Humoral autoimmunity in transplantation

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For many decades transplant rejection was considered to be a consequence of an adaptive immune response directed against disparate allogeneic MHC antigens expressed by the graft. More recently it has become appreciated that transplantation may also trigger cellular and humoral responses directed against self-proteins. Many aspects of this autoimmune response remain, however, poorly understood; not least why autoimmunity apparently occurs much more frequently after transplantation than in the general population and why the response targets such a seemingly diverse range of target autoantigens. Most importantly, it is still not known whether the autoimmune response makes a significant contribution to transplant rejection. By reviewing recent literature relating to transplant-induced autoimmunity and presenting some of our recent findings in experimental murine models, this article hopes to provide some answers to these questions.

Key words: transplant rejection, autoimmunity, cellular, humoral, t cells

Abbreviations

ANA Anti-nuclear autoantibody
GC Germinal centre
Tfh T follicular helper
OVA ovalbumin

1. Introduction

For several decades after the realisation in the 1950s that the adaptive immune response governed outcomes of transplanted organs, rejection was thought to be mediated almost exclusively by cellular mechanisms. However it is now appreciated that humoral responses are at least as important. The investigation of humoral alloimmunity has focused upon the response directed against the disparate classical MHC antigens of the donor, but it is becoming increasingly evident that the antibody response triggered by transplantation also targets autoantigens. The autoantigenic targets are often expressed on the graft and hence these autoantibody responses can in theory contribute to graft damage. Although recent studies have reported a correlation between the development of autoantibody and early graft failure, much is still unknown regarding the role of humoral autoimmunity in responses to solid-organ allografts; not least why transplantation triggers a breakdown in self-tolerance and why certain autoantigens appear to be targeted more commonly. Here I collate clinical and experimental studies from autoimmunity and transplantation to our own recent
work examining autoantibody responses in murine transplant models to address three main questions:

- What is the incidence and spectrum of humoral autoimmunity after transplantation?
- Why does transplantation trigger humoral autoimmunity?
- Do autoantibody responses augment conventional alloimmune responses?

2. The incidence and spectrum of humoral autoimmunity after transplantation

The development of autoantibody following transplantation has been described for every type of solid organ transplant (Table 1). The true incidence, of transplant-induced autoantibody is, however, difficult to assess, because studies generally report only isolated cases or small series of autoantibody-positive patients (1-4) and because it is difficult to distinguish de novo autoimmunity from recurrence of the original disease responsible for failure of native organs (15, 16). Thus the reported incidence varies widely; from 10 to 100% of recipients. One of the most confusing aspects of transplant associated autoantibody is that a surprisingly varied number of target autoantigens have been documented (Table 1). These may either be expressed widely (e.g. nuclear antigens in liver and cardiac transplant patients) or be tissue- or organ-specific (e.g. cardiac myosin in cardiac transplant recipients). This variation exists not just simply between different types of organ transplants, but also within the response to a particular organ (Table 1). Moreover, individual patients may mount responses against multiple target autoantigens (14, 19, 20); a recent study of paediatric renal recipients notably highlighted that autoantibody was generated against, on average, 61% of over 5000 test proteins (14). That such a vast range of autoantigens may be targeted by transplantation-induced humoral autoimmunity is an important observation, as it suggests that autoimmunity is not triggered by specific recognition of key autoantigens, but is instead a non-antigen-specific response to the transplant process itself.

3. Why does transplantation trigger humoral autoimmunity?

Elucidation of the mechanisms responsible for failure of self-tolerance following transplantation first requires consideration of how humoral autoimmune disease develops in non-transplant patients. Notably, autoreactive B cells are readily detectable in the circulation of disease-free individuals (21-24) indicating that central mechanisms, such as clonal deletion and receptor-editing, fail to eliminate all autoreactive B cells. The peripheral population of self-reactive B cells that persists in check by a number of control measures, but of these perhaps the most important is limitation of availability of T cell help. Humoral responses in autoimmune disease are typically class-switched and somatically mutated (25), suggesting their development is a consequence of germinal centre (GC) reactions. The GC response is now known to depend critically upon the provision of help from a recently-described and highly-specialised population of follicular helper T cells (Tfh) which secrete IL-21 and recognise the MHC class II complex of the B cell with high affinity (26, 27). Earlier studies have highlighted the importance of the provision of T cell help in the development of humoral autoimmunity. Simply the provision of T cell help is sufficient in some situations to provoke autoantibody generation (28-32) and recent studies have now established that the GC reactions of autoimmunity are similarly dependent upon autoreactive Tfh (27, 33).

How then is self-tolerance in the helper T cell population broken? A variety of mechanisms have been proposed for how help is generated for autoantibody production. The development of autoimmunity is sometimes clearly linked to an infection that immediately precedes development of overt disease and it has been suggested that the inflammatory triggers associated with infection may break energy in peripheral autoreactive T cells through presentation of target autoantigenic epitopes at much greater frequency than in the resting state (34). In addition, inflammation may generate different T cell epitopes than are normally encountered in the periphery. Such ‘cryptic’ epitopes may be generated because of either breakdown of self-proteins normally sequestered unavailable for immune recognition or changes in protease activity within APCs (35). Formation of neo-antigens through alterations in protein structure from post-translational modification (36) or oxidative stress (37) may also generate a different spectrum of T cell epitopes. Finally, a subset of T cells responding to the infectious agent may provide help through cross-reactive recognition of autopeptide epitope presented by autoreactive B cells (38).

4. T cell help for autoantibody development after transplantation

Because the autoantibody responses provoked by transplantation are frequently IgG class-switched (see Table 1), and thus dependent up-
TABLE 1:
Overview of clinical studies reporting autoimmunity following solid organ transplantation.
Development of humoral autoimmunity has been described following all solid organ transplants. Although reported target antigens vary, and the response in individual patients may target multiple autoantigens, responses are generally IgG class-switched, indicating provision of T cell help. Only a limited number of studies comment upon association with graft outcome (*), with the majority emphasising a possible contribution to chronic rejection.
Abbreviations: Abs, Antibodies; Ag, Antigen; ICAM-1, intercellular adhesion molecule-1; oxLDL, oxidized low density lipoprotein; ACh, Acetylcholine; BOS, bronchiolitis obliterans; AT1R, angiotensin 1 receptor; GAD, glutamic acid decarboxylase; N/E, not examined. Modified from (7).

<table>
<thead>
<tr>
<th>References</th>
<th>Graft</th>
<th>Autoantigen</th>
<th>Ig-Subclass</th>
<th>Rejection</th>
<th>Main Findings</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jurcevic (6) *</td>
<td>Cardiac</td>
<td>Vimentin</td>
<td>IgM</td>
<td>Chronic</td>
<td>28 of 52 (54%) with anti-vimentin autoantibody titres of &gt;100 developed transplant vasculopathy by 5 years (cf 18% incidence (10 of 57) in those without autoantibody).</td>
<td>38 of 109 (35%)</td>
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<tr>
<td>Warraich (8) *</td>
<td>Cardiac</td>
<td>Cardiac myosin (CM)</td>
<td>IgG, IgM</td>
<td>Acute</td>
<td>14 of 15 (93%) recipients transplanted for dilated cardiomyopathy with pre-formed anti-CM IgM autoantibody developed acute rejection; cf. 73% incidence of rejection (36 of 49) in those without autoantibody.</td>
<td>15 of 64 (23%)</td>
</tr>
<tr>
<td>Lawson (9)</td>
<td>Cardiac</td>
<td>ICAM-1</td>
<td>IgG, IgM</td>
<td>N/E</td>
<td>34/50 (68%) recipients developed Anti-ICAM-1 IgM Abs (cf. 1/20 5% of control healthy volunteers).</td>
<td>68%</td>
</tr>
<tr>
<td>Laguens (10) *</td>
<td>Cardiac</td>
<td>Glycolipid</td>
<td>IgG</td>
<td>Acute</td>
<td>13 of 19 (68%) of recipients with high titers of pre-formed anti-skeletal muscle glycolipid Abs developed acute rejection; cf. 15% incidence (4 of 26) in those without autoAb.</td>
<td>19 of 45 (42%)</td>
</tr>
<tr>
<td>Fang (11)</td>
<td>Cardiac</td>
<td>Oxidized LDL</td>
<td>IgG</td>
<td>N/E</td>
<td>Serum levels of anti-oxLDL Abs are inversely related to the extent of ACh-induced, endothelial-dependent coronary artery dilatation.</td>
<td>N/E</td>
</tr>
<tr>
<td>Goers (12) *</td>
<td>Lung</td>
<td>K-α 1 tubulin</td>
<td>IgG</td>
<td>Chronic</td>
<td>Anti-K-α 1tubulin Abs were present in 10 of 36 (28%) of recipients with bronchiolitis obliterans (BOS), and were undetectable in 36 healthy recipients.</td>
<td>10 of 72 (14%)</td>
</tr>
<tr>
<td>Burlingham (13) *</td>
<td>Lung</td>
<td>Collagen (V)</td>
<td>Cellular immunity</td>
<td>Chronic</td>
<td>7 of 24 (29%) of recipients with strong autoreactive T cell responses against Collagen (V) developed severe BOS; cf. 3% incidence (1 of 30) of BOS in those without T cell autoimmunity</td>
<td>24 of 54 (44%)</td>
</tr>
<tr>
<td>Dragun (3) *</td>
<td>Kidney</td>
<td>AT1R</td>
<td>IgG</td>
<td>Acute</td>
<td>Anti-AT1R Abs were found in 16 patients with refractory vascular rejection and malignant hypertension.</td>
<td>N/E</td>
</tr>
<tr>
<td>Joosten (4). *</td>
<td>Kidney</td>
<td>Agrin</td>
<td>IgG</td>
<td>Chronic</td>
<td>Antibodies against anti glomerular basement membrane agrin were present in 13 of 16 (81%) patients with transplant glomerulopathy (TG), but only in 3 of 16 controls with chronic allograft nephropathy without TG.</td>
<td>N/E</td>
</tr>
<tr>
<td>Li (14).</td>
<td>Kidney</td>
<td>Renal pelvis Ags</td>
<td>Not defined</td>
<td>N/E</td>
<td>Integrative genomic analysis of pre and post transplant sera demonstrated development of autoAb against wide spectrum of autoantigen (but mainly renal pelvis antigens) in all 18 paediatric recipients studied</td>
<td>100%</td>
</tr>
<tr>
<td>Dubel (15) *</td>
<td>Liver</td>
<td>Smooth muscle &amp; Nuclear Ags</td>
<td>Not defined</td>
<td>Chronic</td>
<td>Autoantibody present in 10 of 14 (71%) patients with chronic rejection, but in only 11 of 44 (23%) without chronic rejection.</td>
<td>22 of 57 (39%)</td>
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<tr>
<td>Braghi (16) *</td>
<td>Pancreas</td>
<td>Anti GAD / Anti-tyrosine phosphatase</td>
<td>IgG</td>
<td>Chronic</td>
<td>Overall, pre-formed autoantibody (present in 31 of 75 (41%) pancreas recipients) did not influence graft survival, but 4 of 5 patients whose titres rose after transplantation suffered early graft loss.</td>
<td>31 of 75 (41%)</td>
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<tr>
<td>Kalache (17)*</td>
<td>Heart</td>
<td>Cardiac myosin</td>
<td>IgG</td>
<td>Chronic</td>
<td>26 of 40 (65%) of heart transplant recipients with established allograft vasculopathy had detectable serum anti-cardiac myosin IgG autoantibodies, whereas autoantibody was present in only 4 of 32 disease-free recipients. This association was independent of anti-HLA abs.</td>
<td>30 of 72 (42%)</td>
</tr>
<tr>
<td>Porcheray (18)*</td>
<td>Kidney</td>
<td>Various</td>
<td>IgG</td>
<td>Chronic</td>
<td>Sera from 17 of 25 (68%) kidney transplant recipients with a diagnosis of chronic humoral rejection bound carcinoma cell line Hep-2, compared to 3 of 25 (12%) stable recipients. However, the precise target autoantigens varied between patients.</td>
<td>20 of 50 (40%)</td>
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on T cell help, the above processes that break T cell tolerance to self are presumably also operating after transplantation. However, the incidence of humoral autoimmunity after transplantation appears much greater than that of autoimmune disease in general. Similarly, most cases represent de novo autoantibody development rather than recurrence of pre-existing autoimmune disease (3, 4, 6), suggesting either that the processes for activating autoreactive T cells are accentuated or that additional, transplant-specific mechanisms exist. Certainly, extensive damage mediated by aggressive innate and adaptive alloimmune response may release large quantities of autoantigen (Fig 1A, (2, 13, 39)). Alternatively, the T cell response to alloantigen is likely to be much greater than to classical protein antigen, because the ability to uniquely recognise alloMHC ‘directly’ as intact protein on the surface of donor cells (40) results in a 100-1000 fold greater precursor frequency. Thus compared to the T cell response against an infectious agent that triggers autoantibody generation through cross-reactive recognition of autopeptide epitopes on autoreactive B cells, the many more T cell clones activated by a transplant will greatly increase the potential for cross-reactive B cell recognition (Fig 1B). In addition, the autoreactive B cell response is presumably triggered by recognition of donor proteins that are shed from the graft and that express B cell epitopes that cross-react with the equivalent host protein (such as vimentin and myosin). Nevertheless, processing of the donor moiety may, because of individual variations in the precise amino acid structure of these proteins, generate different, and immunogenic, T cell epitopes (Fig 1C, (41)).

5. Autoreactive B cell help from graft-versus-host recognition by passenger lymphocytes

Our recent mouse studies have highlighted a further mechanism for how transplantation triggers delivery of T cell help for autoantibody production (5). We examined the response to murine heart allografts using a model of chronic rejection in which the donor strain, bm12, differs from the recipient strain C57Bl/6 (B6) by only three amino acids in the I-Ab MHC class II locus. As a consequence, bm12 hearts are rejected very slowly when transplanted in-
to B6 recipients, but do develop progressive allograft vasculopathy that is similar to the arterial disease commonly observed in human heart transplants. Histopathological examination of the transplanted hearts, that included the demonstration of endothelial complement deposition, was highly suggestive of humoral vascular damage. Despite being one of the most commonly used models of chronic allograft vasculopathy, these features had not been previously described and were notably absent, and vasculopathy minimal, in heart grafts from B cell deficient recipients. Surprisingly given these features, we were unable to detect any evidence of IgG alloantibody generation against the disparate I-Abm12 class II alloantigen. Instead bm12 heart grafts elicited strong IgG anti-nuclear autoantibody (ANA) 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-centre B cell follicles in recipients’ spleens (Fig 2). Autoantibody was not generated in MHC class II deficient recipients suggesting that the response was T cell dependent, but we were surprisingly unable to establish a role for recipient T cells. Instead autoantibody production was completely abrogated using donors that were either genetically-deficient or depleted of CD4 T cells prior to heart graft removal, indicating that help was provided solely by small numbers of donor CD4 T cells that migrated from the heart graft after transplantation (Fig 2). Autoantibody also did not develop in recipients that lacked MHC class II expression only on B cells, suggesting that cognate interaction between the donor CD4 T cell and host B cell was essential for triggering humoral autoimmunity. This parallels previous work reporting the development of anti-nuclear autoantibodies upon injection of bm12 splenocytes to B6 mice (42).

Abrogation of autoantibody correlated with an absence of immunoglobulin and complement endothelial deposition on the allograft and with attenuation in the features of humoral vascular rejection. However, to confirm the contribution of autoantibody to the development of vasculopathy and eventual graft failure, bm12 heart grafts were transplanted into B6 recipients that had been primed for humoral autoimmunity by injection of bm12 CD4 T cells two weeks previously. Because the injected CD4 T cells were highly purified and did not express MHC class II, we reasoned that CD4 T cell administration is unlikely to sensitize against the disparate I-Abm12 alloantigen. All heart grafts in primed recipients were rejected rapidly (Fig 3).

We concluded that graft-versus-host allorecognition of MHC class II on host B cells by donor CD4 T cells can provide an unusual form of help for generating autoantibody (Fig 1d); autoantibody that in turn plays an effector role in the development of allograft vasculopathy.

**Figure 2:**
Antinuclear autoantibody develops after bm12 heart grafting.
A, Representative images of indirect immunofluorescent staining of HEp-2 cells with weekly sera samples from B6 recipients of either bm12 heart grafts (WT) or syngeneic grafts, or MHC class II deficient (MHCII−/−) recipients of bm12 hearts. B, Graphical depiction (mean±SEM) of fluorescence intensity (grade 1 to 5) for each group. C, Hematoxylin and eosin–stained paraffin sections depicting germinal center secondary follicles within the spleens of recipients of bm12 allografts but not in recipients of syngeneic grafts. D, Representative photomicrographs of HEp-2 staining from different B6 recipients of day 50 bm12 heart allografts revealing distinct staining patterns: (i) coarse-speckled; (ii) nuclear-homogenous; and (iii) cytoplasmic and nuclear homogenous. *P<0.05 cf either recipients of syngeneic hearts or allografted MHCII−/− mice. Modified from (5).
Our study thus highlights that passenger donor CD4 T cells within a heart graft can, surprisingly, contribute to its rejection.

6. Do autoantibody responses augment conventional alloimmune responses?

In our model, help for B cells is unlikely to depend upon the precise peptide presented by the B cell, because every host MHC class II complex will be recognised as a foreign alloantigen by the donor CD4 T cell population. All B cell clones, irrespective of BCR specificity, should theoretically receive an equivalent degree of T cell help, and it was therefore unclear as to why the humoral response triggered following transplantation only targeted select nuclear autoantigens. Presumably nuclear self-antigens differ from conventional immunogens in the activation signals they trigger within B cells and we hypothesised that this essential difference related to availability of target antigen; that constitutive expression of nuclear autoantigen permits continual ligation of the BCR, which in combination with simultaneous helper T cell binding to MHC class II, provides essential signals for plasma cell differentiation. The corollary of this hypothesis; that donor T cell help would provoke conventional antibody responses if ligation of BCR by target antigen occurred simultaneously; was tested by reconstituting T cell-deficient B6 TCR-/- mice with bm12 CD4 T cells that, as a source of target alloantigen for B cell recognition, expressed transgenic H-2Kd (bm12Kd). This prompted strong auto- and anti-Kd IgG alloantibody responses; in contrast, injection with WT bm12 cells generated autoantibody only (Fig 4). Autoantibody and anti-Kd IgG alloantibody responses were also detected following bm12Kd heart transplantation into TCR-KO recipients. Interestingly, whereas bm12Kd CD4 T cells survived indefinitely in T and B cell-deficient RAG-/- B6 hosts; indicating resistance to lysis by NK-cells; they were undetectable once humoral immunity had been provoked by their transfer into B6 TCR-/- mice. This rapid disappearance most likely reflects alloantibody-mediated killing and these experiments thus demonstrate that donor CD4 T cells may initiate a graft-versus-host response that is ultimately responsible for their own destruction; effectively they provoke a suicide response. Given the above findings it is reasonable to consider whether similar donor CD4 T cell-mediated responses are responsible for the development of autoimmunity that has been described in other transplant models (43-45). This has not been formally examined, but certainly, neither the presence of CD4 T cells within peripheral organs such as the heart nor their ability to recognise host cell MHC class II alloantigen is likely unique to the bm12 strain. However, the bm12 to B6 murine model is characterised by remarkably-minimal disparity between the donor and recipient strains and notably, autoantibody did not develop when either bm12 x BALB/c F1 CD4 T cells were transferred to B6 TCR-/- mice. This likely reflects NK cell-mediated killing of the more disparate F1 cells, because unlike bm12 and bm12Kd cells which survived indefinitely upon transfer into B6 RAG-/- mice, the F1 cells were detectable after the first week only if NK cells were simultaneously depleted. Similar depletion of NK cells in TCR-/- mice resulted in strong, rapid anti-Kd alloantibody responses upon injection with
bm12 x BALB/c F1 CD4 T cells, confirming that disparate donor CD4 T cells can also provide help for alloantibody generation, provided that NK cell recognition is circumvented. The development of alloantibody in these animals again coincided with disappearance of the donor CD4 T cell population. Finally, BALB/c x bm12 F1 heart allografts in wild-type B6 mice that were depleted of NK cells also provoked autoantibody and early, augmented alloantibody responses, indicating that adaptive recipient CD8 T cell responses do not develop quickly enough to prevent donor CD4 T cells from provoking GVH-mediated humoral immunity.

7. Summary

Our analysis of the mechanisms responsible for the development of vasculopathy in MHC class II-mismatched heart allografts has revealed several unusual findings. The demonstration that passenger CD4 T cells within the heart graft were present in sufficient numbers to provoke recipient autoantibody responses was surprising. Moreover the dependence on donor CD4 T cells for initiating humoral autoimmunity enabled manipulation of the model to provide strong evidence for the first time of a contributory role for autoantibody in the development of allograft vasculopathy. The concept that passenger donor CD4 T cells within a cardiac allograft can contribute to its rejection 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. This permits extended survival of donor lymphocyte populations by circumventing 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 (46-49). A more searching question is whether similar donor lymphocyte-driven responses are responsible for causing vasculopathy in human recipients of organ transplants? This has not been examined, but is lent support by the recent demonstration that peripheral T cells migrate through non-lymphoid organs (50-52), and by historical transplant studies documenting donor leukocyte ‘micro-chimerism’ in human transplant recipients (53). This micro-chimerism includes populations of donor T cells (54, 55), whose presence is strikingly manifest in the rare, but usually devastating, acute GVH disease that can occur after solid organ transplantation.

Similarly, the observation that graft-versus-host recognition by donor T cells may provoke alloantibody responses independently of the recipient T cell response is of potentially more than academic interest. Strategies that deliberately promote mixed donor/recipient bone-marrow (BM) chimerism achieve robust transplant tolerance in animal (56) and in human recipients (57). Yet the defining characteristic of our model that initiates a destructive autoantibody response is the requirement for co-existence of both donor and lymphocyte populations. The possibility that mixed chimerism can still result in graft rejection through activation of alternative effector mechanisms (that are inherently dependent upon the chimeric state for their development) has not been formally examined, although one study has documented that an imbalance in the proportions of donor and recipient T and B lymphocytes is associated with autoantibody production (58) and notably, allo- and auto-antibody responses have been observed recently in patients in whom BM chimerism has been created to enable immunosuppressive-free kidney allograft engraftment (59).

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