whereas glomerulonephritis, T cell rejection, and drug toxicity were uncommon. Similar data reported by Sellares found that in 315 allograft recipients, who underwent indication biopsies, a large prevalence of cABMR was a cause of graft failure.

Histopathology

Transplant glomerulopathy (TG) and peritubular capillary (PTC) basement multilayering represent the histological hallmark of cABMR. TG is a morphological pattern of chronic kidney injury that lacks detectable immune-complex deposits and is associated with poor kidney transplant outcomes. It primarily is an endothelial pathology affecting kidney microcirculation endothelium, which is seen as duplication (double contours) and/or multilamination of capillary basement membranes, along with substantial replacement of endothelial fenestrations with a continuous endothelial lining. The combination of alloantibody, PTC multilamination, C4d, and TG has been called the ‘ABCD tetrad’ by Halloran and colleagues.

cABMR is characterised by specific and gradually irreversible immune-mediated graft damages that need to be clearly differentiated from isolated interstitial fibrosis/tubular, atrophy, and/or CNI nephrotoxicity, and chronic T cell-mediated rejection (TCMR). To this purpose, a consensus meeting at the National Institutes of Health proposed criteria for cABMR. These elements include:

1) Histological evidence of chronic injury
   • Arterial intimal fibrosis without elastosis
   • Duplication of glomerular basement membrane
   • Multilaminated PTC basement membrane
   • Interstitial fibrosis with tubular atrophy
2) Evidence for antibody action/deposition in tissue
3) Serologic evidence of anti-HLA or other anti-donor antibody

If only two of the numbered criteria are present, then the diagnosis is considered ‘suspicious’ for cABMR.

PATHOPHYSIOLOGY

There is an increased body of evidence suggesting that patients with high quantity anti-HLA antibodies (particularly if they are donor-specific) developed either pre or post-transplant, show a worse outcome. At any given time, approximately 25% of transplant recipients have antibodies against HLA antigens as evaluated with the newest, highly sensitive, and specific techniques for DSA monitoring. Moreover, antibodies against non-HLA have also been implicated in ABMR. Antibodies can mediate endothelial injury through complement-dependent and independent mechanisms by transducing signals that are pro-inflammatory and proliferative. It is clear that preformed, or de novo, HLA-DSAs cause cABMR, but it is less certain what the role and scope of non-HLA antibodies are in mediating graft injury and loss.

One hypothesis is that alloantigen sensitisation occurs from non-HLA polymorphic differences between the donor and recipient (e.g. major histocompatibility complex [MHC] Class I-related chains A and B [MICA, MICB]). Unfortunately the progress in this area has been limited by the lack of validated clinical assays for non-HLA alloantibodies, the confounding presence of HLA-DSAs, and - in the case of MICA antibodies - the lack of proof of specificity. A second hypothesis is that autoantigen sensitisation occurs from the exposure of cryptic epitopes after tissue injury or inflammation (vimentin, K-αtubulin, collagen V, agrin, etc.).

The pathophysiology of cABMR is still not completely understood. Studies suggest that in renal transplantation de novo HLA-DSAs develop post-transplant in up to 25% of non-sensitised patients, often without overt clinical evidence of concurrent rejection. In addition, around 30% of patients on the waiting list have detectable HLA antibodies. In both groups of patients, the presence of these antibodies increases the risk of subsequent cABMR. The development of a histological test to identify antibody-mediated complement activation on transplant biopsies (C4d staining) has provided a way of flagging up potential deleterious interactions between antibody and graft endothelium. In addition, molecular techniques, such as gene expression profiling, have allowed the identification of subclinical endothelial cell damage that can be present even in the absence of complement activation or detectable DSA.
More recently, a study by Lynch and colleagues\textsuperscript{29} described a technique that may allow a more global assessment of B cell reactivity to the allograft. Their results suggest that a humoral response to the allograft may be more frequent than previously appreciated. Antibodies reactive to donor HLA molecules, minor histocompatibility antigens, endothelial cells, red blood cells, or autoantigens can trigger or contribute to rejection even late after transplantation.\textsuperscript{30} Often the immune system provides an integrated response to achieve allograft rejection with T cell-mediated rejection and ABMR being either linked through time or coexisting.\textsuperscript{31} Antibody-mediated injury to allograft is initiated by DSAs binding to HLA antigens or to other targets on the allograft endothelium. If DSAs are complement activating, the classic complement pathway is rapidly activated through IgG binding and activation of C1q.\textsuperscript{32} Alternatively, DSAs can bind endothelial cell targets and stimulate cell proliferation or induce antibody-dependent cell-mediated cytotoxicity (ADCC) with interferon $\gamma$ release.\textsuperscript{20} These processes seem to be more important for the development of a type of chronic antibody-mediated injury that is more dependent on natural killer cells than on complement.\textsuperscript{33} Antibodies can also bind to HLA and other targets, and incompletely activate the complement system without causing apparent injury. This process is referred to as accommodation.\textsuperscript{34}

The clinical significance of cABMR has been increasingly documented in recent years, with some data suggesting that it may represent the leading cause of late allograft loss.\textsuperscript{4} cABMR is a long-term process that develops in sequential steps over months to years.\textsuperscript{35} cABMR has been proposed to arise through a series of stages or states.\textsuperscript{36} The first common event is alloantibody production, followed by antibody interaction with alloantigen resulting in the deposition of C4d in PTC and possibly glomeruli, followed by pathologic changes and graft dysfunction.\textsuperscript{37}

DSAs, particularly HLA antigen Class II antibodies, can cause insidious graft injury, and therefore constitute a central causal factor for TG (Figure 1). The pathogenicity of Class II antibodies has been documented in outstanding papers that demonstrated a significant association between Class II antibodies and risk of developing TG.\textsuperscript{38,39} The international Banff consensus criteria classify TG as cABMR, if the pattern is accompanied by detectable DSAs and diffuse or focal linear C4d positivity in PTCs; 4-6 Mauiyyedi et al.\textsuperscript{40} detected deposition of C4d in PTCs in 61% of chronic

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\includegraphics[width=\textwidth]{image}
\caption{Proposed pathogenetic mechanisms for transplant glomerulopathy. HLA: human leukocyte antigen.}
\end{figure}
rejection biopsy of patients with TG. In addition, a study by Regele et al.\textsuperscript{41} reported the presence of C4d in PTCs in 34% of patients with TG, and this staining presages later TG development.

Pathologic patterns of cABMR are seen in renal biopsies performed either for clinical indications or for protocol, even a long time after kidney transplantation.\textsuperscript{5,42} In addition to reduced immunosuppression and non-adherence, early acute rejection seems to have a relevant role on late cABMR. Several years ago Cosio et al.\textsuperscript{43} documented that in 1-year surveillance biopsies the degree of inflammation at 1-year post-transplant predicts loss of graft function and graft failure independently of function and other variables (Figure 2).

Recently, El Ters et al.\textsuperscript{44} found that early acute rejection, even in the absence of pre-transplant DSAs, increases the risk for alloimmune allograft loss late after transplantation, and the phenotype of this late loss is cABMR. The author hypothesised that the formation of new DSAs, particularly Class II DSAs, can be a consequence of early acute rejection.\textsuperscript{45} El Ters et al.\textsuperscript{44} also found that cABMR was responsible for 43% of allograft loss. Willicombe et al.\textsuperscript{46} in surveillance biopsies performed at 3 years after transplantation, found that, despite excellent serum creatinine values, only one-third of the biopsies were normal and the lesions seemed to correlate with risks for immunological injury.

As mentioned above, the cause of late graft dysfunction and failure seems increasingly linked to the presence of antibodies or antibody-mediated injury. This has been recently documented by the 5-year follow-up data of the patient cohort from the DeKAF study.\textsuperscript{47,48}

Hill et al.\textsuperscript{49} described a new insight on the pathogenesis of cABMR. DSA positive patients show a striking acceleration of arteriosclerosis. Pathologic examination reveals that the inner intima is hypercellular, with actively proliferating myofibroblasts laying down collagen, often overlying older, condensed collagen of pre-transplantation donor origin.

**DIAGNOSIS**

The diagnosis of cABMR is principally based upon the association of deteriorating graft function associated with the pathologic features found
on renal biopsies. Search of circulating DSAs with adequate techniques is also useful. Protocol biopsies may be useful as well-documented by the paper of Wiebe.42

Finding of biomarkers associated with cABMR could be extremely useful. Einecke50 was able to realise a molecular classifier for predicting future graft loss. Sis et al.28 found that several endothelial transcripts (ENDATs) correlated with histopathological lesions of cABMR. Immunoproteasome beta subunit 10 was found to be increased in the graft and in blood samples during cABMR.51 Finally, recent studies52,53 looking at B cell-activating factor (BAFF), a B cell stimulating molecule, showed that the appearance of soluble BAFF levels, early after transplantation, correlates with de novo development of DSAs and, ultimately, progression to chronic active ABMR in paediatric and adult first kidney transplant recipients who were highly desensitised before transplantation.

**THERAPY**

We have clearly documented that cABMR develops in patients with alloantibodies - principally, but not only, DSAs - detectable in the serum. Such antibodies may be present in the recipient before transplantation or may develop after transplantation.

Patients with DSAs Preformed Before Transplantation

Patients waiting for a transplant may be highly immunised and many show detectable DSAs in their serum. Sensitised patients who are DSA-negative with negative complement-dependent cytotoxicity (CDC-XM) may be transplanted safely. They will likely require more immunosuppressive therapy and an induction therapy.54-56

Treatment of cABMR may be distinguished in:

a) Prevention of acute ABMR as main cause of cABMR

b) Treatment of established cABMR

**Prevention**

The different desensitisation protocols apply primarily to DSA-positive patients who are CDC-XM positive. The desensitisation protocols can prevent both acute and cABMR. The majority of the current protocols are modified versions of the high-dose intravenous immunoglobulins (IVIG) initiated at the Cedars-Sinai Medical Center or of the plasmapheresis PP with low dose IVIG initiated at John Hopkins Hospital.57 Jordan et al.58 initially provided high-dose IVIGs (2 g/kg) to cross-match positive recipients, and the patients received a kidney transplant when their CDC T cell XM became negative. Subsequently to improve the results, Vo et al.59,60 decided to use alemtuzumab induction treatment and added rituximab to the protocol.

The other approach to desensitisation comprises the use of PP and low-dose anti-cytomegalovirus IVIG (CMV-IVIG). With such an approach61 Montgomery et al.62 successfully desensitised 211 DSA-positive recipients of living donor kidneys with PP and low-dose IVIG.

Stegall et al.63 added eculizumab during the pre and post-transplant period in DSA positive patients and obtained 7.7% post-transplant acute ABMR, compared with 41.2% in the control group. However, at 2 years after transplantation the incidence of cABMR was similar between the two groups. cABMR remains a major issue when transplanting hyperimmune patients.

In addition to desensitisation, when applicable - in theory - every option available to treat acute ABMR could also be applied to cABMR. However there are no controlled trials for treatment of cABMR reported in literature. The only treatment option with some reported benefit is the combination of rituximab and IVIG.64 To prevent cABMR, patients with preformed DSAs, when treated immediately post-transplantation with a more intensive prophylactic regimen (PP, IVIGs, and anti-CD20), demonstrated a significant decrease in DSA and a decrease in cABMR rate at 1 year.65

For the treatment of established cABMR there are only three case series treated with such combination therapy.66,67 DSAs went down in only some patients and therapy had limited effects in cases with massive proteinuria, more severe peritubular capillaritis, and previous acute rejection. In a recent paper by Ashimine et al.68 it was reported that in 320 patients neither pre-transplant splenectomy nor rituximab treatment had an inhibitory effect on de novo HLA antibody production after renal transplantation during medium term follow-up.

Smaller studies by Billing et al.69 and Smith et al.70 documented a partial response to rituximab
treatment in patients affected by cABMR. Billing et al. documented in 20 paediatric patients that treatment with IVIG and rituximab significantly reduced or stabilised the progressive loss of transplant function with cABMR over an observation period of 2 years, apparently by lowering circulating DSA. Smith et al., in 31 patients affected by cABMR, reported that rituximab followed by standard maintenance immunosuppression showed a therapeutic effect in the treatment of cABMR, which is confined to a subset of treated subjects that cannot be identified as a priori. Very few patients received bortezomib as a rescue treatment for cABMR and proteinuria with mixed results. An interim analysis of a very recent study with eculizumab therapy of cABMR documented an apparent stabilisation of renal function. Taken together these results indicate that any treatment for cABMR using drugs with potential high toxicity should only be performed in the context of a randomised controlled trial.

Patients with de novo DSAs after Transplantation

Several authors reviewed the incidence and impact of de novo DSAs, both in adult and paediatric recipients. The actual 5-year post-transplantation cumulative incidence of de novo DSAs in a low-risk population is 20% (Figure 3). Once DSAs appear, the probability of graft loss within 3 years after DSA appearance is 24% (Figure 4), and respective to patients without DSAs, the relative risk of graft loss is 9-times higher at 1 year after DSA appearance. In a multivariate analysis, the main causes of de novo DSAs were deterioration quotient (DQ) locus mismatches, younger age at transplantation, and transplantation from deceased donors. Others claim for prior non-adherence or history of a clinical acute cellular rejection as causes of de novo DSAs.

If the appearance of DSAs is associated with clinical signs of acute ABMR, the treatment is that of acute ABMR. The main problem is what to do when the appearance of DSAs is not coupled with acute or chronic rejection. Indeed, detection of de novo DSAs in a routine test in patients with stable allograft function represents a step back in the continuum of the natural history of acute and cABMR; it is largely unknown how to treat these patients. To date, prophylactic treatment such as rituximab and splenectomy or eculizumab does not seem to have any effect on DSA appearance.

Monitoring DSAs after transplantation seems to be essential, considering that DSA appearance has a poor prognosis. As procedures including antibody removal by PP, IA, antibody production

Figure 3: The actual 5-year post-transplantation cumulative incidence of de novo DSAs. DSA: donor specific antibodies.
downregulation by B cell, or plasma cell targeting, or complement cascade inhibition have had a very limited success when employed in an advanced phase of cABMR,56,78,79 the prompt removal of de novo DSAs seems to be essential. Notwithstanding, no standard of care on this issue currently exists. To date, only a multicentre antibody removal trial (ART) is ongoing in Italy in a randomised, prospective fashion PP and low-dose CMV-IVIG.80

**CONCLUSIONS**

The lack of improvement in long-term outcomes of kidney transplants has been ascribed to the CNI nephrotoxicity. Indeed, CNIs represent the cornerstone of maintenance immunosuppression in organ transplantation. In the last decade, several studies have challenged this approach; indeed, chronic allograft rejection and death of transplanted patients are now the main causes of long-term graft loss. Chronic rejection has, for a long time, been identified with a lack of adequate T cell control with immunosuppressant. New techniques able to identify circulating antibodies and to reveal their presence and pathogenic role have now allowed us to recognise that in many cases a deficiency in humoral arm control may be the cause of long-term deterioration of graft function. Now both acute and cABMR have been well identified and included in the Banff classification. The early identification and therapeutic approaches to treat cABMR are still limited, and a successful prophylaxis seems the best approach to limit both acute and cABMR. Several studies suggest that monitoring circulating DSAs, protocol, or per cause biopsies and discovering new drugs targeting B cells and complement are the best options for the early identification and control of cABMR.

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**Figure 4: Probability of graft loss within 3 years after de novo DSAs appearance.**

DSA: donor specific antibodies.
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