Donor CD4 T Cells Contribute to Cardiac Allograft Vasculopathy by Providing Help for Autoantibody Production

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Background—The development of autoantibody after heart transplantation is increasingly associated with poor graft outcome, but what triggers its development and whether it has a direct causative role in graft rejection is not clear. Here, we study the development of antinuclear autoantibody in an established mouse model of heart allograft vasculopathy.

Methods and Results—Humoral vascular changes, including endothelial complement staining, were present in bm12 heart grafts, explanted 50 days after transplantation. Alloantibody was not detectable, but long-lasting autoantibody responses developed in C57BL/6 recipients from the third week after transplantation. No autoantibody was generated if donor CD4 T cells were depleted before heart graft retrieval or in recipients that lacked B-cell major histocompatibility complex class II expression, indicating that humoral autoimmunity is a consequence of donor CD4 T-cell allorecognition of the major histocompatibility complex class II complex on recipient autoreactive B cells. An effector role for autoantibody in graft rejection was confirmed by abrogation of humoral vascular rejection, and attenuation of vasculopathy, in B-cell deficient recipients and by development of vascular obliteration and accelerated rejection in recipients primed for autoantibody before transplantation.

Conclusions—Passenger CD4 T cells within heart transplants can contribute to allograft vasculopathy by providing help to recipient B cells for autoantibody generation. (Circ Heart Fail. 2009;2:361-369.)

Key Words: antibodies ■ rejection ■ transplantation ■ allograft vasculopathy ■ autoantibody ■ graft-versus-host-disease ■ allorecognition

Despite improvements in immunosuppressive therapy, the long-term clinical success of heart transplantation is limited by the development of chronic allograft vasculopathy (CAV).1,2 Therapeutic strategies that specifically prevent the development of CAV may prove more successful at prolonging graft survival, but their design is hampered by a lack of understanding of the mechanisms responsible for its initiation and progression.

Clinical Perspective on p 369

CAV manifests as diffuse vascular intimal hyperplastic lesions, consisting primarily of smooth muscle cells, macrophages, fibroblasts, leukocytes, and extracellular matrix formation, which affect the vessels in a concentric fashion.3 Adventitial scarring may additionally cause a fixed restrictive element around the vessel and contribute to the luminal loss.4 A unifying concept has now emerged for how disparate risk factors such as ischemia-reperfusion injury, infection and metabolic abnormalities such as hypercholesterolemia, glucose intolerance, and hypertension promote the progression of CAV. Interferon-gamma, in part through induction of inducible nitric oxide synthetase, is a critical determinant linking impairment in smooth muscle contractility after endothelial injury with later structural changes in CAV.5 Although its etiology is multifactorial, most studies have highlighted that CAV is a culmination of adaptive immune effector responses that are triggered by T-cell recognition of alloantigen1,6; either “directly,” as intact alloantigen on the surface of donor antigen presenting cells (APC) or “indirectly” as self-restricted allopeptide following processing by host APC.7 T-cell responses directed against self-antigens have, however, also become increasingly associated with allograft rejection.8–11 Although often directed against graft-specific antigens, it is unclear whether these autoimmune responses can contribute to graft rejection, because self-restricted autoreactive T cells are unable to bind to peptide autoantigens expressed in the context of donor major histocompatibility complex (MHC) by graft cells. They may...
instead provide help for autoantibody, which, by binding to exposed autoantigen on graft endothelial cells, provides a putative mechanism for stimulating vasculopathy.12,13 In support, autoantibody responses have been reported after transplantation in rodent models10,14 and are associated with early graft failure in human transplant recipients.3,15,16 A causal link between autoantibody and CAV has, however, not been established definitively.

It is also unclear why transplantation should trigger autoimmunity. Animal studies suggest that T-cell allorecognition is required10 and that indirect pathway responses alone are sufficient.17 Most plausibly, host APCs that have been activated after interaction with allopeptide-specific CD4 T cells break T-cell tolerance to autoantigens by copresenting self-peptides in a stimulatory fashion.18 Activated autoreactive T cells may then provide help for generating autoantibody. However, the only study to address simultaneously the kinetics of T- and B-cell reactivity to self- and allo-antigens after heart transplantation reported that although autoantibody against cardiac myosin developed after the T-cell alloimmune response, it preceded antmyosin T-cell autoimmunity.19

Here, we study the development of autoantibody in a mouse model of chronic cardiac allograft vasculopathy, to determine how T-cell help is provided to autoreactive B cells and to clarify the role of autoantibody in allograft rejection. We report the unexpected finding that donor CD4 T cells, present within the heart graft, provide help to recipient B cells for the generation of autoantibody; autoantibody that in turn contributes to CAV development.

Methods

Animals

B6.H-2bm12 (bm12), C57BL/6 (H-2b) (wild-type [WT B6]; MHC class II-deficient [MHCII/H-2b]; recombinase-activating gene-2 knockout [RAG2/H-2b]) mice were bred in-house. CBA/Ca (H-2b) mice were purchased from Charles River Laboratories (Kent, United Kingdom). Bm12 were crossed to RAG2/H-2b to obtain H-2bm12 RAG2/H-2b mice (bm12RAG2/H-2b). Animals were maintained in specific-pathogen-free conditions and all experiments approved by the United Kingdom Home Office under the Animal (Scientific Procedures) Act 1986.

Heterotopic Cardiac Transplantation

Cardiac allografts were transplanted intra-abdominally and rejection defined as complete cessation of palpable myocardial contraction. Grafts were excised at predetermined time points after transplantation, and either stored at −80°C or fixed in 10% buffered formalin. In some experiments, donor mice were lethally irradiated (13Gy) 24 hours before organ retrieval or were injected intraperitoneally with 0.5 mg of depleting anti-CD4 mAb (YTS 191.1) on the sixth and fifth day before retrieval.

Generation of Bone-Marrow Chimeras

To generate chimeric B6/H-2b mice, RAG2/H-2b mice were sublethally irradiated (2Gy) and 20 hours later reconstituted with 2×10⁶ BMCs obtained from MHCCI/H-2b B6 mice, depleted of T cells using anti-CD90 microbeads and Automacs machine separation (Miltenyi Biotec, Surrey, United Kingdom). Control chimeric B6/H-2b mice were generated by reconstituting sublethally irradiated RAG2/H-2b mice with WT B6 BMCS. Chimerism was confirmed by flow cytometric analysis of peripheral blood lymphocytes 8 weeks after reconstitution.

Recipient Immunization

A 20-mer peptide, corresponding to the disparate, hypervariable α-region of the β-chain of the I-A bm12 antigen (EYWNSQPE-FLEQKRAELDTV), was synthesized by standard Fmoc chemistry (Immune Systems, Paignton, United Kingdom; peptide purity 90%). Mice were immunized subcutaneously with 50 μg peptide, emulsified in complete Freund adjuvant.

Autoantibody was induced by intraperitoneal injection of 5×10⁶ splenic bm12 CD4 T cells, purified by Automacs separation, by first eliminating MHC class II positive cells with anti-MHC class II microbeads and then positively selecting CD4 T cells using anti-CD4 microbeads. CD4 T cell purities, assessed by flow cytometry, were >99%, with no detectable I-A bm12. In certain experiments, purified bm12 CD4 T cells were lysed by 3 cycles of freeze-thawing.

Autoantibody was passively transferred by intravenous injection of 200 μL heat-inactivated serum (pooled from B6 recipients of bm12 heart grafts) 3 times weekly for 4 weeks after heart grafting.

Autoantibody and Antibm12 Alloantibody and Determination

Antinuclear autoantibody responses were determined by HEp-2 indirect immunofluorescence (The Binding Site Ltd, Birmingham, United Kingdom),23 by incubation dilutions of test sera on slides coated with HEp-2 cells and detecting bound autoantibody with FITC-conjugated anti-mouse IgG mAb (STAR 70; Serotec). For each slide, 5 random photomicrographs were taken and scored according to immunofluorescence intensity (1 to 5, by an observer blinded to the study groups [E.M.B.]).

Alloantibody responses were assayed using flow cytometry, by adding serial dilutions of test sera to 5×10⁶ bm12 or B6 BM DCs and detecting bound alloantibody with FITC-conjugated anti-mouse IgG mAb (STAR 70; Serotec). Results were expressed as geometric mean-channel fluorescence against serum dilution. Serum from CBA/Ca (H-2b) animals immunized with 1.5×10⁶ bm12 splenocytes emulsified in complete Freund adjuvant was used as positive control.

Histopathologic Examination

Hearts were paraffin-mounted and stained with hematoxylin and eosin or van Giesen. The severity of vasculopathy was scored by a cardiac histopathologist (M.G.), blinded to the study groups, as follows: 0, no vascular damage; 1, <25% vascular occlusion; 2, 25% to 50% occlusion; 3, >50% occlusion but with residual lumen; and 4, no identifiable lumen.

Complement C4d deposition was assessed on cryostat sections by an avidin-biotin-peroxidase technique (Vector Laboratories, Burlingame, Calif), using unconjugated rat anti-mouse C4 mAb (16D2; Abcam Inc., Cambridge, Mass), and rabbit anti-rat IgG (Abcam) as secondary. 16D2 anti-mouse C4 mAb binds to C4, C4b, and C4d, but because C4 is not cell bound, and C4b is short-lived (Abcam) as secondary. 16D2 anti-mouse C4 mAb binds to C4, C4b, and C4d, but because C4 is not cell bound, and C4b is short-lived with an in vitro half-life measured in minutes,25 positive staining represents C4d deposition. Sections were visualized using chromagen 3,3’-diaminobenzidine (Sigma-Aldrich, St Louis, Mo) and counterstained with Harris hematoxylin (BDH, London, United Kingdom). Allograft IgG deposition was similarly detected using biotinylated rabbit anti-mouse IgG (STAR 11B; Serotec, Oxford, United Kingdom).

Statistical Analysis

Graft survival was depicted using Kaplan–Meier analysis and groups compared by log-rank test. Comparison between groups for severity of vasculopathy and autoantibody scores was performed by Mann–Whitney U test as they were non-Gaussian in distribution (GraphPad Prism, GrafItPad Software, Inc, La Jolla, Calif).

Results

The Development of CAV in MHC Class II-Mismatched bm12 Heart Grafts

The immunologic mechanisms responsible for the development of CAV were studied in a well-established mouse
model of chronic heart graft rejection \(^{26-28}\) in which the donor bm12 strain differs from the recipient B6 by only 3 amino acid residues in the MHC class II I-A antigen. Bm12 heart allografts were rejected slowly by B6 recipients (Figure 1A) and along with patchy inflammatory infiltrates (Figure 1B) developed progressive intimal thickening and luminal narrowing (Figure 1C), morphologically resembling human cardiac allograft vasculopathy. Syngeneic B6 grafts and bm12 grafts transplanted into MHCII\(^{-/-}\) recipients survived indefinitely with no evidence of CAV or parenchymal damage (Figure 1).

Rejecting bm12 heart grafts showed additional histologic features (Figure 2) considered pathognomonic of humoral vascular rejection.\(^{29}\) In 13 of 16 bm12 hearts examined, vascular fibrinoid necrosis and/or vascular inflammation was present and associated with C4d complement and IgG endothelial deposition. These changes were not evident in syngeneic grafts nor in bm12 hearts transplanted into either MHCII\(^{-/-}\) mice or B-cell deficient recipients. IgG deposition within bm12 hearts grafted into B6 recipients has been noted previously.\(^{28}\)

**Autoantibody, but not Alloantibody, Develops After Heart Transplantation**

B6 recipients of bm12 heart allografts did not develop detectable circulating IgG alloantibody (Figure 3A). This likely reflects absence of a conformational epitope on the \(\text{I-A}^{\text{bm12}}\) antigen for B-cell recognition, rather than a defect in the provision of T-cell help for alloreactive B cells, because B6 mice, immunized with (CBA/Ca x bm12) F1 splenocytes (to provide additional third-party H-\(2^k\) alloantigens as a source for helper T-cell activation) developed alloantibody directed against CBA/Ca but not bm12 antigens (Figure 3B).

Bm12 heart grafts instead elicited strong IgG antinuclear autoantibody responses in B6 recipients that were detectable from week 3 onwards, were still present 16 weeks after transplantation and were associated with long-lasting germinal-center B cell follicles in recipients’ spleens (Figure 4). Autoantibody was not generated in MHCII\(^{-/-}\) recipients or in recipients of syngeneic grafts. Distinctive patterns of HEp-2 staining have been ascribed to differing specificities of autoantibody\(^{30}\) and although patterns were consistent for individual animals, they varied between animals within groups (Figure 4D), suggesting that the autoantibody response targets multiple autoantigens. Nevertheless, autoantibody was not due to polyclonal, nonspecific B-cell activation; responses against irrelevant ovalbumin protein or third-party H-2\(K^d\) alloantigen did not develop (not shown).

**Figure 1.** MHC class II–mismatched heart grafts are rejected slowly and develop allograft vasculopathy. A, Kaplan–Meier survival curves of cardiac grafts. B, Hematoxylin and eosin–stained paraffin sections of day 50 bm12 heart allografts, depicting cellular inflammatory infiltrates with accompanying areas of myocyte necrosis and replacement fibrosis (i). Infiltrates consist of (ii) plasma cells (arrowed), lymphocytes, and neutrophils. Syngeneic B6 heart grafts (iii), and bm12 grafts in MHCII\(^{-/-}\) B6 recipients (iv), remain disease free. C, EVG-stained paraffin sections of bm12 heart allografts in WT recipients, demonstrating an initially cellular vasculopathy (day 28), which is later more fibrotic (day 50). Syngeneic B6 grafts or bm12 hearts transplanted in MHCII\(^{-/-}\) mice do not develop vasculopathy.

**Figure 2.** Humoral vascular responses are evident in bm12 heart allografts. A, Hematoxylin and eosin–stained paraffin sections of day 50 bm12 heart allografts reveal (i) vascular fibrinoid necrosis and (ii) vascular inflammatory infiltrates (arrowed). Cryostat immunohistochemical staining of day 50 heart allografts in WT recipients demonstrates IgG (B) and C4d (C) vascular deposition, not present in syngeneic grafts or bm12 grafts transplanted into either MHCII\(^{-/-}\) or \(\mu\)MT recipients.
T-Cell Help for Autoantibody Production Is Provided by Donor CD4 T Cells Within the Heart Graft

The absence of isotype-switched autoantibody in MHCII−/− recipients suggests a T-cell–dependent response. To examine the suggested role of indirect pathway T cells in providing help for autoantibody production,17,19 B6 mice were immunized with a synthetic bm12 allopeptide (incorporating the disparate amino-acid residues) 14 days before transplantation. Heart grafts in peptide-primed animals were rejected more rapidly (Figure 5A), highlighting both the immunogenicity of the peptide and its contribution via indirect-pathway recognition to bm12 heart graft rejection. Predictably, given the above finding of a lack of B-cell epitope on the I-A^bm12 alloantigen, peptide priming did not provoke alloantibody (not shown). More surprisingly, however, the autoantibody response was not augmented (Figure 5B), suggesting that indirect pathway T cells do not provide help for its development.

We hypothesized that help is instead provided by passenger bm12 CD4 T cells that migrate from the heart graft after transplantation; as precedent, injection of bm12 splenocytes into B6 mice instigates graft-versus-host responses that generate antinuclear autoantibody.31 B6 mice were therefore transplanted with bm12 heart grafts that lacked CD4 T cells. Three approaches were used. Bm12 RAG2-deficient (bm12RAG2−/−) mice, that lacked T and B lymphocytes, were created and WT bm12 donors were depleted of CD4 T cells, by treating with either 13Gy lethal irradiation or anti-CD4 mAb. Autoantibody production was abrogated in all 3 groups (Figure 5C), confirming a critical requirement for donor CD4 T cells in providing help to recipient autoreactive B cells.

Help for Autoantibody Production Is Provided by Cognate Interaction Between Donor CD4 T Cells and Recipient B Cells

To confirm that donor CD4 T cells provide help for autoantibody production through direct allore cognition of

Figure 3. B6 recipients of bm12 heart grafts do not develop alloantibody. A, Antibm12 alloantibody responses in WT B6 recipients of bm12 heart grafts. Depicted is the analysis of day 50 sera, but no alloantibody was detected at earlier time points (not shown). B, Immunization of B6 mice (n=3) with (bm12×CBA/Ca) F1 splenocytes generates alloantibody (mean±SEM) against CBA/Ca target cells but not against bm12 targets.
MHC class II on autoreactive B cells, but not through soluble factors released after interaction with recipient DCs and macrophages, B6 BM chimeric mice were created that lacked MHC class II expression specifically on B cells, but had otherwise normal APCs (BCII$^{-/-}$ mice, Figure 6A). In contrast to control BCII$^{-/-}$ animals, autoantibody was not detectable in BCII$^{-/-}$ recipients of bm12 heart allografts (Figure 6B), consistent with a requirement for cognate interaction between the T-cell antigen receptor and MHC class II on autoreactive B cells.

An Effector Role for Autoantibody in the Development of CAV

Given the histologic findings demonstrating antibody-mediated vascular damage, we sought to verify that autoantibody played an effector role in allograft rejection. Compared with WT controls, heart grafts from donors either deficient or depleted of CD4 T cells developed less severe vasculopathy (although this only reached statistical significance in the irradiated group) and did not demonstrate endothelial complement deposition (Figure 7A and 7B). Similarly, C4d endothelial staining was not detectable in hearts transplanted into BCII$^{-/-}$ mice, but was present in allografts from control BCII$^{+/+}$ recipients (Figure 6C). Rejection times of heart grafts from anti-CD4 mAb treated and bm12RAG2$^{--}$ donors were, however, comparable with controls, and surprisingly, hearts from irradiated donors were rejected more rapidly (Figure 7C). Notably, allografts from bm12RAG2$^{--}$ and anti-CD4 mAb treated donors contained similar lymphocyte infiltrates as seen in WT hearts (Figure 7D). These infiltrates are typical of acute cellular responses; their presence suggests that conventional recipient T cell alloimmunity can effect graft rejection in the absence of a humoral autoimmune response. In comparison, marked fibrosis, but minimal lymphocytic infiltration, was present in the irradiated hearts suggesting that radiation damage was responsible for their rapid failure.

An effector role for autoantibody is further suggested by marked attenuation of vasculopathy in bm12 hearts transplanted into B-cell deficient μMT animals, with survival of all hearts until explant at day 50 (Figure 8). However, although passive transfer of autoantibody to μMT recipients was associated with focal endothelial C4d staining, neither vasculopathy nor rejection was restored (Figure 8).

We thought that passive transfer was unable to recreate the long-lasting and progressive levels of autoantibody that are present in WT recipients, so as an alternative approach, bm12
hearts were transplanted into B6 recipients already primed for autoantibody by transfer of bm12 CD4 T cells 2 weeks previously (Figure 8D). All heart grafts developed severe vasculopathy, with vascular obliteration, and were rejected rapidly (Figure 8). Because the injected CD4 T cells were highly purified and do not express MHC class II, CD4 T-cell administration is unlikely to have sensitized against the disparate I-Abm12 alloantigen. In support, immunization with lysed, rather than whole, bm12 CD4 T cells, did not accelerate bm12 heart graft rejection, and did not augment the severity of vasculopathy (Figure 8). Similarly, bm12 heart grafts transplanted into CD4 T-cell–immunized recipients survived indefinitely, without developing vasculopathy, and allografts in recipients primed for indirect-pathway responses by I-Abm12-peptide immunization had vasculopathy comparable in severity with WT controls, rather than the vascular obliteration associated with administration of whole bm12 CD4 T cells (Figure 8B). Thus, the accelerated rejection in the recipients that received donor CD4 T cells is the consequence of priming for autoantibody, and not T-cell alloimmunity.

**Discussion**

The results of this study provide important new insights into the development of autoantibody after transplantation and its contribution to allograft vasculopathy. Two major findings emerged: help for autoantibody production is provided through donor CD4 T-cell allore cognition of MHC class II on recipient B cells; and autoantibody contributes to the development of vasculopathy. Our results thus highlight the novel concept that passenger donor CD4 T cells within a cardiac allograft can contribute to its rejection.

Autoimmune responses are increasingly described in recipients of solid organ transplants, but their contribution to graft damage is unclear, because although associated with poor outcome, this may simply indicate nonpathogenic bystander activation that is triggered by a particularly aggressive (and consequently damaging) alloimmune response. Rodent studies have highlighted the contribution of T-cell autoimmunity to graft rejection, but the only convincing evidence of an effector role for autoantibody is the beneficial response to the depletion of autoantibody in renal transplant patients with vascular rejection associated with antiangiotensin receptor autoantibodies. The demonstration of a requirement for donor CD4 T cells in initiating humoral autoimmunity enabled us to distinguish the contribution of autoantibody to CAV from that of host alloimmunity. The histologic features, particularly complement deposition on allograft endothelium, are pathognomonic of humoral vascular rejection, which, given the absence of these features in μMT recipients and our experiments highlighting the lack of anti-bm12 alloantibody responses, are presumably triggered by autoantibody binding. Notably, complement deposition is absent in allografts from CD4 T-cell–deficient donors and is restored in μMT recipients by passive transfer of autoantibody. An effector role for autoantibody is confirmed by the early rejection and development of severe vasculopathy in bm12 hearts transplanted into animals primed for humoral autoimmunity by transfer of donor CD4 T cells. Allosensitization caused by residual I-A^bm12^-
expressing APCs within the transferred T cells is highly unlikely; severe CAV did not occur in either donor T-cell–injected μMT recipients or WT recipients immunized with either bm12-peptide or lysed bm12 CD4 T cells.

Not all of the experiments undertaken here demonstrated a role for autoantibody in the development of CAV. Passive transfer of autoantibody did not restore CAV in B-cell–deficient recipients, but as noted, the amount administered was likely insufficient for the development of CAV, albeit enough to provoke endothelial complement deposition. A similar explanation was provided for the recent observation that passive transfer of anti-MHC class I alloantibody to B-cell–deficient recipients triggered allograft endothelial cell signaling but did not restore CAV. More notably, the severity of CAV, although reduced in heart grafts from CD4 T-cell depleted or bm12RAG2−/− donors, was not statistically different from WT grafts. As explanation, autoantibody likely complements conventional alloimmune responses; all heart grafts (including those from CD4 T-cell deficient donors) contain lymphocytic infiltrates typical of an acute cellular response and vasculopathy still develops in hearts transplanted into B-cell–deficient recipients. A reconciliatory interpretation of our results is that the effector role of autoantibody is largely masked in unmodified recipients by concurrent strong alloimmune responses but becomes apparent on augmentation by injection of donor T cells.

Our findings add to the wider body of work emphasizing the importance of alloantibody in chronic allograft rejection, and although the mechanisms responsible are unclear, binding to target autoantigens on graft endothelial cells is thought to trigger CAV development. A difficulty in postulating a similar role for autoantibody is that the target autoantigens are intracellular. The association between autoantibody and endothelial complement deposition in bm12 hearts transplanted into μMT recipients that received autoantibody intravenously after transplantation (AT μMT) but not in hearts transplanted into μMT recipients. D, Representative indirect immunofluorescent staining of HEp-2 cells with sera sampled weekly after bm12 heart transplantation into μMT recipients, WT recipients, and WT recipients with preformed autoantibody.

Figure 8. Autoantibody contributes to the development of allograft vasculopathy. A, Kaplan–Meier survival curves of bm12 cardiac allografts in WT (n=17), μMT (n=8), and μMT mice passively transferred with autoantibody after transplantation (AT μMT, n=4). Bm12 hearts were also transplanted into WT (preformed, n=5, MST=35 days) or μMT (challenged μMT, n=4) mice that were injected intraperitoneally with bm12 CD4 T cells 2 weeks before transplantation or into WT injected with lysed bm12 CD4 T cells (Lysate-primed, n=4). *P<0.01, when compared with WT recipients, μMT recipients, and challenged μMT recipients. B, Allografts were harvested at day 50 and the severity of vasculopathy (grades 0 to 4) depicted as mean±SEM. Included is the vasculopathy score for bm12 allografts in bm12 peptide-immunized mice (P-primed). *P<0.05. C, Representative photomicrographs depicting focal endothelial complement deposition in bm12 hearts transplanted into μMT recipients that received autoantibody intravenously after transplantation (AT μMT), but not in hearts transplanted into μMT recipients. D, Representative indirect immunofluorescent staining of HEp-2 cells with sera sampled weekly after bm12 heart transplantation into μMT recipients, WT recipients, and WT recipients with preformed autoantibody.
The novel donor CD4 T-cell–dependent mechanism described here for effecting allograft damage constitutes an unusual manifestation of graft-versus-host recognition and is presumably a consequence of the minimal antigen disparity between the bm12 and B6 mouse strains that permits extended survival of donor lymphocyte populations by circumventing host defenses such as NK cell cytolysis. Our findings therefore have immediate relevance to other murine models of CAV that rely on limited donor-recipient antigen disparity to avoid acute rejection.41–44 A more searching question is the extent to which similar donor lymphocyte-driven responses are responsible for causing vasculopathy in human recipients of organ transplants? No study has as yet examined the role of passenger donor lymphocytes in the development of transplant-induced autoantibody. Their involvement is supported, at least theoretically, both by the recent demonstration of organ transplants? No study has as yet examined the role of passenger donor lymphocytes in the development of transplant-induced autoantibody. Their involvement is supported, at least theoretically, both by the recent demonstration of donor T cells,49,50 through nonlymphoid organs, such as the heart,45–47 and by historical transplant studies that document donor leukocyte “microchimerism” in human transplant recipients.48 This microchimerism includes populations of donor T cells,49,50 whose presence is strikingly manifest in the rare, but usually devastating, acute graft versus host disease that can occur after solid organ transplantation.

In conclusion, we report the novel finding that donor CD4 T cells within heart allografts contribute to the development of CAV by providing help to recipient B cells for autoantibody production. To what extent donor lymphocytes contribute to autoantibody development in human heart transplant recipients is currently speculative. Nevertheless, their potential involvement raises the possibility that depleting lymphocytes in transplant organs before engraftment will reduce the severity of CAV and improve long-term graft survival.

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Disclosures
None.

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**CLINICAL PERSPECTIVE**

The major problem associated with heart transplantation is the insidious loss of graft function and eventual failure due to the progressive development of cardiac allograft vasculopathy. Chronic allograft vasculopathy is a different disease process than conventional atherosclerosis and although its etiology remains poorly understood, immune recognition of donor major histocompatibility complex protein is generally considered fundamental in triggering its development. A role for autoimmune responses directed against the recipient’s own proteins has, however, become increasingly recognized. In particular, the development of autoantibodies directed against cytoskeletal proteins, such as vimentin, is associated with aggressive chronic allograft vasculopathy and predictive of early heart transplant failure. Nevertheless, 2 major questions remain unanswered: why are autoimmune responses triggered after transplantation, and how does autoantibody cause graft damage? Using a mouse heart transplant model, we report the novel finding that intragraft donor CD4 T cells provide an unusual form of help to recipient B cells for the production of anti-nuclear autoantibody. Autoantibody was associated with humoral vascular rejection and its contribution to chronic allograft vasculopathy further suggested by the early rejection of hearts when transplanted into mice primed for active autoantibody responses. Whether autoantibody generation in human transplant recipients is similarly dependent on donor T cell can only be answered by prospective clinical studies, but is supported, at least theoretically, by historical transplant studies that document donor leukocyte “microchimerism” in human transplant recipients. This microchimerism includes populations of donor T cells whose presence is strikingly manifest in the rare but usually devastating, acute graft versus host disease that can occur after solid organ transplantation.
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