NEPHROLOGY

Nephrology **21,** Suppl. 1 (2016) 4–8

Mini Reviews

Reviewing the pathogenesis of antibody-mediated rejection and renal graft pathology after kidney transplantation

KUNIO MOROZUMI,[1] ASAMI TAKEDA,[2] YASUHIRO OTSUKA,[2] KEIJI HORIKE,[1] NORIHIKO GOTOH,[2] SHUNJI NARUMI,[2] YOSHIHIKO WATARAI[2] and TAKAAKI KOBAYASHI[3]

[1]Department of Nephrology and Kidney Transplantation, Masuko Memorial Hospital, [2]Department of Nephrology and Kidney Transplant Surgery, Japanese Red Cross Nagoya Daini Hospital, and [3]Department of Renal Transplant Surgery, Aichi Medical University, Nagoya, Japan

KEY WORDS:
antibody-mediated rejection, chronic rejection, de novo DSA, electron microscopic changes of peritubular capillaritis, kidney transplantation.

Correspondence:
Kunio Morozumi MD, Department of Nephrology, Masuko Memorial Hospital 35-28 Takebashi-cho, Nakamura-ku, Nagoya-City, Aichi, 453-8566 Japan. Email: morozumi@masuko.or.jp

doi: 10.1111/nep.12777

Conflict of interest: The authors have no conflict of interest to declare.

ABSTRACT:

The clinicopathological context of rejection after kidney transplantation was well recognized. Banff conferences greatly contributed to elucidate the pathogenesis and to establish the pathologic criteria of rejection after kidney transplantation. The most important current problem of renal transplantation is de novo donor-specific antibody (DSA) production leading chronic rejection and graft loss. Microvascular inflammation is considered as a reliable pathological marker for antibody-mediated rejection (AMR) in the presence of DSA. Electron microscopic study allowed us to evaluate early changes in peritubular capillaries in T-lymphocyte mediated rejection and transition to antibody-mediated rejection. Severe endothelial injuries with edema and activated lymphocyte invaded into subendothelial space with early multi-layering of peritubular capillary basement membrane suggest T-lymphocyte mediated rejection induce an unbounded chain of antibody-mediated rejection. The risk factors of AMR after ABO-incompatible kidney transplantation are important issues. Anti-ABO blood type antibody titre of IgG excess 32-fold before transplant operation is the only predictable factor for acute AMR. Characteristics of chronic active antibody-mediated rejection (CAAMR) are one of the most important problems. Light microscopic findings and C4d stain of peritubular capillary and glomerular capillary are useful diagnostic criteria of CAAMR. Microvascular inflammation, double contour of glomerular capillary and thickening of peritubular capillary basement are good predictive factors of the presence of de novo DSA. C4d stain of linear glomerular capillary is a more sensitive marker for CAAMR than positive C4d of peritubular capillary. Early and sensitive diagnostic attempts of diagnosing CAAMR are pivotal to prevent chronic graft failure.

Rejection of kidney transplantation is primarily mediated by activation of alloreactive T cells and antigen-presenting cells such as B lymphocytes, macrophages, and dendritic cells. Acute allograft rejection is caused primarily by the infiltration of T cells into the allograft, which triggers inflammatory and cytotoxic effects on the graft. The sequence of events that underlies graft rejection is recognition, via MHC class I and II antigens, of the donor's histocompatibility differences. Rejection is currently classified as T-lymphocyte mediated and antibody-mediated type (AMR) both in acute and chronic rejection.[1–3]

Antibody-mediated type can be induced by immunoglobulin G antibodies that bind to antigens on the vascular endothelium, such as class I MHC, ABO, and vascular endothelial cell antigens. Sensitization prior to transplant can occur by pregnancy or blood transfusions. Previous transplantation can also sensitize patients against HLA molecules.

The most important current problem of renal transplantation is de novo DSA production leading chronic rejection and graft loss.

With de novo HLA antibody production, AMR can occur within weeks of the transplant, if immunosuppressive treatment is insufficient. Endothelium of donor graft peritubular and glomerular capillaries display MHC molecules that are the

target for antibody production. In antibody rejection, C4d seems to be a useful marker of complement activation and is seen commonly in peritubular capillaries. [2,3]

Antibody-mediated type is a major cause of late kidney transplant failure. It is important to have an understanding of HLA typing including anti-MHC-class-I-related chain A (MICA). [4] It is now appreciated that biopsy for AMR does not have to include diffuse C4d, but does require a closer look at peritubular capillary microvasculature. In a study conducted by Worthington et al., only five out of 14 patients with renal graft dysfunction and pathology showing C4d deposition in peritubular capillaries were found to have anti-HLA antibodies. [5]

Banff conferences for allograft pathology greatly contributed to elucidate the pathogenesis and to establish the pathologic criteria of rejection after kidney transplantation. [1–3,6–9] Advances in new immunosuppressive agents, new and sensitive methods to detect anti-donor HLA antibody (DSA) and refined Banff classification for renal allograft pathology tremendously improved renal graft survival.

ELECTRON MICROSCOPIC STUDY OF PERITUBULAR CAPILLARY IN EARLY REJECTION

Relationship between acute T-lymphocyte mediated rejection and antibody-mediated rejection due to de novo HLA antibody

The most important current problem of renal transplantation is de novo DSA production leading chronic rejection.

Microvascular inflammation is considered as a reliable pathological marker for AMR in the presence of DSA. Electron microscopic study allowed us to evaluate early changes in peritubular capillaries in T-lymphocyte mediated rejection. Peritubular capillaritis and glomerular capillaritis (glomerulitis) are key histopathologic changes suggesting acute phase of AMR. Double contour of glomerular basement membrane and multi-layering of peritubular capillary basement membrane (MLPTCBM) are reliable marker of CAAMR[8]. There are few reports describing early changes in peritubular capillary endothelial cell injuries and peritubular capillary basement membrane.[10]

Electron microscopic study of renal allograft biopsy before calcineurin era allows us to understand early changes in peritubular capillary in acute rejection. [10] Figure 1 showed changes in peritubular capillary and peritubular capillary basement membrane from very early phase to late phase of restructured peritubular capillary basement membrane with multi-layering. Figure 1a shows invading activated lymphocyte into subendothelial space of peritubular capillary surrounded by interstitial edema and numerous infiltrated lymphocytes in a recipient with very early acute rejection. Figure 1b shows very early changes in peritubular capillary basement membrane splitting with severe edema and subendothelial invading macrophage in acute rejection biopsy taken 21 days after operation. Figure 1c shows early multi-layering of peritubular capillary basement membrane (MLPTCBM) and many infiltrated activated lymphocytes between disrupted basement membranes with severe edema. Figure 1d shows well developed circumferential MLPTCBM and subendothelial invaded lymphocyte. Interestingly, early changes in MLPTCBM have disappeared and almost normal peritubular capillary restored in some of follow-up protocol biopsies[10]. Severe endothelial injuries with subendothelial edema and activated lymphocyte invaded into subendothelial space concomitant with early MLPTCBM are common findings of acute rejection and elapsed and/or repeated rejection. De novo DSA develops during these rejection processes. These findings suggest T-lymphocyte mediated rejection induce an unbounded chain of antibody-mediated rejection.

ABO-incompatible kidney transplantation and antibody-mediated rejection

ABO-incompatible renal transplantation are well-established as a commonplace treatment in Japan.[11] The incidence of acute AMR has dramatically decreased under sufficient desensitization immunosuppressive protocol. [11] However, the risk factors of AMR after ABO-incompatible kidney transplantation are important issues. Optimal target ranges of anti-blood type antibodies for prophylactic purpose of AMR are important issues.

Figure 2 shows the impact of anti-blood type A/B starting titres on AMR. The Anti-ABO blood type antibody titre of both IgG and IgM class excess 32-fold justly before transplant operation is the only predictable factor for AMR. Pretreatment for desensitization is important to reduce anti-ABO blood antibody levels 16-fold or less than 16-fold.

Acute AMR has occurred in up to 33% of cases undergoing ABO incompatible kidney transplantation. Current incidence of acute AMR is less than 10% in Japan under sufficient desensitization protocol. The incidence of acute AMR is very low one month after transplant operation. However, among cases with AMR in the early post-operative period, prevalence of transplant glomerulopathy is 22% at one year. [12] Transplant glomerulopathy suggests the presence of CAAMR. The resistance of the graft to AMR in spite of the presence of antibodies against donor endothelium is called accommodation. Accommodation is commonly noted in ABO-incompatible kidney transplantation. The study to elucidate the mechanisms of accommodation in ABO-incompatible kidney transplantation was reported.[13] There are several proposed mechanisms to describe this phenomenon. Decrease in antigen-antibody interaction, difference in antigen expression, decreased susceptibility of injury to endothelium and complement components are possible mechanisms. In addition the presence of diffuse C4d without capillary damage or inflammation infers that accommodation may include resistance to the terminal complement cascade in ABO-incompatible kidney transplantation. It has been found that there is more graft loss

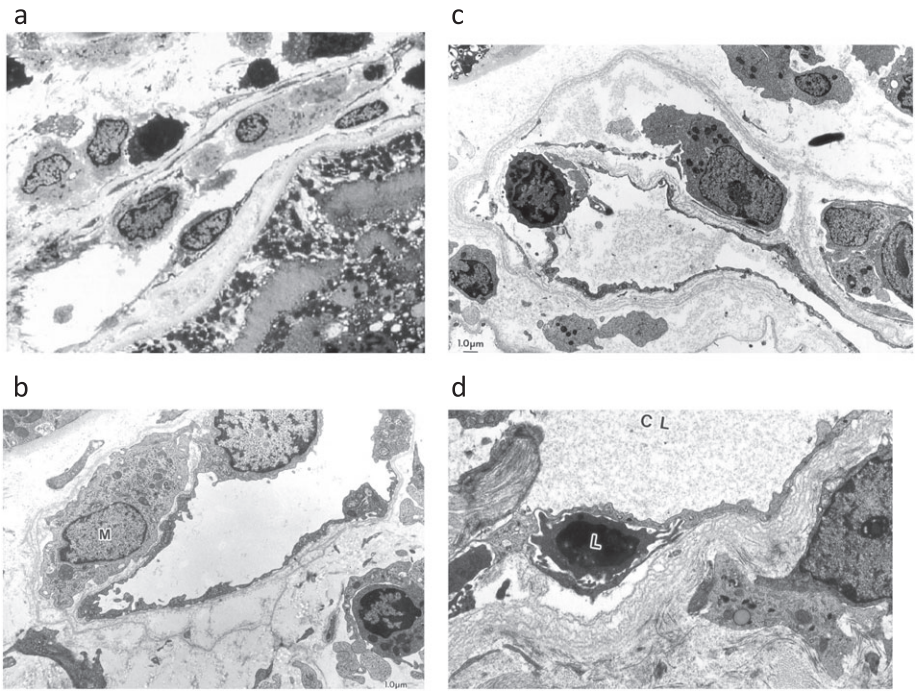

Fig. 1 Changes in peritubular capillary and peritubular capillary basement membrane from very early phase to late phase of restructured peritubular capillary basement membrane with multi-layering (a) Invading activated lymphocyte into subendothelial space of peritubular capillary; (b) Very early changes in peritubular capillary basement membrane splitting with severe edema and subendothelial invading macrophage; (c) Early multi-layering of peritubular capillary basement membrane (MLPTCBM) and many infiltrated activated lymphocytes between disrupted basement membranes; (d) Well developed circumferential MLPTCBM and subendothelial invaded lymphocyte.

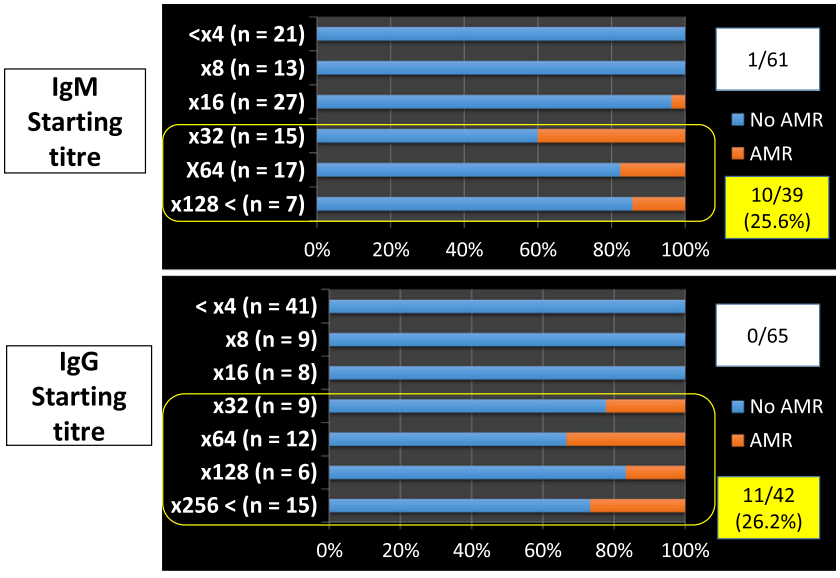

Fig. 2 Impact of Anti-A/B antibody starting titre on active antibody-mediated rejection (AAMR) episodes.

with positive C4d AMR in HLA-sensitized patients, which may be due to differences in biological responses between non-self-carbohydrate antigens (blood type antigens) and non-self-peptide antigens (HLA antigens). We have still unsolved problems to understand the pathogenesis of AMR in ABO-incompatible kidney transplantation.

WHAT KINDS OF DSA CAN CAUSE ANTIBODY-MEDIATED REJECTION?

Consensus guidelines on the testing and clinical management issues associated with HLA and non-HLA antibodies in transplantation are reported. A comprehensive list of recommendations is

provided covering the technical and pretransplantation and posttransplantation monitoring of HLA antibodies in solid organ transplantation. The recommendations are intended to provide state-of-the-art guidance in the use and clinical application of recently developed methods for HLA antibody detection when used in conjunction with traditional methods.

Pre-transplant DSA was associated with AMR at multiple mean fluorescence intensity (MFI) cutoffs. [14] No correlation was seen between acute T-cell mediated rejection (CMR) and pre-transplant DSA at any of the same MFI cutoffs.

Complement fixation by donor-specific HLA antibodies (DSA) is a primary mechanism for antibody-mediated damage of organ allografts. C1q-binding DSAs have a higher risk of rejection and graft loss compared to C1q-DSA.

The C1q binding activity by de novo DSA in patients with AMR largely reflects differences in antibody strength.[15] The C1q assay does not appear to distinguish functionally distinct DSA with clinical significance.

It is unclear whether all donor-specific antibodies (DSA) can cause chronic antibody-mediated rejection (AMR). Subclinical stage before manifestation of renal dysfunction may be a critical period for reversing AMR. The issues to identify factors related to the development of subclinical AMR and to clarify the characteristics of de novo DSA are significant. About 40% of patients with de novo DSA demonstrated biopsy-proven subclinical AMR, leading to progressive graft injury. [16] Significant subclinical AMR-related factors were younger recipients, history of acute T cell-mediated rejection and DSA class II, especially DR-associated DSA. Mean fluorescence intensity (MFI) values of DR-associated DSA were significantly high.

Although short-term graft survival has been improved by recent desensitization protocols including B cell depletion therapy, little is known about risk factors of chronic antibody-mediated rejection in anti-HLA DSA positive recipient in comparison with ABO-incompatible recipients. Protocol biopsies within 1 year revealed subclinical CAMR in 36% of HLA-DSA, 5% of ABO-incompatible group, although clinical acute AMR was observed in 8% and 3%, respectively. [17] The incidence of CAMR was not different between class I and class II DSA. Most of class I DSA (94%) changed to negative 1 year after RTx, whereas 77% of class II DSA remained positive. In addition, the remaining DRB ± DQB DSA caused CAMR in 80% of patients, while DQB DSA alone did not cause CAMR. Sustained class II (DRB ± DQB) DSA detection after RTx may pose a potential risk for developing CAMR, but negative change in class I DSA could also elicit CAMR. [17]

Table 1 Characteristics of suspected chronic active antibody-mediated rejection (CAAMR) based on cg and peritubular capillary basement membrane (PTCBM) by LM

	A-HLA-Ab	PTC-C4d	GC-C4d	g	ptc	cg	ptcbm	cv
1	Class I	(–)	(–)	3	3	1	1	0
2	NA	(–)	(–)	2	1	1	1	0
3	DSA	(–)	(+–)	1	2	1	1	0
4	DSA	LDw	(–)	0	1	1	1	0
5	(–)	(–)	(+)	1	2	1	1	0
6	(–)	LFw	(–)	0	1	1	1	0
7	DSA	LDw	(+–)	1	1	2	1	0
8	(–)	LD	NA	2	1	2	1	0
9	DSA	(–)	(–)	2	2	2	1	0
10	DSA	(–)	(+)	2	2	2	1	0
11	NA	(–)	(–)	2	3	3	1	1
12	Class II	(–)	(+)	2	0	3	1	0
13	DSA	LD	(++)	1	1	3	1	0
14	DSA	LD	(++)	1	1	3	1	0
15	DSA	LFw	(+)	2	2	3	1	0
16	Class II	(–)	(–)	1	1	3	1	1

HOW CAN WE EASILY MAKE A DIAGNOSIS OF ANTIBODY-MEDIATED REJECTION?

Significance of the histopathologic findings suggesting CAAMR and C4d depositions on peritubular and/or glomerular capillaries

Feucht described C4d as a marker for AMR;[18] however, this was not uniformly used until the report by Racusen from Banff conference for allograft pathology. [2] AMR was an entity recognized and C4d staining took key place of morphological classification of AMR. However, a few studies have indicated C4d staining is around 93–96% specific, but 31–95% sensitive. [2]

There has also been evidence for C4d negative AMR, and the limitations of C4d as a marker for AMR are well recognized.[19] High endothelial-associated transcripts genes (ENDATs) expression and endothelial activation with DSA was found to be strongly associated with the presence of transplant glomerulopathy by Sis. [20] Interestingly, fewer than 50% of the biopsies performed with DSA and ENDATs had diffuse C4d staining in peritubular capillaries. C4d negative form of AMR is less severe than C4d positive AMR but is associated with chronic changes of transplant glomerulopathy.

To validate the morphologic criteria for chronic active antibody-mediated rejection, the background of graft biopsies showing glomerular double contour, thickened peritubular capillary basement membrane, glomerulitis, peritubular capillaritis and chronic vasculopathy are studied (Table 1). Among 16 recipients suspected with chronic active antibody-mediated rejection by light microscopic findings, 8 of 14 recipients had DSA and 3 of 6 no-DSA recipients had non-DSA anti-HLA antibodies. C4d positive peritubular capillary wall was noted in 5 of 16, C4d positive glomerular capillary was 8 of 16 biopsies. C4d positivity of any capillary wall was noted in 10 of 16 biopsies. C4d positivity of glomerular capillary wall might be a better marker of chronic antibody-mediated rejection than C4d positivity on peritubular capillary.

CONCLUSION

Late AMR is a major cause of late kidney transplant failure. Chronic active antibody-mediated rejection is a major cause of graft loss as well as patient death with functioning graft. Early

and sensitive diagnostic attempts are pivotal to prevent chronic graft failure.

We focused on several issues concerning antibody-mediated rejection in this article. Unsolved problems of antibody-mediated rejection have remained. Clear-cut diagnosis of pathologic condition may be difficult in some of recipients with chronic graft injuries.

Clinicopathological criteria for chronic rejection are well established by Banff conference for allograft pathology; however, the convenient term chronic allograft nephropathy remains as an indistinct cause of graft loss in the late post-transplantation period for a while.

REFERENCES

1. Racusen LC, Solez K, Colvin RB *et al*. The Banff 97 working classification of renal allograft pathology. *Kidney Int.* 1997; **51**: 1476–80.
2. Racusen LC, Colvin RB, Solez K *et al.* Antibody-mediated rejection criteria—an addition to the Banff '97 classification of renal allograft rejection. *Am. J. Transplant.* 2003; **3**: 708–14.
3. Solez K, Colvin RB, Racusen LC *et al.* Banff '05 Meeting Report: differential diagnosis of chronic allograft injury and elimination of chronic allograft nephropathy ('CAN'). *Am. J. Transplant.* 2007; **7**: 518–26.
4. Duquesnoy RJ, Mostecki J, Hariharan J *et al.* Structurally based epitope analysis of major histocompatibility complex class I-related chain A (MICA) antibody specificity patterns. *Hum. Immunol.* 2008; **69**: 826–32.
5. Worthington JE, McEwen A, McWilliam LJ *et al.* Association between C4d staining in renal transplant biopsies, production of donor-specific HLA antibodies, and graft outcome. *Transplantation* 2007; **83**: 398–403.
6. Solez K, Colvin RB, Racusen LC *et al.* Banff 07 classification of renal allograft pathology: updates and future directions. *Am. J. Transplant.* 2008; **8**: 753–60.
7. Sis B, Mengel M, Haas M *et al.* Banff '09 meeting report: antibody mediated graft deterioration and implementation of Banff working groups. *Am. J. Transplant.* 2010; **10**: 464–71.
8. Mengel M, Sis B, Haas M *et al.* Banff 2011 Meeting report: new concepts in antibody-mediated rejection. *Am. J. Transplant.* 2012; **12**: 563–70.
9. Haas M, Sis B, Racusen LC *et al.* Banff 2013 meeting report: inclusion of c4d-negative antibody-mediated rejection and antibody-associated arterial lesions. *Am. J. Transplant.* 2014; **14**: 272–83.
10. Oikawa T, Morozumi K, Koyama K *et al.* Electron microscopic peritubular capillary lesions: a new criterion for chronic rejection. *Clin. Transplant.* 1999; **13** (Suppl. 1): 24–32.
11. Takahashi K, Saito K. Present status of ABO-incompatible kidney transplantation in Japan. *Xenotransplantation* 2006; **13**: 118–22.
12. Tanabe T, Ishida H, Horita S *et al.* Decrease of blood type antigenicity over the long-term after ABO-incompatible kidney transplantation. *Transpl. Immunol.* 2011; **25**: 1–6.
13. Takahashi K. A new concept of accommodation in ABO-incompatible kidney transplantation. *Clin. Transplant.* 2005; **19** (Suppl 14): 76–85.
14. Wu P, Jin J, Everly MJ *et al.* Impact of alloantibody strength in cross-match negative DSA positive kidney transplantation. *Clin. Biochem.* 2013; **46**: 1389–93.
15. Yell M, Muth BL, Kaufman DB *et al.* C1q Binding Activity of De Novo Donor-specific HLA Antibodies in Renal Transplant Recipients With and Without Antibody-mediated Rejection. *Transplantation* 2015; **99**: 1151–5.
16. Yamamoto T, Watarai Y, Takeda A *et al.* De Novo Anti-HLA DSA Characteristics and Subclinical Antibody-Mediated Kidney Transplantation. *Transplantation* 2015 Dec 4. [Epub ahead of print].
17. Yamanaga S, Watarai Y, Yamamoto T *et al.* Frequent development of subclinical chronic antibody-mediated rejection within 1 year after renal transplantation with pre-transplant positive donor-specific antibodies and negative CDC crossmatches. *Human Immunol.* 2013; **74**: 1111–18.
18. Feucht HE, Schneeberger H, Hillebrand G *et al.* Capillary deposition of C4d complement fragment and early renal graft loss. *Kidney Int.* 1993; **43**: 1333–8.
19. Haas M. C4d-negative antibody-mediated rejection in renal allografts: evidence for its existence and effect on graft survival. *Clin. Nephrol.* 2011; **75**: 271–8.
20. Sis B, Jhangri GS, Bunnag S, Allanach K, Kaplan B, Halloran PF. Endothelial gene expression in kidney transplants with alloantibody indicates Antibody-mediated damage despite lack of C4d staining. *Am. J. Transplant.* 2009; **9**: 2312–23.