Background—Activation of T cells induces immunoglobulin (Ig)M-to-IgG B-cell isotype switching via costimulatory regulatory pathways. Because rejection of transplanted organs is preceded by alloantigen-dependent T-cell activation, we investigated whether B-cell isotype switching could predict acute cellular rejection and the subsequent development of transplantation-related coronary artery disease (TCAD) in cardiac transplant recipients.

Methods and Results—Among 267 nonsensitized heart transplant recipients, switching from IgM to IgG anti-human leukocyte antigens (HLAs) antibodies directed against class II but not against class I antigens was associated with a shorter duration to high-grade rejection, defined as International Society for Heart and Lung Transplantation grade 3A or higher (P<0.001), a higher cumulative rejection frequency (P=0.002), accelerated development of TCAD (P=0.04), and decreased late survival (P=0.03). Conversely, the persistence of IgM anti-HLA antibodies against class II but not against class I antigens for >30 days and the lack of IgG isotype switching were associated with protection against both acute rejection (P=0.02) and TCAD (P=0.05). Alloisotype switching coincided with T-cell activation, as evidenced by increased serum levels of soluble CD40 ligand costimulatory molecules. Finally, a case-control study showed that reduction of cardiac allograft rejection by mycophenolic acid was accompanied by reduced CD40 ligand serum levels and the prevention of IgM-to-IgG anti-HLA class II antibody switching.

Conclusions—T-cell–dependent B-cell isotype switching and the consequent production of IgG anti-HLA class II antibodies are strongly correlated with acute cellular rejection, a high incidence of recurrent rejections, TCAD, and poor long-term survival. Detecting this isotype switch is a clinically useful surrogate marker for in vivo T-cell activation and may provide a noninvasive approach for monitoring the efficacy of T-cell targeted immunosuppressive therapy in heart transplant recipients. (Circulation. 2005;112:2468-2476.)

Key Words: antibodies ■ coronary disease ■ transplantation

The long-term success of cardiac transplantation is currently limited by the high incidence of transplantation-related coronary artery disease (TCAD), a complication principally related to antecedent episodes of high-grade allograft rejection and/or ongoing immune responses against donor human leukocyte antigens (HLAs). Between 42% and 88% of cardiac transplant recipients produce anti-HLA antibodies at some point after transplantation, and when these are first detected within the first year after transplantation, 5-year heart allograft survival is reduced from 91% to 78%. This is the result of both an increase in episodes of acute rejection and the development of accelerated TCAD. Adverse outcomes have been predominantly associated with the immunoglobulin (Ig) G isotype of anti-HLA antibodies, and switching of isotypes from IgM to IgG alloantibodies has been reported to increase the risk of acute and chronic renal and liver allograft rejection.

The risk of developing high-grade acute cellular rejection is greatest during the first 3 months after cardiac transplantation, when most alloreactive recipient T-cell clones recognize foreign HLA molecules directly. With adequate immunosuppression, over time there is a reduction in the frequency of recipient T-cell clones directly recognizing immunodominant alloantigenic determinants derived from donor HLA class II molecules and a gradual shift to indirect recognition of multiple, new epitopes from these molecules, ie, presented by antigen-presenting cells such as dendritic cells, macrophages, and B cells. These processes, termed...
intermolecular and intramolecular spreading, respectively, are postulated to involve close T-cell and B-cell cooperation and to be associated with an increased risk for long-term graft loss. Consequently, a major objective in cardiac transplantation is to develop immunomodulatory therapies that induce an early and long-lasting state of T-cell and B-cell immune nonreactivity, or tolerance, to donor HLA.

Because IgM-to-IgG isotype switching by B cells is a process that is tightly regulated by both T-cell costimulatory signals and recognition of cognate antigen, in the present study we examined (1) whether there was a correlation between B-cell isotype switching from IgM to IgG antibodies directed against HLA molecules and the complications of cellular rejection and accelerated TCAD, (2) whether the development of isotype switching was correlated with systemic markers of T-cell activation, and (3) whether isotype switching could be prevented by novel, more potent immunosuppressive regimens that reduce T-cell–mediated rejection. Our results indicate that isotype switching and the production of IgG anti-HLA class II antibodies are associated with an increased risk of recurrent rejections, progression to TCAD, and poor long-term survival and that this presumably reflects insufficient immunosuppression of T-cell/B-cell cooperation. Detecting this isotype switch is a clinically useful surrogate marker for in vivo T-cell activation and may provide a noninvasive approach for monitoring the efficacy of T-cell targeted immunosuppressive therapy in heart transplant recipients.

Methods

Patients

Between January 1992 and January 2000, 475 adult patients received heart transplants at the Columbia-Presbyterian Medical Center, New York, NY. The study included 267 previously unsensitized primary heart transplant recipients. Patients who were sensitized before transplantation with anti-HLA antibodies (n=22), who received a second transplant (n=52), or who were supported with left ventricular–assist devices before transplantation (n=90) were excluded from the study. In addition, patients without follow-up data for posttransplantation anti-HLA antibody production (n=31) and those who died within the first month after transplantation (n=33) were excluded. All perioperative deaths were due to nonimmune causes, with the exception of 2 patients who died of acute rejection within the first week after transplantation. The demographic characteristics of patients who were excluded from analysis were similar to those of the studied cohort.

Immunosuppressive Regimen

All patients who received transplants before 1996 were given a triple immunosuppression regimen consisting of cyclosporine, steroids, and azathioprine (AZA). Cyclosporine (Neoral, Novartis Pharmaceuticals) was administered preoperatively and then 1 to 2 mg/kg daily as a continuous infusion until the patient could receive oral medications. Cyclosporine doses were titrated to a whole-blood trough level of 300 to 350 ng/mL. All patients received 4 mg/kg AZA preoperatively and then 2 mg/kg intravenously until they were able to receive oral medications. All patients who received transplants after 1996 received mycophenolate mofetil (MMF) given at a starting dose of 1000 mg twice daily, instead of AZA, in combination with cyclosporine and steroids. Intraoperative methylprednisolone was administered at a dose of 1 g intraoperatively and then at 125 mg q8h for 3 doses. Prednisone was given in tapering doses of 1 mg · kg⁻¹ · d⁻¹ postoperatively to 0.1 mg · kg⁻¹ · d⁻¹ at the fourth month. Rejection episodes were treated with oral or intravenous steroid pulses of 100 mg/d for 3 days followed by a tapered dose for 1 week to the baseline dose. Nonresponders were treated with cytolytic therapy (OKT3 or ATGAM).

HLA Typing

Serological typing of HLA-A and HLA-B loci was done by standard microcytotoxicity techniques. HLA-DR typing was performed by analysis of serology samples and DNA techniques with sequence-specific oligonucleotide primers and polymerase chain reaction.

Anti-HLA Antibodies

At the time of each endomyocardial biopsy, serum was screened for complement-mediated lytic activity, in the presence or absence of dithiothreitol, against T and B lymphocytes from a panel of 70 of the most frequently encountered HLA class I and II antigens. In addition, donor-specific alloantibodies were assessed. Persistent serum reactivity after dithiothreitol treatment identified IgG alloantibodies, whereas the loss of reactivity identified IgM alloantibodies. Anti-HLA class I antibodies were identified when serum reacted with both T-cell and B-cell panels. Anti-HLA class II antibodies were identified when serum reacted with B-cell but not with T-cell panels. Antibodies against both HLA class I and II antigens were identified when serum reacted against both panels and when B-cell reactivity exceeded T-cell reactivity by ≥2-fold. The presence of autoantibodies was excluded by autologous serum cross-match with recipient T cells and B cells. Overall, the use of these combined criteria identified anti-HLA class I and II antibodies with a high degree of sensitivity and specificity.

Detection of IgM-to-IgG Anti-HLA Antibody Switching

Development of isotype switching was considered to have occurred at the time when IgG anti-HLA antibodies were first detected in the serum of a transplant recipient whose previous samples demonstrated either no anti-HLA reactivity or only the presence of IgM anti-HLA antibodies.

Detection of Soluble CD40 Ligand Production

ELISA was used to detect levels of soluble CD40 ligand (Chemicon) from patient sera. Absorbance was measured spectrophotometrically within 10 minutes after adding the phosphoric acid stop solution (450 nm as the primary wavelength and 620 nm as the reference wave length).

Acute Rejection

Rejection was diagnosed by routine endomyocardial biopsy: weekly for the first 4 weeks, then every 2 weeks for the next month, monthly for 4 months, every 2 months for the next 6 months, every 3 months for the next 6 months, and then every 6 to 12 months. The biopsy fragments were graded according to the International Society for Heart and Lung Transplantation criteria. High-grade cellular allograft rejection was defined pathologically as grade 3A or 3B.

Transplantation-Related CAD

The diagnosis of TCAD was based on the results of annually performed coronary angiograms and defined as (1) discrete lesions resulting in >50% obstruction of the proximal or middle portions of major graft vessels or (2) diffuse, concentric narrowing of the whole vessels, including their branches. Patients were not given routine vasodilators before coronary injections. In patients with possible diffuse CAD, intimal thickening was documented by vascular ultrasound. All coronary angiograms were compared with the previous year’s films to detect the presence of luminal irregularities, discrete stenoses, loss of third-order branches, or “pruning” of vessels. Because of concerns related to donor-transmitted CAD, our center generally did not accept hearts from female donors >45 years and male donors >40 years with angiographic evidence of CAD in >1 major vessel. In hearts from younger donors, baseline angiograms were not routinely performed. Hearts explanted before retransplantation and postmortem heart autopsy specimens were ex-
aminated for evidence of vessel occlusion and irregularities, ischemic damage, and the presence of acute cardiac rejection.

The results of annual coronary angiography were available for 222 patients. Three patients with a follow-up period <3 months and 2 patients diagnosed with donor-transmitted coronary artery disease shortly after transplantation were excluded from this analysis.

Statistical Analyses
Survival curves were estimated with the Kaplan-Meier method and compared by log-rank tests. The Kaplan-Meier estimates describing the time to the first episode of high-grade acute rejection used the development of anti-HLA antibodies as a time-varying covariate, wherein each patient was coded as a nonproducer until the time when relevant antibodies were detected. Patients who developed anti-HLA antibodies before the first rejection episode had the time to rejection censored at the time when anti-HLA antibodies were first detected. At that time, these patients were recategorized to the antibody producers group, and the time origin for the time-to-rejection curve was set to the day when anti-HLA antibodies were detected. All patients were followed up until the first episode of high-grade acute rejection or the day of the last negative endomyocardial biopsy. Patients who developed anti-HLA antibodies after the first acute rejection episode were treated as nonproducers of alloantibodies for this analysis. Because most cases of anti-HLA antibody production were detected before the first annual coronary angiography, Kaplan-Meier estimates describing the time to development of TCAD used the development of IgM and IgG anti-HLA antibody switching as a static variable.

The Cox proportional-hazards model was used for multivariable analyses. Separate Cox models were developed to identify risk factors for high-grade acute rejection, TCAD, and IgG anti-HLA class II isotype switching. The multivariable Cox models evaluating the effects of covariates on the risk of high-grade acute rejection used anti-HLA antibody isotype switching as a time-varying covariate, whereas analysis of the risk for progression of TCAD used antibody detection as a static variable. In addition, to accommodate the uncertainty of the time when TCAD developed between annual angiograms, a discrete-time version of the proportional-hazards model was used according to the EXACT option in the SAS PHREG procedure. Covariates that achieved a significance of $P<0.15$ in individual analyses were entered into the multivariable model by stepwise selection to produce a final set of risk factors. The risk factors analyzed included donor and recipient ages; gender; ethnicity; pretransplantation diagnosis of heart disease; donor-recipient complete mismatch at HLA-A, HLA-B, and HLA-DR loci; ischemic time; and triple immunosuppressive regimen with MMF versus AZA.

Because nonfatal morbid events such as cellular rejection can occur repeatedly in the same patient, cumulative frequencies for these events were modeled by the method of Wei et al., which takes into account the fact that repeated episodes may be correlated within each patient. We did not control for multiple comparisons; therefore, type I errors are inflated above their nominal level of 0.05. Results were considered significant for $P<0.05$. Data were analyzed with SAS software version 7.1 (SAS Institute, Inc).

Results
High Frequency of B-Cell Isotype Switching and Production of IgG Anti-HLA Antibodies in Cardiac Transplant Recipients
Of the 267 transplant cases studied, 80% were male, 80% were white, and the mean ± SD age was 51.1 ± 12.4 years. The major causes of heart disease were CAD (41%) and idiopathic cardiomyopathy (41%). The 1- and 5-year graft survival was 95% and 79%, respectively, with a mean follow-up of 3.1 years (range, 0.1 to 7 years).

IgG anti-HLA alloantibody class switching was detected in 54% (143/267) of studied patients, 20% (53/267) produced IgM anti-HLA antibodies only, and 26% (71/267) did not demonstrate anti-HLA antibody production. Among patients who switched isotype, 20% (29/143) developed IgG anti-HLA class I antibodies, 40% (57/143) produced IgG anti-HLA class II antibodies, and 40% (55/143) of recipients demonstrated IgG antibodies against both classes of HLA molecules. As illustrated in Figure 1, by 1 year after transplantation, 28% of patients had developed anti-HLA class I antibodies and 44% had developed anti-HLA class II antibodies. In the majority of patients who switched alloantibody isotype during follow-up, most switching occurred during the first year after transplantation: 86% (74/86) and 88% (100/114) of anti-HLA class I and II producers, respectively. Isotype switching against MHC class I molecules occurred earlier (median, 15 days; range, 2 to 1539 days) than against class II molecules (median, 71 days; range, 5 to 1294 days).

B-Cell Isotype Switching and Production of IgG Anti-HLA Class II Antibodies Are Correlated With the Risk for High-Grade Acute Rejection After Cardiac Transplantation
As shown in Figure 2, B-cell isotype switching and the production of IgG antibodies directed against HLA class II but not against class I molecules were correlated with an increased risk of high-grade acute rejection. Acute rejection.
developed in 53.4% (47/88) of patients who switched isotype to IgG anti-HLA class II compared with only 30.4% (80/263) of nonswitchers (log-rank $P<0.03$). By multivariable analysis, 2 factors were correlated with the increased risk of high-grade acute rejection: switching to IgG anti-HLA class II molecules (relative risk [RR] = 3.30, 95% confidence interval [CI] = 2.18 to 4.99, $P<0.001$) and an AZA-based immunosuppressive regimen relative to one based on MMF (RR = 1.71, 95% CI = 1.20 to 2.44, $P<0.003$). Moreover, producers of IgG anti-HLA class II antibodies experienced a higher incidence of recurrent rejections (23%, versus 12% of nonswitchers, $P=0.06$), and their overall cumulative frequency of high-grade rejection within the first year after transplantation was higher (0.86, versus 0.51 in nonswitchers, $P=0.002$).

In contrast to these findings, isotype switching against HLA class I molecules was not correlated with rejection by multivariable analysis (RR = 0.95, 95% CI = 0.60 to 1.51, $P=0.95$). Producers and nonproducers of anti-HLA class I antibodies did not differ with respect to the overall cumulative frequency of rejections (0.81 versus 0.63, $P=0.16$) or the development of recurrent rejections (18% versus 17%, $P=0.84$).

B-Cell Isotype Switching and Production of IgG Anti-HLA Class II Antibodies Are Correlated With the Risk for TCAD and Decreased Survival

Because recurrent episodes of acute rejection are known to be associated with and predispose to accelerated TCAD, we next investigated whether there was a correlation between a posttransplantation alloantibody isotype switch and progression to TCAD. As shown in Figure 3, the presence of IgG antibodies directed against MHC class II but not against class I molecules was correlated with the risk for TCAD. Among recipients who switched isotype to IgG anti-HLA class II antibodies, TCAD developed within a median of 2.0 years, compared with 3.1 years in nonswitchers (log-rank $P=0.02$). Multivariable analysis confirmed that the isotype switch and the presence of IgG antibodies directed against MHC class II molecules were risk factors associated with accelerated development of TCAD.

Figure 2. A and B, B-cell isotype switching and production of IgG anti-HLA class II antibodies increases the risk of the first high-grade acute rejection after cardiac transplantation. Development of IgG anti-HLA antibodies in this analysis was treated as a time-varying covariate, wherein each patient was coded as a nonproducer of IgG anti-HLA antibodies until the day when the antibodies were detected. Patients who developed IgG anti-HLA antibodies were recategorized to the antibody producers group, and their time to rejection as antibody nonproducers was censored, as described in Methods. Abbreviations are as defined in text.

Figure 3. A and B, Increased risk for TCAD accompanying the B-cell isotype switch is correlated with the presence of IgG anti-HLA class II but not class I antibodies. The analysis included only those patients with available results from the annual angiograms who had no evidence of donor-transmitted CAD. Abbreviations are as defined in text.
TCAD (RR = 1.47, \( P = 0.04 \)). All patients in our study switched the anti-HLA antibody isotype against class II molecules before the development of TCAD. However, later episodes of isotype switching against HLA class II molecules after the first 10 weeks after transplantation (median time to anti-HLA class II antibody switching) portended a higher risk of TCAD (RR = 1.99, \( P = 0.003 \)) than did earlier episodes (RR = 1.23, \( P = 0.40 \)) when compared with nonswitchers. No significant associations were seen between earlier and later episodes of isotype switching against HLA class I molecules in comparison with nonproducers of these antibodies.

Because recurrent rejections and accelerated progression of TCAD predict poor long-term allograft survival, we next sought to investigate the impact of antibody switching on patient survival beyond the first year after transplantation. Among 206 one-year survivors of heart transplantation, those who switched antibody isotype to IgG anti-HLA class II had a lower 5-year survival (91\% [96/105]) than controls (81\% [81/100], log-rank \( P = 0.03 \)). There was no significant difference in survival of patients who did and did not switch antibody isotype against IgG MHC class I molecules.

**Persistence of IgM Anti-HLA Antibodies Against Class II but Not Against Class I Molecules for >30 Days Protects Against High-Grade Acute Rejection and TCAD**

We next investigated whether individuals who demonstrated a persistence of IgM antibodies directed against HLA molecules for at least 30 days and who did not type-switch to IgG antibodies were protected from acute rejection and TCAD. Patients who switched isotype and produced IgM anti-HLA antibodies were excluded from this analysis. The results showed that the persistent production of IgM anti-HLA antibodies directed against class II (log-rank \( P = 0.02 \)) but not against class I molecules (log-rank \( P = 0.23 \)) was associated with protection against acute rejection (Figure 4). Only 17\% (4/23) of patients with persistent production of IgM anti-HLA class II antibodies had a high-grade rejection during follow-up compared with 39\% (56/144) of those who did not produce these antibodies. Moreover, as illustrated in Figure 5, persistent production of IgM anti-HLA antibodies against class II (log-rank \( P = 0.05 \)) but not against class I (log-rank \( P = 0.47 \)) molecules was associated with protection against TCAD. During the follow-up period, 51\% (14/28) of patients with persistent IgM anti-HLA class II antibodies developed TCAD compared with 74\% (71/96) of patients who did not produce these antibodies.

**Increase in Circulating Soluble CD40 Ligand Coincided With B-Cell Isotype Switching From IgM to IgG Anti-HLA Class II Antibodies**

Because B-cell immunoglobulin isotype switching and production of IgG antibodies are regulated by interactions between CD40 ligand (CD40L) on activated T cells and CD40 on the B-cell surface, we measured serum levels of soluble CD40L in 4 groups of patients as an indirect estimator of T-cell dependent B-cell activation. The 4 groups consisted of (1) 8 heart transplant recipients on the day when IgG anti-HLA class II antibodies first appeared in their sera; (2) 6 heart transplant recipients who never developed anti-HLA antibodies of the IgG isotype at a time after transplantation that was matched for duration to the patients in group 1; (3) 4 end-stage heart failure patients listed as status I for heart transplantation whose sera were obtained on the day of listing; and (4) 4 healthy volunteers who served as controls. Soluble CD40L serum concentrations were significantly increased in all patients who underwent heart transplantation compared with both status I heart transplant candidates (0.139 ng/mL) and healthy individuals (0 ng/mL). Moreover, soluble CD40L levels were 2-fold higher in the sera of patients on the day when their B cells switched isotype to produce IgG anti-HLA class II antibodies than in the sera of nonswitchers at similar times after transplantation (0.67 versus 0.31 ng/mL, \( P < 0.012 \)).

**Immunosuppressive Regimen Influenced the IgM-to-IgG Anti-HLA Class II Antibody Switch**

We next sought to identify clinical risk factors for anti-HLA class II isotype switching. By individual analysis, the only
risk factor identified was modulation of the triple immunosuppressive regimen used. Multivariable analysis confirmed that substitution of MMF for AZA in patients who were also receiving steroids and cyclosporine significantly reduced the risk of developing IgG anti-HLA class II antibody production, which was 1.9-fold higher for AZA- than for MMF-based regimens \((P=0.004,\) the Table). Moreover, treatment with a regimen containing AZA rather than MMF was a stronger risk factor for production of IgG anti-HLA class II antibodies than more conventional risk factors for alloreactivity, such as donor-recipient mismatches at HLA-A, HLA-B, or HLA-DR loci.

**Multivariable Analysis of Risk Factors for Antibody Isotype Switching to IgG Anti-HLA Class II Antibodies Within the First Year After Transplantation**

<table>
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<th>Predictors of Antibody Isotype Switching to IgG Anti-HLA Class II</th>
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<th>(RR)</th>
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Case-Control Study Demonstrated That MMF Prevented T-Cell Activation and B-Cell Anti-HLA Antibody Isotype Switching

To directly evaluate the effect of MMF on anti-HLA class II isotype switching, we performed a case-control study comparing alloantibody production in 30 cardiac transplant recipients matched according to age and sex. These patients participated in a prospective pilot study conducted in 1994 that compared MMF (cases) versus AZA (controls) as part of a triple immunosuppressive regimen. Treatment with MMF delayed the onset of B-cell isotype switching and anti-HLA class II antibody production, with only 29% of MMF-treated recipients developing anti-HLA class II antibodies by the end of the first year compared with 55% of AZA-treated patients \((P=0.25)\). Notably, whereas both groups had undetectable levels of soluble CD40L at the time of transplantation, MMF treatment prevented the significant rise in soluble CD40L levels seen at 6 months in patients treated with AZA \((0.01 \text{ versus } 0.36 \text{ ng/mL, } P<0.001)\). Because MMF has previously been reported to significantly reduce the risk of acute, high-grade cellular rejection compared with AZA,25 our results indicate that the mechanism of this protective effect involves prevention of T-cell activation and of IgG anti-HLA class II isotype switching.

**Discussion**

In this study, we have shown that de novo isotype switching of IgM to IgG anti-HLA antibodies directed against class II antigens was associated with earlier and more frequent high-grade cellular rejections, an increased risk of accelerated TCAD, and decreased long-term survival after heart transplantation. In contrast, a lack of isotype switching with persistent production of IgM anti-HLA class II antibodies was associated with a decreased incidence of acute cellular rejection and protection against the development of TCAD. Moreover, alloantibody isotype switching was associated with higher levels of soluble CD40L ligand. A case-control study showed that reduction of cardiac allograft rejection by MMF was accompanied by both reduced CD40L serum
levels and prevention of IgM-to-IgG anti-HLA class II antibody switching. These results indicate that IgM-to-IgG anti-HLA class II antibody switching is the result of concomitant T-cell activation and B-cell stimulation via CD40L-CD40 interactions.

The relation identified in this study between cellular rejection and production of IgG antibodies against MHC class II antigens may reflect a heightened activity of recipient T cells, which recognize processed forms of soluble HLA alloantigens released from the graft in context of self-MHC. Such T cells, engaged in the indirect recognition pathway, produce lymphokines required for the growth and maturation of alloantibody-producing B cells and cytotoxic T lymphocytes. Activation of B cells by soluble MHC class II products, particularly HLA-DR molecules, and the subsequent presentation of multiple HLA-DR allopeptides by self-B cells to CD4 T cells has been postulated to initiate the cascade of intramolecular and intermolecular epitope spreading and diversification of the immune response, resulting in the amplification of T-cell responses, recurrent high-grade cellular rejections, and the early development of TCAD. In this view, an amplification loop of increased alloreactivity and activation of B cells set up by the primary T-cell recognition of donor HLA class II peptide would result in B-cell isotype switching and surface expression of IgG antibodies against donor MHC class II molecules, which would then bind cognate antigen and allow the B cell to present diverse peptides in the context of self-MHC class II molecules to the CD4 T cells. Because injury of the allograft during acute rejection is an important source of soluble alloantigens, this may explain the high incidence of IgG anti-HLA class II antibody switching directly after the first rejection occurrence and the subsequent high frequency of recurrent rejections.

B-cell isotype switching to IgG antibodies against MHC class I antigens does not appear to have the same adverse effects on allograft outcome. One possible explanation may be that MHC class I antigens expressed by the graft are recognized predominantly by the recipient’s CD8+CD28+ T-suppressor cells. Activation of the suppressor cell pool has been shown to inhibit activation of HLA class II–reactive T-helper cells and thereby maintain quiescence after organ transplantation.

The observed patterns of progressive intramolecular and intermolecular T-cell HLA-DR epitope spreading in cardiac transplant recipients with recurrent rejections and accelerated TCAD, as well as the increased frequency of cellular rejection reported when IgG antibodies against MHC class II antigens are present in sensitized patients before transplantation, provide support for the concept of a B-cell/T-cell amplification loop.

Although it is generally agreed that HLA disparity is correlated with the strength of alloreactivity, our study has shown no association between B-cell anti-HLA class II antibody switching and the degree of HLA-DR mismatch. The ability of B cells to switch isotype in response to T-cell–presented HLA-DR molecules relies on the intact function of all elements of the T- and B-cell interaction, such as CD4+ T-cell ability to recognize HLA-DR antigens, express appropriate surface receptors, and produce sufficient numbers of cytokines to stimulate B-cell differentiation. In transplant recipients, the function of many of these components is inhibited by the action of immunosuppressive drugs, the efficacy of which may vary in individual patients, depending on some genetic determinants (eg, variation in cytokine genes) and other unknown factors. The heterogeneity of alloresponses to allografts with the same degree of HLA-DR disparity has been previously shown. Therefore, escape of B-cell activation in immunosuppressed patients and generation of anti-HLA class II antibody switching would identify patients with heightened responses to donor MHC molecules, irrespective of the degree of HLA-DR mismatch. This test would therefore allow assessment of individual allore sponsiveness independent of routinely monitored immunosuppressive drug levels and donor MHC disparity.

After alloantigen recognition, activated T cells in transplant recipients upregulate CD40L and amplify the alloreactive response by ligating CD40 molecules, not only on antigen-presenting cells but also on allograft endothelial cells. Increased T-cell expression of CD40L has been associated with acute and chronic renal allograft rejection, and interruption of the CD40L-CD40 costimulatory pathway results in long-term survival of heart, kidney, liver, and skin allografts in animal models of transplantation. Because IgM-to-IgG isotype switching by B cells is tightly regulated by both T-cell costimulatory signals and recognition of cognate antigen, our results implicate CD40L-CD40 interactions in the induction of B-cell isotype switching after organ transplantation.

Several lines of evidence support the idea that monitoring recipient sera for switching of IgM to IgG anti-HLA class II antibodies can provide a clinically useful parameter for evaluating the efficacy of T-cell directed immunosuppressive therapy. First, we have previously shown that induction of IgG anti-HLA class II antibodies is accompanied by intragraft T-helper cell activation and interleukin-2 receptor expression. Second, we recently showed that use of a humanized monoclonal antibody capable of selectively blocking the interleukin-2 receptor on T cells without cellular activation reduced both cellular allograft rejection and the formation of IgG anti-HLA antibodies. Third, we have demonstrated that a triple immunosuppressive regimen containing MMF, which is superior to that containing AZA for prevention of T-cell activation and cellular rejection, is also superior for prevention of posttransplantation B-cell isotype switching of IgM to IgG antibodies directed against MHC class II molecules.

Collectively, these observations indicate that monitoring cardiac allograft recipients for de novo production of IgG anti-HLA class II antibodies may provide a simple indirect assessment of whether inhibition of CD4 T-cell alloactivation in a given patient is adequate and may be used as a surrogate marker in clinical trials for determining the efficacy of novel T-cell immunomodulatory therapies. Moreover, detection of de novo production of IgG anti-
HLA class II may allow relatively early posttransplantation identification of patients at increased risk for poor long-term outcomes who would benefit from either modification of their current immunosuppressive regimen or institution of more effective immunomodulatory therapies aimed at slowing or preventing the progression of TCAD.

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