Absence of donor MHC antigen expression ameliorates chronic kidney allograft rejection

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Absence of donor MHC antigen expression ameliorates chronic kidney allograft rejection.

Background. In previous studies, we have demonstrated that a subset of mouse kidney allografts has prolonged survival without any immunosuppressive treatment. Chronic rejection (CR) develops in these long surviving grafts. The pathologic features of CR in this model are similar to CR in human kidney grafts.

Methods. To explore the role of donor major histocompatibility complex (MHC) antigens in the development of CR, we performed vascularized kidney transplants using kidneys from donor mice that lack expression of both MHC class I and II antigens (MHC−/−).

Results. Survival was significantly improved in recipients of MHC−/− allografts. This enhanced survival was associated with higher glomerular filtration rate (GFR) in MHC−/− allografts (4.92 ± 0.54 cc/min/kg) compared to controls (2.19 ± 0.63 cc/min/kg; P = 0.004). The typical histologic features of CR were markedly reduced in MHC−/− allografts. Semiquantitative histopathological scores for MHC−/− grafts (13.3 ± 2.1) were significantly lower than in control allografts (19.0 ± 1.0; P = 0.04). Along with this improvement in structural abnormalities, significantly fewer CD4+ T (38.3 cells/mm²) vs. 75.0 cells/mm²; P = 0.008), CD8+ T cells (38.7 vs. 96 cells/mm², respectively; P = 0.008) and macrophages (60 vs. 134 cells/mm², respectively; P = 0.04) infiltrated MHC−/− allografts compared to controls. The levels of intragraft cytokine mRNA expression also were reduced in MHC−/− allografts compared to control allografts. Finally, serum alloantibodies were virtually undetectable in recipients of MHC−/− kidney allografts.

Conclusions. Cell surface expression of donor MHC antigens promotes the development of CR. Donor antigen expression promotes the accumulation of infiltrating cells in the graft and the development of donor specific alloantibodies. Abrogation of these responses is associated with improved graft survival and reduced CR in MHC−/− grafts.

Keywords: chronic rejection, kidney, transplantation, MHC, mouse, antibody, major histocompatibility complex antigens, late graft failure.

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Despite dramatic improvements in the field of transplantation, chronic rejection (CR) remains the leading cause of late graft failure [1]. While the pathophysiology of CR has been debated, immunologic factors have been felt to play a critical role in the development of CR. For example, CR has been strongly correlated with acute rejection episodes [2–4] and the impact of acute rejection on CR development has been rising despite the introduction of newer immunosuppressive treatments [5]. Furthermore, several studies have demonstrated improved graft survival for living related allografts compared to cadaveric grafts [5, 6]. On the other hand, the failure of CR to improve with immunosuppression suggests non-immunologic factors are involved. These factors may include disparity between donor and recipient size, drug nephrotoxicity, hypertension, and hyperlipidemia [reviewed in 7]. Thus, the relative importance of antigen-dependent mechanisms has not been quantitated.

The rejection of transplanted tissues is triggered by the recognition of donor major histocompatibility complex (MHC) antigens. This recognition process activates humoral and cellular effector responses leading to graft injury and destruction. Antigen recognition occurs via one of two mechanisms. In direct recognition, foreign antigens are presented by foreign antigen presenting cells (APCs). In indirect recognition, recipient T cells recognize processed foreign antigens presented by recipient APCs. It is believed that the direct pathway predominantly mediates early acute rejection, as the graft contains donor-derived APCs, expressing a high density of MHC molecules available for presentation [reviewed in 8]. In contrast, indirect recognition may be more significant in chronic rejection, where donor APCs are no longer present [9], a process that may be resistant to standard immunosuppression. However, there is mounting evidence that indirect recognition may be important in acute rejection. For example, animals immunized with donor MHC class II peptides rejected their grafts in an accelerated...
fashion compared to recipients that had not been immunized [10]. Further, skin allografts lacking MHC class II are rapidly rejected, via indirect recognition of donor MHC class I [11]. However, the relative roles of direct versus indirect allorecognition in chronic rejection have not yet been addressed.

In previous studies, we demonstrated that mouse kidney allografts transplanted across complete MHC disparities survive for prolonged periods of time [12, 13]. Renal allografts in mice have severely reduced function compared to non-rejecting isografts and develop a histologic picture consistent with chronic rejection by 6 weeks post-transplant [14]. In this study, we examined the effect of reducing the inherent immunogenicity of the graft on the course of CR. Our studies suggest that cell surface expression of donor MHC antigens contribute to the extent and severity of CR. Reduction in donor alloantigen expression suppresses the development of donor-specific humoral responses and improves graft function and survival.

METHODS

Animals

Major histocompatibility complex class I- and II-deficient mice (MHC−/−) were obtained by breeding inbred AB−/− deficient mice (H-2b) [15] with inbred β2-microglobulin-deficient mice (H-2b) [16]. C57BL/6 (H-2b) mice purchased from Jackson Laboratory (Bar Harbor, ME, USA) and used as control donors fully expressing MHC antigens. [BALB/c × DBA/2J]F1 mice (Jackson Laboratory) were used as recipients in all experiments. All mice were maintained in the Durham VA Animal Facility under AALAC guidelines.

Mouse kidney transplantation

Vascularized kidney transplants were performed in mice as previously described [13]. Briefly, animals were anesthetized with isoflurane and the donor kidney, ureter, and bladder were harvested en bloc, including the renal artery with a small aortic cuff and the renal vein with a small caval cuff. These vascular cuffs were anastomosed to the recipient abdominal aorta and vena cava, respectively, below the level of the native renal vessels. Total ischemic time averaged 35 to 40 minutes. Donor and recipient bladders were attached dome to dome. The right native kidney was removed at time of transplant and the left native kidney was removed through a flank incision four days later. Overall surgical mortality was approximately 20% and there were no significant differences in peri-operative mortality between the experimental groups.

Three experimental groups were evaluated. In the control allograft group, donor kidneys from C57BL/6 mice (H-2b) were transplanted into fully allogeneic [BALB/c × DBA/2J]F1 animals (H-2b). In the experimental MHC−/− allograft group, kidneys from MHC−/− mice (H-2b) were transplanted into [BALB/c × DBA/2J]F1 recipients. In the non-rejecting isograft control group, kidneys from [BALB/c × DBA/2J]F1 donors were transplanted into their littermates. Transplanted mice were studied at six weeks following transplantation.

Measurement of kidney transplant function

To accurately assess kidney transplant function, clearances of inulin and para-aminohippurate (PAH) were measured as previously described [13] and used as measures of glomerular filtration rate (GFR) and renal plasma flow (RPF), respectively.

Evaluation of allograft histomorphology

Following the renal hemodynamic studies, animals were sacrificed and kidneys were macrodissected. Sections of transplant kidney were fixed in formalin and stained with hematoxylin-eosin, Masson-trichrome and periodic acid-Schiff stains. A renal pathologist who was masked to the experimental groups evaluated the sections.

Immunohistology

Immunopathology was performed as previously described [14]. Briefly, kidney tissue was embedded in OCT (Sakura, Torrance, CA, USA), snap frozen in pre-cooled 2-methylbutane, and stored at −80°C. Tissue sections (4 μmol/L) were cut with a cryostat, and mounted on positively charged microslide cover glasses (Superfrost Plus, Fisher Scientific, Pittsburgh, PA, USA) and stored at 80°C in an airtight box. Sections were then air-dried, and fixed for 10 minutes in 4°C acetone (HPLC grade; Fisher Scientific). Sections were then post-fixed for two minutes in 100 mmol/L Tris-buffered 1% paraformaldehyde containing 1 mmol/L ethylenediaminetetraacetic acid (EDTA), pH 7.2, and rinsed with phosphate buffered saline (PBS) pH 7.2. Primary antibodies included fluorescein-5-isothiocyanate (FITC)-conjugated polyclonal primary antibodies consisting of affinity purified goat anti-murine IgG (FC, μ-specific; ICN Biomedicals, Costa Mesa, CA, USA), affinity purified goat F(ab′)2 anti-murine IgM (μ-specific; ICN), and goat anti mouse CD3 (ICN) or rat monoclonal antibodies TIB126 (anti-MHC class I), TIB120 (anti-MHC class II), GK1.5 (anti-CD4), and 3.155 (anti-CD8) (prepared as hybridoma supernatants; American Type Culture Collection, Rockville, MD, USA), 30-H122 (anti-Thy 1.2; Boehringer Mannheim, Indianapolis, IN, USA), RA36B2 (anti-B220), and M1/70HL (anti-CD11b/Mac-1; BD Pharmingen, San Diego, CA, USA). For the non-labeled primary antibodies, binding was detected with affinity-purified fluorescein isothiocyanate-labeled goat anti-rat IgG, followed by a secondary layer of affinity-isolated FITC-rabbit anti-goat IgG (Cappel, Durham, NC, USA).
Primary antibody was applied to sections for 45 minutes at room temperature. Sections were rinsed and coverslipped with a medium consisting of 25 mg/mL 1,4-diazabicyclo[2.2.2]octane (DABCO), 0.5 mg/mL 4,6-diamidino-2-phenylindole (DAPI), and 50% glycerol in PBS, pH 8.6, and stored in the dark at 4°C. Digital images were obtained utilizing a high-resolution CCD digital camera (SPOT II; Diagnostic Instruments, Sterling Heights, MI, USA), and software mounted onto an epifluorescent microscope.

**Quantitative immunohistology**

Quantitative immunohistology was performed as previously described [17]. Cells were quantitated using a 1-cm² grid divided into 100 1-mm² squares placed in the eyepiece of a Leitz DMRB microscope (Leica, Heidelberg, Germany). Cells were counted in ten randomly selected squares and were scored with the ×40 objective. Final counts were expressed as the number of cells per square mm (mean ± SEM). An individual who was masked to the experimental groups evaluated all sections.

**RNA isolation**

Total cellular RNA was obtained using standard methods [18]. Briefly, after the renal clearance study, a portion of the allograft cortex was removed, snap frozen in liquid nitrogen and stored at −70°C until use. The frozen tissue was homogenized in ice-cold 4 mol/L guanidinium isothiocyanate (Sigma Chemical Company, St. Louis, MO, USA) using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY, USA). The samples were layered onto a 5.7 mol/L cesium chloride gradient and centrifuged at 174,000 × g at 20°C for 18 hours in a Beckman L7 ultracentrifuge (Beckman Instruments, Palo Alto, CA, USA). The RNA pellets were resuspended in 0.3 mol/L sodium acetate, pH 6.0 (Sigma), extracted once with 1:1 phenol:chloroform, and precipitated in ethanol. The dried pellet was redissolved in diethylpyrocarbonate- treated water and the concentration and purity of the RNA was determined from the absorbance at 260 nm and 280 nm. Samples were stored at −70°C until use.

**RNase protection assay**

Cytokine transcripts were quantified using a modification of the RNase protection assay previously described [19] using a multiprobe template set (BD Pharmingen, San Diego, CA, USA). Riboprobes were prepared from MCK template sets containing the cDNA for interleukin (IL)-1α, IL-1β, IL-2, interferon-γ (IFN-γ), IL-4, IL-5, IL-6, IL-10, tumor necrosis factor-α (TNF-α), IL-12p40, IL-12p35, IL-15, and chemokines RANTES (regulated upon activation, normal T cell expressed and secreted), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein (MIP)-1α and MIP-1β and L32 using the In Vitro Transcription kit (BD Pharmingen). Ten micrograms of total RNA were assayed in each sample using the RPA kit as outlined by the manufacturer. The protected fragments were size-fractionated on the Quick-Point rapid DNA sequencing system (Novex, San Diego, CA). Dried gels were placed on Kodak XAR film (Rochester, NY, USA), with intensifying screens, for 24 to 120 hours at −70°C. The intensity of autoradiographic signal was quantitated by laser densitometry (Molecular Dynamics, Sunnyvale, CA, USA). The results were expressed as arbitrary densitometry units that correspond to the area under the densitometric peak. Results were normalized for levels of L32 expression.

**Alloantibody analysis**

Alloantibody production was measured in recipient serum using indirect flow cytometry. Splenocytes were isolated from class I-deficient (H-2b) or class II-deficient mice (H-2b). One × 10⁶ cells were stained per sample. Cells were treated with Fc-Block (BD Pharmingen) per the manufacturer’s directions, prior to antibody staining, and then stained with phycoerythrin-conjugated anti-B220 (BD Pharmingen). Cells were then exposed to serial dilutions of recipient serum at 4°C for 30 minutes. Bound IgG was then detected by treating cells with FITC-conjugated rat-anti-mouse IgG1 (BD Pharmingen). Cells were analyzed by flow cytometry, as described above, evaluating the percent of FITC-positive events in the B220⁺ and B220⁻ populations.

**Statistical analysis**

Data are presented as the mean ± standard error of the mean, except for survival data, which are also presented as median survival. For the hemodynamic studies, data points for each animal represent the mean of the values measured during two clearance periods. Statistical significance was assessed using the Student t test or Wilcoxon rank sum test as appropriate.

**RESULTS**

**Prolonged survival following transplantation of MHC−/− kidney allografts**

As shown in Figure 1, recipients of control allografts had a median survival of 8.5 days (range 1 to 42 days), consistent with results of our previous studies [12, 13]. In contrast, survival of MHC−/− allografts was significantly prolonged, with median survival of 42 days (range 2 to 42 days; P = 0.003; Table 1). While only 20% of control allograft recipients survived to the study endpoint of 6 weeks, nearly 80% of recipients of MHC−/− allografts survived to this time point. Thus, reduced expression of donor MHC antigens on a kidney allograft improves graft survival.
Fig. 1. Survival of kidney allograft recipients. Recipients were followed out to six weeks following transplantation. Recipients of MHC−/− allografts (○) had a significantly improved rate of survival than recipients of control allografts (●), with a median survival time of 42 days versus 8.5 days, respectively (P = 0.003). Animals were sacrificed at day 42 for renal hemodynamic studies.

Table 1. Survival of recipients of kidney allografts

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Survival days</th>
<th>Median survival days (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control allograft</td>
<td>1, 1, 1, 1, 3, 5, 5, 6, 7, 8, 8, 8, 9, 9, 11, 13, 14, 15, 16, 16, 19, 20, 37, 42, 42, 42, 42, 42, 42</td>
<td>8.5 (15.3 ± 2.6)</td>
</tr>
<tr>
<td>MHC−/− allograft</td>
<td>2, 6, 42, 42, 42, 42, 42, 42</td>
<td>42 (34.4 ± 5.1)</td>
</tr>
</tbody>
</table>

Renal function is preserved in MHC-deficient allografts

We performed hemodynamic measurements as a quantitative measure of kidney injury. As shown in Figure 2, 6 weeks following transplantation, GFR in isografts (6.45 ± 0.53 cc/min/kg; N = 5) was similar to that seen in uninephrectomized mice (7.98 ± 1.62 cc/min/kg) [20]. In rejecting control allografts, GFR was substantially depressed (2.19 ± 0.63 cc/min/kg; N = 5) compared to isografts (P < 0.01). In contrast, GFR in MHC−/− allografts (4.92 ± 0.75 cc/min/kg; N = 9; P = 0.004) was significantly higher than control allografts. Further, while GFR in MHC−/− allografts tended to be lower than isografts, this difference did not achieve statistical significance.

Improved histopathology in MHC-deficient allografts

As shown in Figure 3A, the histology of isografts six weeks following transplantation was essentially normal, with little fibrosis or inflammation. In contrast, as we have previously described [14], control allografts (Fig. 3B) demonstrated the characteristic features of chronic rejection including: extensive interstitial and periglomerular fibrosis, perivascular and medial fibrosis, mesangial cell hyperplasia and membranous thickening, as well as a diffuse, patchy, chronic inflammatory cell infiltrate. MHC−/− allografts similarly demonstrated chronic rejection seen in control allografts. However, the severity of these changes was markedly reduced (Fig. 3C). To estimate the severity of these histopathological abnormalities, semiquantitative scoring of graft sections was performed with cumulative scores generated for the individual grafts. The mean histologic score in MHC−/− allografts was 13.3 ± 2.1, significantly lower than the score in control allografts (19.0 ± 1.0; P = 0.02). The diminished severity of histologic changes was most notable for reductions in tubular atrophy, and vasculopathy.

Immunohistologic and morphometric analyses of kidney allografts

The cell surface phenotypes of graft infiltrating cells were examined by immunofluorescent staining and quantitated by morphometric analysis. Infiltrates in both control and MHC−/− allografts groups consisted predominantly of T cells, as shown in Figure 4 A and E. The numbers of infiltrating T cells were reduced in MHC−/− grafts. Both CD8+ T cells (Fig. 4G; 38.7 ± 2.4 cells/mm²) and CD4+ T cells (Fig. 4F; 38.3 ± 4.4 cells/mm²) were significantly reduced in MHC−/− grafts compared to controls (Fig. 4C, 96 ± 11.4 CD8+ T cells/mm²; P = 0.008; and Fig. 4B, 75 ± 6.6 CD4+ T cells/mm², P =
Despite this reduction in T cell infiltration, the relative number of CD4 to CD8 cells was similar in both controls and MHC−/− allografts (CD4/CD8 0.95 and 0.81, respectively). Numerous macrophages were present in both allografts groups, but again, the numbers in MHC−/− grafts (Fig. 4H; 60 ± 6.9 cell/mm²) were less than controls (Fig. 4D; 134 ± 23.0 macrophages/mm², \( P = 0.04 \)). Thus, the absence of donor MHC expression significantly reduces the immune cell infiltrate in kidney allografts.

**Cytokine mRNA expression is reduced in MHC−/− kidney allografts.**

To characterize in situ T cell function, intragraft expression of cytokines at 6 weeks post-transplantation was evaluated by RNase protection assay (RPA; described in the Methods section). Expression of pro-inflammatory cytokines IL-2, IFN-\( \gamma \), IL-1\( \beta \), TNF-\( \alpha \), and IL-6 were similar between allograft groups. However, expression of Th2 cytokines IL-4, IL-5, and IL-10 were 32 to 72\% lower in MHC−/− allografts compared to controls (Table 2). Expression of transforming growth factor (TGF)-\( \beta 2 \) and TGF-\( \beta 3 \), fibrogenic cytokines posited to be important in the development of CR [14, 21], were modestly, but significantly enhanced in MHC−/− allografts compared to controls (\( P = 0.05 \)), while TGF-\( \beta 1 \) was similar to controls. Thus, the absence of MHC antigens on donor tissue significantly alters the intragraft expression of Th2 cytokines. Furthermore, despite enhanced expression of some TGF-\( \beta \) isoforms, the severity of CR was reduced in MHC−/− allografts.

**Alloantibody production is absent in recipients of MHC−/− allografts**

Anti-donor antibodies in the serum of transplant recipients were measured by indirect flow cytometry. Significant titers of anti-donor MHC class I H-\( 2b \) (Fig. 5A) were detected in recipients of control allografts but not MHC−/− grafts. Similarly, antibody against MHC class II I\( A^b \) (Fig. 5B) was detected in controls but not MHC−/− grafts. Isografted recipients demonstrated neither MHC class I nor II antibodies.

To further analyze the functional nature of serum antibody production, sections of allografts were stained to detect IgG, IgM and C3. In control allografts, there was substantial IgG within glomeruli and on tubules, with occasional cytoplasmic staining (Fig. 6E). IgM was expressed heavily throughout the glomerulus (Fig. 6F),
with modest C3 within the glomerulus and tubules (Fig. 6G). Thus, donor alloantigen stimulates the production of antibodies that are deposited within the graft and associated with complement deposition. In contrast, detectable IgG was virtually absent in all MHC−/− allografts (Fig. 6A). Only faint deposition of IgM was detected in the mesangium and along glomerular capillaries (Fig. 6B). Complement (Fig. 6C) was seen faintly and diffusely along basement membrane and segmentally within the mesangium, but at levels that were substantially less than in control allografts. This is consistent with previous studies suggesting that T cells and macrophages during the course of rejection.

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DISCUSSION

In these studies, the combined absence of donor expression of MHC class I and II antigens dramatically improved the function and survival of kidney allografts and reduced the histologic severity of chronic rejection. Additionally, there were marked differences in the character of the immune response toward MHC−/− allografts, including reduced numbers of infiltrating T cells and macrophages, reduced expression of Th2 cytokines, and significantly, the absence of a detectable humoral response toward the allograft. Thus, abrogation of donor MHC expression reduces the immunogenicity of the graft and strongly influences the character and severity of chronic rejection of kidney grafts. Similarly, the parenchymal damage and injury in MHC−/− grafts during acute allograft rejection three weeks after transplantation were significantly reduced compared to wild-type allografts (abstract; Mannon et al, J Am Soc Nephrol 8:660A, 1997). The diminished early injury likely leads to a reduction in the later development of fibrosis and atrophy. Thus, in the context of previous studies, our results support the notion that responses directed toward MHC antigens expressed on donor cells make a significant contribution to the pathogenesis of CR.

One of the most striking differences in the immune response of recipients to MHC−/− allografts was the virtual absence of alloantibodies. While the role of antibody in acute rejection has been debated, there is substantial evidence supporting the detrimental effects of anti-donor antibodies [reviewed in 22]. Alloantibodies may mediate injury, either directly, or through activation of other pro-inflammatory mediators. The role of pre-formed anti-donor antibodies in graft dysfunction in hyperacute rejection is incontrovertible [22, 23] and the subsequent development of anti-donor antibodies following transplantation can be harmful [22, 24–27]. Moreover, the absence of anti-donor antibodies has been associated with reduced incidence of chronic rejection [28–31]. Based on these studies, we posit that the absence of alloantibodies in recipients of MHC−/− allografts results in the protection against CR observed in MHC−/− allografts, which translates in improved graft and recipient survival. Moreover, these data support the critical importance of intact donor antigen expression on the development of a humoral alloimmune response.

Similar to the effect on the humoral response, the cellular immune responses to MHC-deficient allografts also were attenuated compared to controls. In the infiltrates of MHC−/− allografts, there are strikingly fewer T cells and macrophages during the course of rejection. This is consistent with previous studies suggesting that donor MHC expression regulates the accumulation of immune cells within the grafts. For example, reduction of donor MHC class I expression reduced the quantity of CD8+ T cells infiltrating the graft [13], while the absence of MHC class II alters CD4+ T cell infiltration [32]. In this case, deficiency of all MHC antigens caused a proportional reduction in both CD4+ and CD8+ T cells. Impaired intra-graft accumulation of lymphocytes may be due to reduced antigen load to the extent that graft antigen serves as a stimulus for T cell migration. Alternatively, donor dendritic cells lacking MHC class I and class II may be unable to adequately activate lymphocytes in peripheral lymphoid tissues and thereby facilitate their ability to home to the graft [33]. The relative contribution of MHC antigens on parenchymal cells versus dendritic cells to the regulate accumulation of lymphocytes in the allograft cannot be determined by the current studies. However, this issue could be addressed by rescuing cell-specific expression of MHC antigens in deficient mice and studying the contribution of MHC expression on individual cell populations to regulate T cell infiltration into allografts. Thus, the absence of donor MHC antigens affects both humoral and cellular components of the immune response to an allograft.

Cytokine expression, which reflects the nature and intensity of the intra-graft immune response, also was substantially altered in MHC-deficient kidney allografts. For instance, we found modest but significant reductions in Th2 cytokines IL-4, IL-5 and IL-10 in MHC−/− allo-
grafts compared to controls. This is similar to our findings in allografts with isolated MHC class II deficiency [32]. A predominant Th1 response has been suggested to promote rejection and graft injury [34–37], and Th2 responses have been associated with prolonged graft survival and tolerance induction [36–38]. Our data are consistent with recent studies that have argued against a strict functional delineation of Th1 cytokines as detrimental and Th2 cytokines as beneficial in rejection [39–42]. While the cytokine milieu is altered in MHC−/− allografts, it is difficult to quantitate the relative contribution of these changes to the improved function and survival of MHC−/− grafts. Alternatively the absence of alloantigen to stimulate an appropriate allo-antibody within the recipient may be a primary mechanism determining the improved function and prolonged survival of the MHC-deficient grafts.

Up-regulation of TGF-β has been associated with the development of acute rejection [43, 44] and CR in a variety of animal models [14, 45] and in humans [reviewed in 46]. We have previously found that this up-regulation was blunted in MHC class I deficient or class II deficient kidney allografts and yet had no impact on the pathological changes in CR [14]. Interestingly, in this study, we found no difference in intragraft mRNA expression for TGF-β1 between groups and modest but significant increases in the expression of TGF-β2 and β3 isoforms in MHC−/− allografts compared to controls. While the biological effects of these isoforms are associated with the promotion of chronic graft injury in humans [21, 47], our results support the notion that there may be altered receptor expression within allograft groups, or expression of other factors that lead to collagen deposition within CR grafts in this model.

While the functional and histopathological manifestations of CR are reduced in MHC−/− grafts, they are not completely absent. The persistence of significant chronic injury despite the absence of donor MHC antigens may be due to minor antigen disparities between donor and recipient. Additionally, free Dβ heavy chain on the cell surface of MHC−/− mice is sufficient to induce a cellular immune response. In studies of β2-microglobulin-deficient mice, Dβ class I heavy chains are present on the cell surface of lymphocytes and other tissues [48]. These chains induce T cell cytotoxicity [48, 49] and allow for the positive selection of a small number of CD8+ T cells within the thymus [50, 51]. In skin transplant models, Lee et al have demonstrated that such “leakiness” of class I expression results in CD8+ T cell allorecognition and rejection of skin allografts, dependent on CD4+ T cells sensitized indirectly to donor-derived peptides [11, 52, 53]. While these mechanisms depend on direct allorecognition, we cannot exclude the contribution of indirect recognition. Finally, non-immunologic contributions to CR, such as disparity between donor and recipient renal mass and size, do not appear to be operating, as isografted animals have normal graft histology and function.

The elimination of MHC antigen expression by gene targeting is not practical as a clinical maneuver in kidney transplantation. Nonetheless, our studies suggest that reducing donor MHC expression may be useful in ameliorating graft injury. These approaches might include small molecules or antisense oligonucleotides that may target regulatory proteins in MHC expression such as Class II transactivator [54] or that target proteins that regulate peptide loading onto MHC such as TAP (transporters associated with antigen processing) [42]. Furthermore, we suggest that gene targeting approaches that more effectively alter MHC expression by disrupting of class I heavy chains [42, 55], or by deleting transcription factors that regulate MHC expression [56] might have an even more profound effect in animal models.

In conclusion, absence of normal expression of MHC on a donor organ graft protects against the development of CR. There is a profound absence of humoral responses toward these grafts, and a reduction in cellular responses toward allografted tissue. These results demonstrate the
strength of MHC disparity in inducing CR. Strategies to eliminate or reduce MHC expression should improve long-term graft outcome.

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**Fig. 6. Alloantibody deposition in renal allografts detected by immunofluorescence.** MHC−/− allografts demonstrated little IgG (A), IgM (B), and C3 (C). In control allografts, IgG (E), IgM (F), or C3 (G) were present within glomeruli.
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