

Sensitization after Kidney Transplantation

Enver Akalin* and Manuel Pascual[†]

*Renal Division and Recanati/Miller Transplantation Institute, Mount Sinai Medical School, New York, New York; and

[†]Transplantation Center, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland

Kidney transplant recipients may develop *de novo* anti-HLA and non-HLA antibodies after transplantation. Although these antibodies may be donor-specific or non-donor-specific, their presence may increase the risk for acute and chronic rejection, thereby decreasing allograft survival. The introduction of more sensitive and specific methods to detect anti-HLA antibodies, such as Flow Specific Beads and FlowPRA, both before and after transplantation, will help to define immunologically high-risk kidney transplant recipients. Thus, posttransplantation monitoring of anti-HLA antibody production will allow the identification of kidney transplant recipients who might be at increased risk for late allograft failure. Moreover, knowledge of alloantibody status after transplantation may help to guide the appropriate use of immunomodulatory agents to down-regulate anti-HLA antibody production.

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Pretransplantation unsensitized kidney transplant recipients may develop *de novo* donor-specific (DS) anti-HLA antibodies, non-donor-specific (NDS) anti-HLA antibodies, or DS non-HLA antibodies after transplantation. The development of *de novo* antibodies may increase the risk for acute and chronic rejection and decrease allograft survival (1–4). Because of insufficient routine posttransplantation monitoring of *de novo* antibody production, the exact incidence of humoral alloimmune responses after kidney transplantation is still uncertain. In this review article, we discuss recent studies that have investigated the development of *de novo* alloantibodies in kidney transplant recipients and its effect on allograft outcome.

Incidence and Allograft Outcome

The frequency of anti-HLA antibodies detected after kidney transplantation is extremely variable, ranging between 1.6 and 60% (5–23) (Table 1). This variability among studies is the result of multiple factors, including the type of assays used (*e.g.*, less sensitive techniques such as complement-dependent cytotoxicity [CDC] cross-match assays, compared with more sensitive methods such as FlowPRA [One Lambda, Canoga Park, CA] or Flow Specific Beads [Luminex, Canoga Park, CA]), the type of immunosuppression used, the type of patient population analyzed (*e.g.*, randomly selected patients or selected group of patients with acute or chronic rejection), or variable times of sample collection (*e.g.*, starting late after transplantation may exclude the patients who lost the allograft early as a result of humoral rejection). Moreover, in some studies, DS alloantibodies were measured, whereas in others, only panel reactive antibodies (PRA) were analyzed. In recent years, the addition of

anti-human globulin–CDC and flow-cytometry (FC) cross-match along with solid-phase assays (ELISA, Flow Specific Beads, and FlowPRA) into tissue typing techniques has further enhanced the ability to detect anti-HLA antibodies. Flow Specific Beads and FlowPRA are membrane-independent flow cytometric techniques that utilizing purified HLA antigens coupled to micro-particles (24). These methods can identify anti-HLA antibodies that were missed by CDC methods (25). To define “*de novo*” anti-HLA antibodies exactly, a patient’s pretransplant alloantibody status should be studied by the most sensitive assays. A significant number of studies using retrospective FC cross-match tests revealed an increased risk for early acute rejection episodes, primary nonfunction, and/or decreased allograft survival in patients who had pretransplantation CDC-negative but FC-positive cross-match results (3). Thus, it seems that using more sensitive techniques to detect pretransplantation anti-HLA antibodies may improve the allograft outcome by decreasing the risk for early acute humoral rejection episodes.

Worthington *et al.* (18) investigated retrospectively 112 kidney transplant recipients, who lost their allograft and were waiting on the transplant list, for the presence of anti-HLA antibodies by ELISA and compared them with 123 patients who had received kidney transplants during the same period but had functioning allografts for >5 yr. Overall, 50.9% of patients who lost their allografts had produced anti-HLA antibodies, compared with 1.6% in stable kidney transplant recipients. In a recent study by Cardarelli *et al.* (23), 11% of stable outpatient renal transplant recipients, >6 mo after transplantation, had circulating anti-HLA antibodies. However, only 4.4% of patients had *de novo* DS anti-HLA antibodies. A similar study screened 1229 kidney transplant recipients, who were at least 1 yr after transplantation, for anti-HLA antibodies by CDC, ELISA, and Luminex Flow Beads (Luminex) (21). The results showed that 5.5% had DS, 11.3% had NDS, and 83% had no anti-HLA antibodies. In a multivariate analysis, HLA-DR matching, pretransplantation sensitization, and acute rejection

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Address correspondence to: Dr. Enver Akalin, Mount Sinai School of Medicine, One Gustave L. Levy Place, Box 1104, New York, NY 10029. Phone: 212-659-8003; Fax: 212-348-2474 E-mail: enver.akalin@msnyuhealth.org

Table 1. Posttransplantation sensitization and clinical outcomes in pretransplantation unsensitized kidney transplant recipients^a

Reference	Methods	Time Posttransplantation Sera Collected	CXM and/or Anti-HLA Ab	Clinical Outcome
Martin <i>et al.</i> , 1987 (5)	CDC	0 to 5 yr	171 negative 63 positive (50% class I, 11% class II, 36% both)	81% GS (1 yr) 48% GS (1 yr)
Scornik <i>et al.</i> , 1989 (6)	FC	0 to 6 mo	13/48 (27%) AR patients had anti-HLA Ab None of 22 patients w/o AR had Ab	
Suciu-Foca <i>et al.</i> , 1991 (7)	CDC	0 to 12 mo	28 negative 30 positive	71% GS (4 yr) 45% GS (4 yr)
Halloran <i>et al.</i> , 1992 (8)	CDC	0 to 3 mo	51 negative 13 positive (class I)	41% AR 100% AR
Davenport <i>et al.</i> , 1994 (9)	CDC	0 to 92 mo	28 negative 36 positive	Better kidney function 44% graft loss
Lobo <i>et al.</i> , 1995 (10)	AHG-CDC	0 to 12 mo	47 negative/no AR 63 negative/AR 17 positive/AR	100% GS (1 yr) 72% GS (1 yr) 24% GS (1 yr)
Crespo <i>et al.</i> , 1998 (11)	CDC FC	0 to 3 mo	30 steroid-sensitive AR 51 steroid-insensitive AR	0% DSA 37% DSA
Christiaans <i>et al.</i> , 1998 (12)	CDC FC (retrospective)	0 to 6 mo	123 negative 8 positive 113 negative 14 positive	37% AR, 86% GS (1 yr) 100% AR, 38% GS (1 yr) 35% AR, 60% GS (5 yr) 80% AR, 35% GS (5 yr)
Muller-Steindart <i>et al.</i> , 2000 (13)	CDC Flow PRA (retrospective)	0 to 2 mo	50 negative 4 class I 4 class II	39% AR, 92% GS (3 yr) 40% AR, 60% GS (3 yr) 50% AR, 25% GS (3 yr)
Supon <i>et al.</i> , 2001 (14)	AHG-CDC ELISA	1 wk to 11 yr	160 negative 1 class I 15 class II	49% AR 100% AR 60% AR
Piazza <i>et al.</i> , 2001 (15)	FC Flow PRA	0 to 12 mo	91 negative 29 positive 7 class I, 2 class II, 12 both classes I and II	13% AR, 99% GS (2 yr) 62% AR, 66% GS (2 yr)
Pelletier <i>et al.</i> , 2002 (16)	Flow PRA	1 mo to 9.5 yr	179 negative 50 positive 5 class I, 34 class II, 11 both classes I and II	4% AR, 97% GS (8 yr) 44% AR, 66% GS (8 yr)
Lee <i>et al.</i> , 2002 (17)	ELISA	3 mo to 8 yr	80 negative 28 positive	20% graft loss 50% graft loss
Worthington <i>et al.</i> , 2003 (18)	CDC ELISA	1 mo to 16 yr	1.6% of 123 stable patients had anti-HLA Ab 51% of 112 failed transplants had anti-HLA Ab	
Terasaki and Ozawa, 2004 (19)	CDC/FC ELISA	1 yr	1421 negative 244 positive	8.6% graft failure in 1 yr 3.0% graft failure in 1 yr
Zhang <i>et al.</i> , 2005 (20)	ELISA/FC Flow Beads	0 to 104 wk	10 negative 19 positive (11 DS) (5 class I, 3 class II, 3 both classes I and II)	10/11 had ACR and/or AHR
Hourmant <i>et al.</i> , 2005 (21)	CDC/ELISA Flow Beads	1 to 30 y	1021 negative 68 positive (DS, 1 class I, 67 class II) 140 positive (NDS)	Better GS and kidney function
Mizufani <i>et al.</i> , 2005 (22)	CDC Flow PRA	6 mo to 12 yr	46% of 26 stable patients had anti-HLA Ab 72% of 39 failed had Ab (15 class I, 25 class II)	
Cardarelli <i>et al.</i> , 2005 (23)	ELISA	6 to 392 mo	221 negative 28 positive	Better kidney function 11 DS, 17 NDS (3 class I, 6 class II, 2 both classes I and II)

^aACR, acute cellular rejection; AHG, anti-human globulin; AHR, acute humoral rejection; AR, acute rejection; CDC, complement-dependent cytotoxicity; CXM, cross-match; DSA, donor-specific antibody; FC, flow cytometry; GS, graft survival; NDS, non-donor-specific; PRA, panel reactive antibody.

were significantly associated with the development of both DS and NDS anti-HLA antibodies, and patients with alloantibodies demonstrated lower graft survival, poorer allograft function, and more proteinuria. An international cooperative study involving 23 kidney transplant centers conducted a prospective clinical trial to determine the effects of anti-HLA antibodies on kidney allograft survival (19). The overall frequency of anti-HLA antibodies was 20.9% in kidney transplant recipients. It is interesting that among 2185 unsensitized patients, 14.7% developed *de novo* anti-HLA antibodies during 1 yr of follow-up. The results of this important study also revealed that, whereas 8.6% of allografts in patients with *de novo* anti-HLA antibodies failed at 1 yr, only 3.0% of allografts in patients without alloantibodies failed, thus emphasizing the need to identify better the humoral responses after kidney transplantation.

Most studies have demonstrated a significant relationship between the development of *de novo* anti-HLA antibodies and acute rejection episodes. Piazza *et al.* (15) prospectively screened 120 unsensitized kidney transplant recipients for DS anti-HLA antibodies at 1 yr after transplantation by FC cross-match and FlowPRA. Overall, 24.2% had developed DS anti-HLA antibodies, and most of them were detected within the first 3 mo after transplantation. Patients with anti-HLA antibodies had higher incidence of acute rejection episodes (62 *versus* 13%), more allograft failure (34 *versus* 1%), and higher creatinine levels (2.5 ± 1.3 *versus* 1.7 ± 0.5 mg/dl) at 2 yr after transplantation compared with patients without anti-HLA antibodies. Recently, Zhang *et al.* (20) prospectively followed 49 immunologically high-risk kidney transplant recipients for anti-HLA antibodies by ELISA and Luminex Flow Beads. All recipients were pretransplantation CDC and FC T and B cross-match negative, with the exception of one patient with a weak positive T cell FC cross-match positivity. A total of 22.4% of recipients developed *de novo* DS and 38.8% developed NDS anti-HLA antibodies after transplantation. Among the patients with *de novo* anti-HLA antibodies, most developed acute humoral rejections. Crespo *et al.* (11) studied 81 patients who had acute rejection episodes within 3 mo after transplantation and demonstrated that, whereas none of the steroid-sensitive patients had DS anti-HLA antibodies, 37% of patients with steroid-insensitive acute rejection had circulating alloantibodies. A total of 95% of patients with alloantibodies had widespread peritubular C4d staining in their allograft biopsies. The importance of C4d staining as a marker of anti-HLA antibody-mediated allograft injury is discussed in detail by Dr. Racusen in this issue. It will be important to analyze whether the incidence of humoral responses after kidney transplantation will decrease with the use of modern induction immunosuppressive strategies, as the rates of acute cellular rejection have decreased recently to <20% in many centers.

Type of Antibody and Its Effect on Allograft Outcome

The type of *de novo* anti-HLA antibody (class I or II) and its relationship to the development of acute or chronic rejection have been studied by various investigators. An earlier study by Martin *et al.* (5) showed that most *de novo* anti-HLA antibodies

were anti-HLA class I (50% class I alone and 36% both classes I and II). In the early 1990s, Halloran *et al.* (8) studied class I anti-HLA antibodies by the CDC method in 64 patients within the first 3 mo after transplantation. Whereas all 13 patients with *de novo* class I antibodies had acute rejection episodes, only 41% of patients without class I antibodies developed acute rejection episodes. Patients with alloantibodies had more severe rejections, and rejections occurred earlier and led to higher graft loss. In the study by Worthington *et al.* (18), 60% of patients with alloantibodies had developed these antibodies before allograft failure. Seventeen patients developed class I, 14 developed class II, and three developed both classes I and II anti-HLA antibodies. There was no information about the relationship between acute rejection episodes and alloantibodies in these patients. In the study by Zhang *et al.* (20), five patients showed class I, three showed class II, and three showed both classes I and II anti-HLA antibodies. Pelletier *et al.* (16) studied posttransplantation alloantibodies in 277 kidney transplant recipients by FlowPRA and showed that 18% of the recipients developed *de novo* anti-HLA antibodies. A total of 10% of these antibodies were class I, 68% were class II, and 22% were both classes I and II. In that study, only class II anti-HLA antibodies were associated with previous acute rejection episodes and were found to be an independent risk factor for chronic allograft rejection. The authors did not report whether anti-HLA antibodies were DS or not. Lee *et al.* (17) prospectively followed 139 kidney transplant recipients for the development of anti-HLA antibodies at 3 mo, 6 mo, and yearly after transplantation for 8 yr by ELISA. Whereas all 29 patients with biopsy-proven chronic rejection had developed anti-HLA antibodies, only 27% of stable patients without chronic rejection were found to have posttransplantation anti-HLA antibodies. Martin *et al.* (26) investigated anti-HLA antibodies by FlowPRA in 20 kidney transplant recipients who underwent transplant nephrectomy. It is interesting that whereas 42.1 and 31.6% had DS antibodies in their sera at 1 yr after transplantation and at the time of nephrectomy, respectively, 73.6% of nephrectomy eluates and 73.6% of postnephrectomy serum samples showed DS antibodies, demonstrating that in some cases, DS anti-HLA antibodies were bound to the allograft and not detectable in serum. Patients developed both classes I and II antibodies, indicating that both types of alloantibodies may be involved in the pathogenesis of chronic allograft nephropathy (CAN). A retrospective analysis of 420 sera from 263 unsensitized renal transplant recipients by ELISA demonstrated 1.4% class I and 6% class II anti-HLA antibodies (14). Fourteen patients with class II anti-HLA antibodies had acute and seven had chronic rejection. In contrast to two previous studies, 77% of all acute and chronic rejections occurred in patients without detectable HLA antibodies. From these studies, it seems that both anti-HLA class I and class II alloantibodies can be associated with acute and chronic allograft rejection in kidney transplantation.

Occasionally, the development of NDS anti-HLA antibodies (without DS) can be observed, but their significance is not clear. Some authors have suggested that in such cases, both DS and NDS anti-HLA antibodies may develop; however, DS antibodies are bound and absorbed to the allograft and NDS antibodies

are found in the circulation (2,17,26,27). A recent article showed that 26 of 27 patients who did not have previous DS antibody produced DS antibody after allograft nephropathy (27).

The clinical significance of IgM type anti-HLA antibodies in relation to allograft rejection is not clear. Whereas earlier studies using CDC methods and FC suggested that IgM antibodies are not detrimental to the graft (28), a recent study found that a positive T cell IgM FC cross-match at the time of transplantation could be a risk factor for allograft rejection (29). A prospective study of cadaveric kidney transplant recipients by FC demonstrated that 40% of the patients with acute rejection had IgG type antibodies, whereas only 9% of the nonrejecting patients developed alloantibodies that were exclusively IgM type (6). Despite lack of definitive studies, most centers find that pretransplantation DS IgM type anti-HLA antibodies are not relevant as risk factors for acute rejection, and the same might be true after transplantation. Also, detection of IgM by FC is difficult, so FC is not as sensitive as CDC for the detection of IgM and only very high titer of IgM can be detected by FC.

MHC class I chain-related antigen A (MICA) and B (MICB) are two members of highly polymorphic HLA class I genes, which are located in close proximity to the HLA-B locus and encode for cell-surface glycoproteins, including endothelial cells, monocytes, gut epithelium, and fibroblasts but not lymphocytes (30). MICA and MICB expressions were demonstrated in kidney and pancreas allografts with acute and chronic rejection and acute tubular necrosis (31). Mizutani *et al.* (22) compared 39 previously unsensitized kidney transplant recipients who rejected their allografts with 26 recipients with functioning allografts. Both groups had at least 1000 d of graft survival. Detection of anti-HLA antibodies was made by FlowPRA and that for MICA antibodies by cytotoxicity on recombinant cell lines. The incidence of IgG anti-HLA antibodies plus MICA antibodies was higher in patients who rejected their allografts compared with stable patients (77 versus 42%). When patients with IgM anti-HLA were included in the analysis, 95% of patients with failed transplants had antibodies compared with 58% in patients with functioning grafts. The authors proposed that, because MICA and MICB are highly polymorphic antigens and are present on endothelial cells, they may be important targets for allograft rejection as well, in addition to anti-HLA antibodies.

Rarely, acute humoral rejection episodes have been demonstrated in kidney transplant recipients without DS anti-HLA antibodies in their serum. This could be due partly to lack of using sensitive techniques to demonstrate the presence of low levels of anti-HLA antibodies but also due to non-HLA antibodies, which might be directed against endothelial cells, monocytes, or angiotensin II receptors. Opelz (32) recently reported that HLA-identical sibling transplant recipients with no pretransplantation PRA had significantly higher (72.4%) 10-yr allograft survival than recipients with 1 to 50% PRA (63.3%) or recipients with >50% PRA (55.5%), indicating the possible relevance of NDS HLA immunity in kidney allograft outcomes. The targets for antibodies that cause late graft failure could be minor histocompatibility antigens, or PRA reactivity may indicate a general state of heightened immune responsiveness as a

result of previous alloimmunization. Anti-endothelium/monocyte antibodies have been shown to be associated with hyperacute rejection, although this is probably rare (33). Two previous studies showed higher anti-endothelial cell antibodies in patients with a failed renal transplant as a result of chronic rejection compared with patients with functioning allografts (34,35). A recent intriguing study also revealed antibodies targeting angiotensin II type 1 receptor in kidney transplant recipients with “vascular rejection,” who did not have detectable DS anti-HLA antibodies (36). Joosten *et al.* (37) demonstrated antibodies to glomerular basement membrane–heparan sulfate proteoglycan agrin in the sera of patients with transplantation glomerulopathy, a sign of chronic allograft rejection. Although these data point toward a potential role for non-HLA antidonor antibodies in allograft rejection, more work will be needed to analyze whether these non-HLA antibodies are secondary to immune-mediated allograft injury or have a primary role as well. Overall, a majority of studies in the field indicate that the most relevant antibodies in kidney transplantation remain anti-HLA alloantibodies, either anti-class I or anti-class II antibodies.

Mechanisms of Development of *De Novo* Anti-HLA Antibodies

In view of the significant relationship between acute rejection and the incidence of *de novo* anti-HLA antibodies, the generation of alloantibodies after transplantation might be the result of T cell sensitization during or after an acute rejection episode. Indeed, the activation of the humoral immune response to donor antigens by CD4+ T cell-dependent mechanisms has been shown in animal models (38). Significant numbers of animal and human studies have provided evidence that T cell recognition of processed alloantigen *via* the indirect pathway is a key factor that initiates and maintains progression of chronic allograft rejection (39,40). When studying alloantibody production, it should be emphasized that the detection of Ig class-switched alloantibody is inevitably associated with the clinical expansion of T cells with indirect allospecificity, *i.e.*, only indirect pathway T cells can provide “help” for allospecific B cells (39–41). Thus, *de novo* anti-HLA antibody production may be the culmination of an early and specific T–B cell cooperation. The development of *de novo* alloantibodies through T cell-independent B cell mechanisms requires further investigation. Salama *et al.* (42) also demonstrated that antigen-specific regulatory CD4+CD25+ T cells can suppress alloimmune responses to donor HLA peptides in renal transplant recipients. The importance of T regulatory cells on the development of *de novo* alloantibodies also requires further investigation. In general, there is a need for more mechanistic studies on the processes that initiate the production of *de novo* alloantibodies to understand better the pathogenesis of progressive immunologic injury (acute and subacute) that leads to late kidney allograft loss in some recipients.

Treatment of *De Novo* Anti-HLA Antibodies

The best therapeutic approach for kidney transplant recipients with allograft rejection associated with *de novo* donor-

specific alloantibodies will depend on the clinical condition: Episode of early acute *versus* late chronic humoral rejection. In cases of severe acute humoral rejection, the treatment needs to be started rapidly to prevent the risk for allograft loss. Removal of DS alloantibodies with effective control of alloantibody production and the reversal of acute rejection is now possible, as shown by Pascual and colleagues and others since the mid-1990s (4,11,43–50). Therapeutic strategies, including combinations of plasmapheresis (or immunoadsorption), intravenous Ig (IVIG), and Rituximab (anti-CD20), along with tacrolimus and mycophenolate mofetil, have been used successfully to treat severe and “refractory” acute humoral rejection—that is, acute humoral rejection that is resistant to both steroid and antilymphocyte therapy.

IVIG preparations have been used in the treatment of a variety of autoimmune and systemic inflammatory conditions because of their immunomodulatory properties. Recent clinical experience has also shown that IVIG is useful in the field of transplantation (48). IVIG inhibits the *in vitro* anti-HLA lymphocytotoxicity of sera from highly sensitized patients, *in vivo* decreases the titer and potency of anti-HLA antibodies, and its use before transplantation may increase the transplantation rates of patients who are on the waiting list with high PRA titers (>50%) (52). High-dose IVIG (2 g/kg) treatment can abrogate positive CDC T cell cross-matches and allows successful kidney transplantation (51). We have used IVIG and Thymoglobulin induction treatment in CDC B cell and/or FC T and/or B cell cross-match kidney transplant recipients (53,56,57). Eleven patients had pretransplantation DS anti-HLA antibodies by Flow Beads (three class I, three class II, and five both classes I and II). Posttransplantation follow-up of these antibodies at 6 mo to 1 yr after transplantation showed that four patients had lost pretransplantation class II antibodies and two had lost class I anti-HLA antibodies. The mechanisms of IVIG are diverse and act on different components of the immune system. An initial mechanism is probably due to anti-idiotypic interactions with anti-HLA antibodies after the infusion of IVIG. However, in a recent study that investigated the mechanisms of IVIG in 23 sensitized patients, it was shown that IVIG inhibits complement activation, but there was no significant contribution of the anti-idiotypic effect (54). It is interesting that the immunomodulatory effects of IVIG treatment can persist beyond its half-life, indicating the presence of ongoing and sustained active inhibitory mechanisms.

Despite the increased interest in using IVIG in highly sensitized kidney patients before transplantation, its use in kidney transplant recipients with *de novo* anti-HLA antibody production remains to be studied. We recently reported a case of a patient with CAN and positive C4d staining on biopsy and *de novo* DS specific anti-HLA antibodies and her response to IVIG treatment (55). Our patient had received a kidney transplant from her non-HLA identical sister. PRA level was 0%, and there was no DS anti-HLA antibody by Luminex Flow Beads. She underwent allograft biopsy 2 yr after transplantation because of worsening kidney function, which showed grade II CAN (per Banff classification) with positive C4d staining. Repeat Flow Beads demonstrated both class I and II anti-HLA antibodies.

She was treated with 200 mg/kg IVIG in 2 consecutive weeks. The patient’s creatinine level returned to her baseline 1 mo after the last IVIG treatment, and repeat measurements of DS anti-HLA antibodies were negative. The dose of IVIG used in kidney transplant recipients for desensitization and/or the treatment of acute humoral rejection varies between 100 mg/kg and 2.0 g/kg, where lower dose was used in conjunction with plasmapheresis to abrogate CDC T cell cross-match positivity (49,52). We used a 100- to 500-mg/kg dose in CDC T cell-negative but CDC B and/or FC T/B cell cross-match-positive patients (53,56,57). The exact dose to use for patients with *de novo* anti-HLA antibodies is not clear.

Anti-CD20 mAb therapy aiming at depleting B cells (and, thereby, possibly suppressing alloantibody production) may be an interesting option and also has been added as a component of rescue therapy in some isolated cases (58,59). However, this approach remains to be investigated further before it can be recommended as a standard therapy of humoral rejection because of its additional costs and its potential for overimmunosuppression in patients who already are heavily immunosuppressed. The efficacy of immunosuppressants, used alone or in combination, in controlling alloimmune humoral responses in patients with late allograft dysfunction remains to be investigated prospectively. To date, data obtained from small series indicate that the combination of tacrolimus and mycophenolate mofetil therapy can suppress effectively short- and long-term anti-donor antibody production in recipients with acute or late alloantibody-mediated allograft dysfunction (43,49,60). However, no formal controlled clinical trial has demonstrated the superiority of this combination over others (*e.g.*, tacrolimus-sirolimus, cyclosporine-mycophenolate). It seems important to note that, ideally, appropriate immunomodulatory interventions should occur before the arterial or glomerular lesions of CAN develop.

It also can be noted that co-stimulation by CD40–40L, CD28–B7, and B7h-ICOS is crucial for generation of a humoral immune response and alloantibody production. Blocking these pathways was shown to decrease antidonor antibody responses in animal models (61–63). The potential beneficial effect of blocking co-stimulation pathways on the development of *de novo* anti-HLA antibodies in clinical transplantation will require further studies.

Recent studies indicated the development of anti-HLA antibodies with Thymoglobulin (64) and Campath-1H (65) induction treatment. This observational finding in small series requires further investigation to determine the real incidence of *de novo* anti-HLA antibodies after induction treatment with Campath-1H and Thymoglobulin. Finally, there are occasional kidney transplant recipients who develop *de novo* anti-HLA antibodies but continue to have apparent stable allograft function for many months, suggesting a state of “accommodation.” However, Terasaki proposed that for some patients, there may be a long delay between alloantibody detection in serum and allograft injury or failure (2). Therefore, patients who have detectable circulating alloantibodies and maintain stable allograft function may require serial protocol biopsies to rule out subclinical (humoral and/or cellular) allograft injury. The effect

of controlling antidonor alloimmune humoral responses on allograft survival in these patients is not clear, and it also will need to be addressed in well-designed prospective clinical trials.

Conclusion

The introduction of more sensitive and specific methods to detect anti-HLA antibodies before transplantation might better discriminate between immunologically low- and high-risk kidney transplant recipients. The development of *de novo* anti-HLA alloantibodies has been associated with acute or chronic allograft rejection. Therefore, sequential posttransplantation monitoring of anti-HLA antibodies in serum, *e.g.*, every 3 mo for the first year and then annually, would be useful to identify patients who are at higher risk for allograft failure. Controlling alloimmune humoral responses with appropriate immunomodulatory drug regimens undoubtedly will play an important role, particularly in the current era of “immunosuppression minimization” strategies. Such strategies, although tempting to reduce drug side effects, indeed carry the risk for increasing the likelihood of posttransplantation sensitization in stable kidney transplant recipients. Future prospective studies with serial protocol biopsies and monitoring anti-HLA antibodies will define more precisely the contribution of the development of anti-HLA antibodies on allograft injury and graft survival. These prospective studies also may identify when *de novo* antibodies develop after transplantation and the difference between early and late development of anti-HLA antibodies on allograft pathology. These studies eventually would serve as a basis to design future trials for the prevention and the treatment of *de novo* anti-HLA antibodies using new immunomodulatory agents.

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