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Humoral autoimmunity after solid organ transplantation: germinal ideas may not be natural

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Key words: Autoimmunity; Germinal centre; natural antibody; chronic rejection; allograft vasculopathy

Abbreviations

APCs	antigen presenting cells
ACPAs	anti-citrullinated protein antibodies
ADCC	antibody-dependent cellular cytotoxicity
AECA	antiendothelial cell antibody
AMR	antibody mediated rejection
BAFF	B cell activating factor
BCL6	B cell lymphoma 6
BCR	B cell receptor
BOS	bronchiolitis obliterans syndrome
CM	cardiac myosin
ColV	collagen V
DAMPs	Damage Associated Molecular Patterns
DGF	Delayed Graft Function
DSA	donor specific antibody
FDCs	follicular dendritic cells
GC	germinal centre
GVH	graft-versus-host
HSCT	Haematopoietic stem cell transplantation
IRI	ischaemia reperfusion injury
ITAMs	immunoreceptor tyrosine-based activation motifs
LLPCs	long lived plasma cells
MAC	membrane attack complex
mHC	minor Histocompatibility Complex (mHC)
NAb	Natural Antibody (NAb)
OSEs	oxidation specific epitopes
PRRs	pattern recognition receptors
SAP	SLAM-associated protein
SHM	somatic hypermutation
SLE	systemic lupus erythematosus (SLE)
TAA	transplant associated autoimmunity
TFH	T follicular helper
TFR	T follicular regulatory
TGP	transplant glomerulopathy
TLR	toll like receptor (TLRC)
TLT	tertiary lymphoid tissue

Abstract

Non-HLA antibody responses following solid organ transplantation have become increasingly emphasised, with several large clinical series suggesting that such responses contribute to late graft failure. Many of the responses described recognise both recipient and donor moieties of the target antigen and thus represent auto-, rather than allo- immunity. Within this rapidly evolving field, many questions remain unanswered: what triggers the response; how innate and adaptive humoral autoimmunity integrate; and most pressingly, how autoimmunity contributes to graft damage and its relationship to other effector mechanisms of graft rejection. This review summarises recent clinical and experimental studies of humoral autoimmunity in transplant rejection, and considers some of the answers to these questions.

1. Introduction

A link between autoimmunity and transplantation was first suggested as part of the early murine work characterising the nature of transplant tolerance. It was noted that parental strain mice that were injected intraperitoneally with F1 splenocytes subsequently accepted skin grafts from the F1 strain (1), but that growth was retarded in the recipient mice, in association with splenomegaly and evidence of ongoing immunological responsiveness between the F1 donor and parental recipient (2, 3). Lambert et al subsequently reported the development of anti-DNA autoantibody and a systemic lupus erythematosus (SLE)-like disease in such neonatal tolerant mice, with humoral autoimmunity seemingly dependent upon interactions between parental strain CD4 T lymphocytes and F1 strain B cells (4-6).

An association between solid organ transplantation and recipient autoimmune-like manifestations in the recipient was similarly suggested in the early era of clinical transplantation, but has been increasingly emphasised over the last decade, as techniques for detecting and characterising recipient autoantibody responses have improved. This focus suggests that humoral autoimmune response may make meaningful contributions to acute and chronic transplant rejection. There are any many aspects of the response that remain poorly understood, not least: whether the responses observed are truly autoreactive or directed against mismatched minor Histocompatibility Antigens (mHC); the mechanisms by which B cell self-tolerance is broken; and the extent to which autoimmunity independently causes graft rejection, as opposed to augmenting other effector mechanisms. Here, we review published data reporting the incidence of transplant associated autoimmunity (TAA) following solid organ transplantation, consider the mechanisms responsible for generating autoantibody, and discuss how humoral autoimmunity may contribute to graft rejection.

2. What is the Evidence for Autoreactivity in Transplantation?

2.1 Historical Perspective

Transplant associated autoimmunity (TAA) was first described in clinical practice in the late 1970s, in relation to haematopoietic stem cell transplantation (HSCT) (7-9). Patients suffering from chronic graft-versus-host (GVH) disease suffered widespread collagen deposition, resulting in severe scleroderma, joint contractures, pulmonary fibrosis, intestinal malabsorption, and Sjogren's syndrome (10). These features were associated with generation of humoral autoimmunity directed against a variety of self-antigens (11).

The possibility that autoimmunity may also occur following solid organ transplantation was first proposed in the early 1990s, by Dunn and colleagues (12, 13). They reported the development of anti-endothelial and cardiac tissue-specific IgG antibody that bound self as well as allo-determinants,

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and that was related to more severe rejection episodes, although only small numbers of patients were included in their studies. Autoantibody was usually assayed by incorporating tissue lysates as the target in indirect immunofluorescence assays, and thus the precise antigens targeted by the humoral response were not identified. Over the following decade, sporadic studies reported anti-endothelial or tissue-specific IgM / IgG humoral immunity following cardiac (14), kidney (15, 16), liver (17) and lung (18) transplantation, generally in association with either acute or chronic rejection, albeit patient numbers were again too small to establish a link definitively. A common controversy in these early studies that persists to the present day (19) was whether the non-HLA antibodies described represented allo- (targeting only donor antigen (15)) or auto- (targeting recipient) immunity. Recipient humoral immunity was however typically assayed by capture with 3rd party cell lines (16) or umbilical vein (14), suggesting that autoreactive responses were being assessed.

2.2. Characterisation of autoantigens targeted after transplantation

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By the early 2000s, studies had begun to use ELISA or cDNA libraries to identify more precisely the self-antigens targeted by humoral autoimmunity (20-26). These studies often revealed that the target autoantigens were expressed specifically in the donor organ, for example: vimentin intermediate filament (24), myosin motor protein (23, 25), and skeletal muscle glycolipid (21) following heart transplantation; glutamic acid decarboxylase (GAD) enzyme and islet antigens following pancreas transplantation (22); and glomerular basement membrane protein agrin in renal transplant recipients (20), with the latter study by Joosten et al. demonstrating a strong correlation with anti-agrin autoantibody and the development of transplant glomerulopathy. That graft-specific autoantigen was frequently identified as a target of TAA supports a contributory role for autoantibody in graft rejection, but it should be noted that this apparent focus may simply reflect the deliberate selection of graft-related autoantigens for capture in ELISA; use of cDNA libraries suggested a broader humoral autoimmune response (26). Contemporaneous experimental transplant studies were nevertheless beginning to report a conributory role in graft rejection for, initially, T cell autoimmunity (27-30), but also, latterly, autoanitbody (31-35).

Interest in TAA was considerably heightened by the publication of two papers that provided strong evidence that autoimmunity contributed directly to graft rejection (36, 37). In the first, Dragun et al (36) described the correlation of malignant hypertension with refractory vascular rejection, but without evidence of anti-HLA antibody, in 16 kidney transplant patients. All patients were found to have circulating IgG antibody directed against the angiotensin II type 1 receptor (AT1R; a G-protein-coupled receptor expressed on endothelial cells), and it was postulated that agonistic binding of the antibody to its receptor on graft endothelial cells triggered vasconstriction and vascular rejection. In support, treatment with plasmapheresis and the AT1R antagonist losartan improved graft function in 7 of the cohort (36). In the second paper, Burlingham et al (37) reported that post-transplant immune responses against fibrillar collagen V (ColV) self-protein were generated in 44% of a cohort of 54 routinely followed lung transplant recipients, and that this was associated with an approximate 10 fold increased risk in developing severe bronchiolitis obliterans syndrome (BOS) The Burlingham paper examined CD4 T cellular immnity against ColV, but Mohanakumar and colleagues subsequently reported the development of anti-ColV humoral autoimmunity in lung transplant recipients, often in association with autoantibody responses against Kα1 tubulin (38, 39).

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Humoral immunity against non-HLA antigens has been increasingly recognised over the last decade. Small volume clinical studies have continued to report an association between post-transplant anti-vimentin IgG antibody responses and the development of renal transplant glomerulopathy (TGP) (40, 41), although no association with outcomes for heart transplantation was found in the multicentre

'Clinical Trials in Organ Transplantation' (CTOT–05) study of 200 heart transplant recipients, nor in its CTOT-18 follow up (42, 43). The CTOT-18 Stehlik study did nevertheless report that patients with humoral immunity against cardiac myosin (CM) at the time of transplantion or at 12 months' follow up were 2.9 times more likely to reach the study composite end-point (death, retransplantation, coronary stent placement, myocardial infarction, or cardiac allograft vasculopathy). This mirrors an earlier, single-centre report that anti-CM humoral immunity was independently associated with cardiac allograft vasculopathy (44). The Mohanakumar group continued to examine the development of humoral immunity against Kα1 tubulin and ColV following lung [Hachem, 2012 #4754} and reported that either pre-existing (39, 45) IgG autoantibody against anti ColV/ Kα1 tubulin or *de novo* generation after transplantation (46) was associated with early graft dysfunction and an approximately threefold increased risk of developing BOS. Notably, only 3 of 15 (20%) autoantibody-positive patients who successfully cleared autoantibody following B cell depletion therapy progressed to BOS, compared to 27 of the 38 (71%) whose autoantibody persisted despite treatment (46), albeit patients were often also sensitised against donor HLA, making the relative contribution of humoral autoimmunity to graft rejection difficult to gauge. Parallel studies examining the development of TGP reported an association with detectable circulating autoantibody against collagen IV (a basal lamina protein) and the matrix glycoprotein, fibronectin (47). Although widely expressed, both proteins are produced within the kidney (48), and one of the recurring themes of the Mohanakumar's studies is the apparent targeting of TAA onto autoantigens expressed within the transplanted organ.

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Following the original Dragun observations, studies have continued to focus on the humoral autoimmune response against AT1R, predominantly following renal transplantation (49-56), with one case reported of apparent hyperacute rejection mediated by anti-AT1R autoantibody (52). These studies generally detail an association between pre-formed or de novo anti AT1R IgG autoantibody and increased rates of acute antibody mediated rejection (AMR (50, 54)) and / or development of TGP and early graft loss (49, 51, 56), although this is not a universal finding (57). This variability in the published literature may reflect a number of factors: that different studies use different cut-off values of anti-AT1R to designate positivity; that the impact of humoral autoimmunity against ATIR on graft failure may not become evident until at least three years after transplant (55), and that this impact is strongly influenced by the concurrent development of DSA (49, 51). The largest series to date, recently published by Lefaucheur and colleagues (53), details that 27.3% of their study cohort of 1845 renal transplant recipients developed anti-AT1R IgG antibody after transplant, and that its development was an indepedent predictor of graft failure on multivariate analysis, with seven year graft survival rates of 76.4% for those recipients with detectable anti-AT1R antibody vs 84.8% for those without, and with significantly poorer graft survival observed in those autoantibody-positive indivuals who had also developed DSA.
Aside from AT1R, it seems likely that many cell surface receptors could potentially act as targets for the TAA response; autoantibodies against endothelin-1 type A receptor have, for example, been detected in conjunction with anti-AT1R antibody in heart (58) and intestinal (59) transplant recipients. Using cultured human umbilical vein as assay to capture the panoply of endothelial reactive antibody, Sun and colleagues reported that 12.6% of a cohort of 174 kidney transplant recipients developed de novo antiendthelial cell antibody (AECA) after transplantation and that this was associated with an increased incidence of acute rejection, and poorer kidney graft function at three years, although differences in actual graft survival were modest (60). A similar relationship between AECA and rejection episodes following kidney transplantation was described recently by Sanchez-Zapardiel (61). Using a proteomics approach, Jackson and colleagues (62) identified four autoantigens (endoglin, Fms-like tyrosine kinase-3 ligand, EGF-like repeats and discoidin I-like domains 3, and intercellular adhesion molecule 4) as potential targets of humoral immunity following kidney transplantation, and reported an increased incidence of AMR in patients with

circulating AECA, although, as with the Mohanakumar studies above, patients were also highly sensitised against donor HLA antigen; longer term graft function or outcome was not influenced by AECA status.

2.3 Unbiased characterisation of the transplant associated autoantibody response

Unbiased characterisation of the humoral response in kidney transplant recipients, generally using a protein microarray approach, have revealed that multiple different non-HLA antigens may be targeted (63-67). Using this approach, Sarwal and colleagues have reported that paediatric kidney transplant recipients develop humoral responses directed against a wide variety of kidney-related antigens, predominantly those expressed within the renal pelvis and outer cortex. There was minimal cross-reactivity with heart or pancreatic-derived proteins, suggesting that the humoral response is driven by antigen-specific recognition (65). A follow-up study identified 38 *de novo* non-HLA antibody responses associated with the development of 'chronic allograft injury' and highlighted that baseline responsiveness against four targets (CXCL9; CXCL11; IFN-gamma; and glial-derived neurotrophic factor) predicted chronic injury at 24 months (67). A similar approach by Dinavahi et al reported that post-transplant *de novo* humoral reactivity against non-HLA antigens was associated with TGP, but that the responses differed for each individual (66). Simultaneously, Zorn and colleagues reported that chronic AMR following kidney transplantation was associated with broad autoreactivity, but again with largely unique profiles for each patient (64). To exclude the potential confounding from sensitisation against donor HLA antigen, Delville et al. have recently identified 38 kidney transplant recipients with early acute microvascular rejection, but without detectable DSA, and report that 89% of these patients had circulating IgG antibody that bound to a glomerular endothelial line, whereas minimal reactivity was observed in healthy transplant recipients (19). Test sera did not bind to a human arterial line, suggesting that the target antigens are kidney-specific and expressed constitutively within the transplant.

Whereas the broad repertoire of the autoantibody profile revealed by the above protein microarray studies may reflect a state of general B cell hyper-responsiveness with multiple clonal involvement, it could also be explained by activation of a more restricted subset of polyreactive B cells, with single clones capable of binding a variety of different antigens, as first suggested by Zorn and colleagues (68). Polyreactivity extended to the recognition of elements of apoptotic, but not viable, Jurkat cells (69), and their subsequent study of 300 kidney transplant recipients revealed that pre-formed IgG antibody against apoptotic cell determinants was an independent predictor of late kidney graft loss (70). As discussed below, such polyreactivity is consistent with an innate B cell response, and the group have recently described that in a large cohort of 635 renal transplant recipients, the development of 'natural' IgG antibody in the first year after transplantation was associated with an approximate 14% reduction in kidney graft survival at 7 years, with particular poor outcomes observed in those who concurrently developed anti-HLA DSA (71).

3. Humoral Autoimmunity or Alloantibody?

Published papers on antiendiothelial antibody responses have not generally categorised the target proteins identified as autoantigens, and certainly, polymorphisms are found outwith the MHC loci (such as at the *AGTR1* gene that encodes for AT1R), raising the possibilty that AECA is instead a manifestation of conventional cellular alloresponses (presumably indirect-pathway CD4 T cell (72)) directed against minor Histocompatibility Complex (mHC) antigens that are mismatched between donor and recipient. In support, two recent publications have highlighted that mismatches at mHC antigens make a more deleterious impact on kidney transplant outcomes than has conventionally been considered (73, 74). Against this, some of the identified non-MHC targets of the humoral

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4. What Mechanisms are Responsible for Breaking B Cell Tolerance to Self?

From the above, it can be seen that humoral autoimmune responses have been described in relation to almost all types of solid organ transplants, and at various times before and after transplantation. This raises the question whether there is a universal mechanism, or a number of different competing processes, that operate to generate autoantibody. We will firstly consider how tolerance may be broken in conventional B2 B cells, because disordered regulation of this subset has generally been perceived as the main trigger for humoral autoimmune diseases, but will then consider the contribution of 'innate' B1 cell responses, reflecting the recent focus on the possible pathogenesis of this subset (78).

4.1 Loss of tolerance to self in conventional B2 B cells

Escape from central mechanisms

For conventional B2 follicular B cells, an intractable consequence of the random recombination of the variable (V), diversity (D) and joining (J) genes is the expression of B cell receptors on immature B cells that potentially recognise self. Wardemann et al. have reported that between 55 and 75% of immature B cells in the bone marrow are autoreactive (79). Early studies incorporating B cell receptor (BCR) transgenic mice highlighted the role of central deletion in control of such autoreactive clones (80-83). BCR knock-in technology however, subsequently demonstrated that immature B cells first attempt to escape autoreactivity through receptor editing, wherein the re-expression of recombinase activation gene (RAG) avoids deletion through sequential exchange of downstream κ, and then, λ light chain sequences (84-86). A number of congenital, B cell-intrinsic pathologies are associated with a break-down in central tolerance, and release of higher proportions of potentially autoreactive immature B cells into the periphery (reviewed in (87)). For example, polymorphisms in the protein tyrosine phosphatase nonreceptor type 22 (PTPN22) gene polymorphisms are linked to different autoimmune diseases, with the major risk allele conferring altered BCR signalling that results in defective central selection (88).

Breakdown of peripheral anergy

Even in healthy individuals, central mechanisms of receptor editing and deletion within the bone marrow are inexact, and potentially self-reactive B cells - typically those binding with reduced avidity to soluble self-antigen (82) (but also, to a lesser extent, those binding membrane-bound autoantigen (89)) are released from bone marrow as transitional B cells. B cell receptor ligation of self-antigen at this stage results in a state of 'anergy', characterised by down-regulation of surface IgM, attenuated BCR signal transduction, and exclusion from the B cell follicle (83, 90-93). As opposed to a half-life of several weeks for mature follicular B cells, excluded anergic B cells survive for only a few days.

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Anergy is characterised by reduced calcium flux upon BCR stimulation (92, 94), and although continued receptor binding with target self-antigen is important for its maintenance (94, 95), the anergic state is not thought a consequence of diminished signalling from receptor unavailability, because it persists when only a relatively small proportion (~20%) of the B cell surface receptors are engaged (96). Instead, in contrast to naïve cells, wherein Lyn activation principally triggers phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) and generates phosphatidyl inositol-3 kinase (PI_3-kinase), Lyn signalling from chronic autoantigen stimulation in anergic B cells phosphorylates inhibitory motifs on co-receptors such as CD22. This recruits the inositol phosphatases, SHIP-1 and PTEN, and the tyrosine phosphatase SHP-1, which antagonise formation of PI_3-kinase, and inhibit B cell activation (91, 97).

Although much of our understanding of B cell anergy was provided initially by study of murine BCR transgenic models, anergic B cells are readily identifiable in healthy humans and are predominantly autoreactive (98). Indeed, recent data from Cambier et al. suggest that ~40% of circulating non-memory B cells are autoreactive (99). As to why so much energy is invested in producing a large population of B cells that are potentially harmful to the individual, a degree of autoreactivity possibly enables humoral responses to be generated against pathogens that try and evade immune recognition by mimicking the host (100). Autoimmune disease is generally avoided because upon BCR ligation, diminished downstream NF-κB signalling preferentially directs autoreactive B cells to the germinal centre (GC) reaction (rather than seeding extrafollicular foci), with eventual selection of progeny that have somatically mutated away from autoreactivity into the plasma cell compartment – a process that has been termed clonal redemption (100-103).

Notwithstanding, B cell anergy can be broken, most notably by provision of CD4 T cell help to the anergic B cell (104-106). Assuming simultaneous binding of target autoantigen to the BCR, this results in differentiation to an antibody secreting cell and production of typically, anti-nuclear, autoantibody (105). Self-reactivity of Ig receptors is not absolute, but rather a spectrum of autoreactivity exists (91), with those B cell clones that react most strongly to self-antigen exhibiting a more profound anergic state (such as absent IgM expression), whereas those clones with only slight cross-reactivity to self (possibly a large proportion of the circulating naïve B cell population) show only minimal anergic features (reduced calcium flux upon BCR ligation). Availability of B cell activating factor (BAFF) is important in determining whether transitional B cells with modest self-reactivity are destined for anergic follicular exclusion and early death or mature normally as naïve B cells within the follicle (107, 108), with excess BAFF levels supporting follicular maturation.

The extent of the contribution of anergic B cells to autoimmune disease remains unclear, but there is evidence (109-111) that '9G4' idiotype B cells, that are normally anergic in healthy individuals, can expand and differentiate to plasma cells with specificity for anti-nuclear autoantigen in patients with SLE. More recently, using a B cell immunomics approach, Sanz and colleagues have demonstrated that flares of SLE are associated with expansions of IgD^{+ve} $CD27^{-ve}$ activated naïve cells that are autoreactive and likely undergoing plasmablast transformation within extrafollicular foci (112-114). Such cells had not undergone somatic hypermutation (SHM) - thus their germline VDJ recombination encoded for autoreactivity – and although not examined specifically, it seems likely that they were derived from an anergic population.

Autoreactivity from dysregulation of the follicular B cell compartment

Autoreactive B cells are also generated as an almost inevitable consequence of T-dependent antibody responses (reviewed in (115-118) -see Figure 1). Such reactions are characterised by an initial cognate interaction between the antigen-specific helper T cell and B cell at the interfollicular

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zone. This triggers an early burst of B cell proliferation, with two discrete differentiation pathways then available for the B cell: it either migrates to form extrafollicular foci within the lymph node bridging channels and splenic red-pulp, or returns to the follicle to seed a germinal centre response.

The extrafollicular response provides the first line of adaptive pathogen defence, and is characterised by rapid production of short-lived, low-affinity antibody – B cells entering the extrafollicular pathway express BLIMP-1 almost immediately. Hence, although cognate help from CD4 T cells is necessary at initial T-B encounter for subsequent formation of extrafollicular foci (119), the requirement for help thereafter is less clear. In certain autoimmune conditions, extrafollicular foci appear to be much more long-lasting, and can even mediate affinity maturation through SHM (120-123). This may explain the description of a separate subset of CD40L^{hi} PSGL-1^{lo} CXCR4^{+ve} extrafollicular helper CD4 T cells in certain models of chronic autoimmunity (124). As discussed above, recent evidence suggests that extrafollicular foci may be an important source of pathogenic autoantibody during SLE flares (reviewed in (113)).

The GC reaction contrasts markedly, in that GC foci take longer to develop, and are characterised by the production of high affinity and durable antibody responses. These are a consequence of the generation and deposition to the bone marrow of affinity-matured long lived plasma cells (LLPCs) that can potentially survive and secrete immunoglobulin for the life of the individual. GCs also produce non-secretory memory B cells that respond rapidly upon re-exposure to target antigen by either differentiating into plasma cells or seeding new GC reactions (125). Memory B cells are also long-lived, and their survival is independent to that of the LLPC fraction (126, 127).

Performing such precise and varied function requires sophisticated organisation, both temporally and anatomically, and a clearer understanding of GC function has only been achieved in the last decade (reviewed in (128-130)). Following initial encounter with the T cell at the T-B border, those B cells destined to become GC B cells begin to upregulate B cell lymphoma 6 (BCL6), and downregulate EBI2 and sphingosine 1-phosphate receptor (131, 132). This facilitates migration to seed the centre of the B cell follicle, at around day 4 after antigen encounter, with a single B cell sufficient to initiate the GC response – this maintains clonal identity within the GC. T cell help for the GC response is provided by a highly-specialised subset of T follicular helper (T_{FH}) cells, which express the master transcription factor BCL6, and are characterised by expression of CXCR5, PD-1 and ICOSL (reviewed in (133, 134)). The T_{FH} cell subset originate from those 'pre-T_{FH} cells' that engage initially with the B cell at the T-B border, and other than those T cells with highest affinity for target antigen appear preferentially selected (135-137), the factors governing T_{FH} cell differentiation remain unclear.

By day 7, the GC has established its characteristic light and dark zone configuration. Within the dark zone, CD86^{lo}CXCR4^{hi} centroblasts undergo rapid division, coupled with AID-directed mutation of the VDJ sequence. Attracted by a CXCL13 gradient, centroblasts transit to the light zone, acquire a CD86^{hi}CXCR4^{lo} centrocyte phenotype, and encounter and acquire target antigen presented by follicular dendritic cells (FDCs). Four different cell fates are available to the centrocyte: apoptosis; re-entry into the DZ for further cycling; differentiation to LLPCs; or deposition as IgM or IgG memory B cells. Intracellular signalling is re-wired within centrocytes, such that BCR ligation is polarised towards phosphorylation of inhibitory SH2 domain-containing phosphatase-1 (SHP-1) and SH2 domains (138). This accentuates the requirement for survival signals provided by encounter with T_{FH} cells, present at limiting numbers to ensure that higher affinity B cell clones that encounter and then present a greater quantity of target antigen outcompete lower affinity clones for essential T cell help. High affinity centroblasts are imprinted to divide more rapidly and reside longer upon return to the dark zone (139, 140).

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The precise cues that prompt memory cell or plasma cell deposition from the GC remain unclear. High affinity B cell clones appear to be preferentially deposited as LLPCs, providing effective circulating antibody for strong, immediate binding upon repeat pathogen challenge. It is now understood that memory B cells may be formed throughout the GC response, with predominantly non-mutated IgM memory and mutated IgG memory deposited at early and late time points respectively (141). The lower affinity variants may exhibit cross-reactivity with newly encountered pathogens that share structural similarities to the initial target antigen, with the opportunity for these clones to initiate a new GC and to undergo further SHM-based selection.

The random nature of the nucleotide mutations engendered by AID activity risks generating newly-autoreactive centroblasts. Several mechanisms inhibit the development of autoreactivity within the GC (Figure 1). Encounter of soluble target antigen within the GC (presumably not in the context of the FDC) causes massive apoptosis and involution of the GC, in a T cell-independent fashion (142, 143). GC B cell clones that develop SHM-mediated self-reactivity may be similarly deleted, assuming that their target autoantigen is ubiquitously, and non-selectively, expressed within the GC environs. Autoreactive B cell clones are also unlikely to internalise sufficient of the original target antigen from the FDC to compete successfully for help from the antigen specific T_{FH} cell subset. The rewiring of BCR signalling in GC B cells, by accentuating the requirement for essential T_{FH} cell factors, may ensure death of autoreactive B cells that would otherwise have received positive, pro-survival signalling via their BCR alone. Precise focusing of T cell help to the target antigen may be further ensured by a second population of *foxp3*-expressing T follicular regulatory (T_{FR}) cells within the follicle (144). These act in an antigen-specific fashion (145), but may function principally to prevent the development of autoimmunity (146, 147), possibly via FAS-mediated killing of 'rogue' GC B cells (148). Finally, the massive levels of cell cycling and cell death within the GC risk exposing self-antigens (that would otherwise be sequestered intracellularly) to immune recognition. This risk is normally obviated by tingible body macrophages, which rapidly clear apoptotic cells in a non-inflammatory fashion; breakdown in this system is associated with autoimmunity (149).

How these various mechanisms coordinate to prevent autoimmunity requires clarification, but sophisticated murine models have demonstrated that the GC response is inherently geared to select against autoreactivity. For example, Brink et al. have reported that autoreactive clones that are generated within the GC fail to be selected, even if those clones exhibit increased affinity for the original target antigen (103). The same mechanism enables anergic, autoreactive B cells to enter the GC and generate antibody that exclusively recognises foreign pathogen (100). Notwithstanding, dysregulation of the GC response and generation of highly mutated, class-switched autoimmune variants is considered one of the main drivers of autoimmune disease (reviewed in (117)). For example, reverting anti-nuclear autoantibodies obtained from patients with SLE to their germ-line sequence frequently loses reactivity to self (150, 151). Various mechanisms, such as disordered ICOS signalling (152), reduced feedback from inhibitory FcgammaRIIB receptors (153, 154), or loss of T cell regulation (155, 156) can initiate GC autoimmunity, but Degn et al. (157) have reported that once triggered, autoreactive GCs are self-sustaining and may, with time, diversify (128), wherein the focus of the response gradually expands to incorporate autoantigens that were not initially targeted. Such diversification would potentially generate a broad panel of mutated autoreactive plasma cells that have high affinity for self and that reside long term in the bone marrow.

4.2 Innate B cells and reactivity to self

A large proportion of circulating autoantibody is thought to be a product of the innate B cell lineages (158), comprised of B1 cells (159), and to a lesser extent marginal zone B cells (160), that are responsible for the production of 'natural' antibody (NAb). Such antibody is characteristically

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composed of unswitched IgM isotype and is polyreactive, although what constitutes NAb continues to be debated (161). The innate B cell lineage is an evolutionary forerunner of the adaptive immune system, and appears to be relatively conserved from its first origins within cartilaginous fish (162). Critically, NAb is produced at birth from precursors within the fetal liver in a T-independent fashion, and without the presence of obvious exogenous antigen. Despite the polyreactive binding patterns, B1 cells express a restricted, self-reactive BCR repertoire, with approximately a third of the B1 population expressing a CDR3 sequence specific for phosphatidylcholine (163). Phosphatidylcholine – the major membrane phospholipid – is representative of a wider class of polyunsaturated fatty acids that are particularly prone to oxidative stress and their perioxidation results in the generation of oxidation specific epitopes (OSEs – reviewed in (164)). OSEs are expressed on dying cells and in inflammatory lesions (165), and their recognition by IgM NAb is thought to maintain homeostasis and prevent autoimmune disease. Natural antibodies also cross react against diverse moieties expressed on various microbes, such as Gram-negative bacteria, and hence provide an important first-line of defence against pathogens (162). Although generally protective, NAb responses can class switch (typically to IgG3), most likely in response to BCR ligation and concurrent intracellular toll like receptor (TLR) signalling (166), and may contribute to the pathogenesis of autoimmune disease. For example, natural anti-citrullinated protein antibodies (ACPAs) recognise various modified proteins, such as vimentin, type II collagen, and histones, in which arginine has been replaced by citrulline post-translationally (167). Circulating ACPAs are diagnostic for rheumatoid arthritis, and are frequently class-switched and hypermutated (168).

5. How do Transplant Associated Autoantibody Responses Effect Graft Rejection?

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Thus the breakdown in B cell tolerance to self is complex, with a variety of overlapping mechanisms responsible, but with some presumably dominant either in individual patients or in particular autoimmune disease states. Given the diverse patterns of humoral autoimmunity described in human transplant recipients, it seems similarly likely that different processes govern its generation in different patients or for different organs. Whether these different processes all impact to the same extent on graft rejection, and ultimately on graft outcome, is not known, and is an important area for further research, because of the potential to inform development of therapeutic strategies that target humoral autoimmunity in anticipation of improvements in graft outcome. In this section we consider how different autoantibody responses may contribute to allograft rejection.

5.1 The contribution of innate B cell humoral autoimmunity to allograft rejection

Although IgM B1 cell responses provide an immediate defence against a variety of pathogens (158, 162, 169-171), they also perform important homeostatic functions by binding OSEs, such as phosphorylcholine PC) and its degradation product, malondialdehyde (MDA), that are expressed by apoptotic cells, thereby promoting anti-inflammatory phagocytosis and preventing autoimmune disease development (165, 170, 172-174). It therefore seems unlikely that natural IgM antibody makes a meaningful contribution to graft rejection, and notably, in the See study, it was specifically the early development of IgG natural antibody that was associated with poorer long term kidney graft survival (71). Class-switched NAb responses are poorly understood (169, 175) - not least because a memory phenotype can develop without apparent requirement for either CD4 T cell help or BCR signalling (176) - and their role in homeostasis remains unclear (165, 177), with, if anything, the balance of evidence suggesting a protective role in prevention of native atherosclerosis (178-180). Against this, Tay et al have reported that transfer of purified IgG from atherosclerotic mice can provoke arterial disease in naïve mice (181). Although this study provides support for a pathogenic role for natural IgG responses in allograft vasculopathy, exactly how such responses influence graft rejection are not known. Natural IgG responses are predominantly composed of IgG1 and IgG3

isotypes and are thought to fix complement, which may explain the reported association in the See study between post-transplant IgG NAb production and increased complement C4d deposition in the transplanted kidney (71). As occurs, for example following caspase-dependent cleavage of vimentin (182) or myosin protein (183), 'stress' to the allograft by either surgical trauma or ischaemia reperfusion injury may provoke translocation of intracellular proteins as blebs to the cell surface (184-186), with either citrullination (typically to vimentin and / or myosin) or endothelial peroxidation generating epitopes for natural IgG recognition. Classical pathway complement activation from C1q recognition of bound IgG NAb could conceivably result in progressive graft damage through direct endothelial injury by the C5b-9 membrane attack complex (MAC) on graft endothelium, even when formed at sub-lytic levels (187-189).

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Complement activation in relation to bound IgG NAb may also mediate graft damage by augmenting signalling via pattern recognition receptors (PRR) within a variety of innate and adaptive immune cells. Within these cells, Toll like receptor (TLR – the prototypical PRR) signalling triggers intracellular NFκB signalling and induces expression of a variety of inflammatory genes. This leads to secretion of proinflammatory cytokines, neutrophil recruitment and maturation of antigen presenting cells, which ultimately potentiates many aspects of the adaptive alloimmune response (190-192). Experimental transplant models have confirmed the critical role that TLR signalling plays in transplant rejection (193-195). Immediately following transplantation, TLRs recognise Damage Associated Molecular Patterns (DAMPs) that are inevitably expressed as a consequence of donor brain death, cold ischaemia, ischaemia reperfusion injury, and innate allorecognition (196). A variety of DAMPs have been identified, and several, such as vimentin (197), myosin (198), and High Mobility Group Box protein-1 (HMGB1 (199)), are commonly targeted in humoral autoimmunity. For example, vimentin is now known to be a principal regulator of the NLRP3 inflammasome (200), and is upregulated within murine cardiac allografts undergoing chronic rejection (201). Stress-related citrullination of vimentin (202) and myosin (203), possibly in relation to release from the allograft within exosomes (204), will theoretically generate epitopes for recognition by natural ACPAs (although citrullination of vimentin is perhaps not required for autoantibody binding (41)). Many other DAMPS are similarly considered to represent OSEs for NAb engagement (205). It seems likely that NAb binding may further enhance DAMP signalling pathways, for example, by attracting complement C3b opsonin and C3-5a anaphylatoxins. Clinical evidence is lacking, but IgM NAb has been reported to be a critical initiator of murine intestinal IRI, with binding to myosin heavy chain associated with local complement activation (183, 206). This is in keeping with the wider body of experimental and clinical evidence suggesting a role for local complement activation in mediating IRI (reviewed in (207)).

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Assuming that potentiation of DAMP signalling is a major mechanism for how IgG NAb responses mediate poorer longer-term allograft survival, one would predict that detectable NAb is associated with both more severe IRI and with augmented T and B donor-specific alloimmunity. In support, Yang et al. have recently shown that pre-formed anti-LG3 antibody was associated with more severe delayed graft function (DGF - considered to be a manifestation of IRI), and that one year kidney graft function was poorer, but only for those patients with anti-LG3 antibody who experienced DGF (208). Yang also reported that passive transfer of anti-LG3 resulted in microvascular complement deposition and exacerbated murine renal IRI (77), mirroring findings in human kidney transplant recipients (209, 210). The relationship between IgG NAb and recipient alloimmunity is more difficult to assess, and it is particularly difficult to untangle whether NAb is simply an epiphenomenon, or does truly synergise with host cellular and humoral alloimmunity in effecting graft rejection. Thus, a number of studies have reported that post-transplant humoral autoimmunity is associated with the development of DSA (45, 46, 62), but fewer have identified an effector role independent from DSA,

let alone a possible synergistic impact (49, 53, 71). Perhaps most convincingly, Lefaucher and colleagues' study of 1845 renal transplant recipients (53) reported that the development of anti-AT1R autoantibody was an independent predictor for graft failure, and that seven year graft survival was poorer for patients who developed anti-AT1R reactivity and DSA (60.2%; 95% CI, 50.4–68.7) than those who either developed anti-AT1R (81.9%, 95% CI, 77.3–85.7) or detectable DSA (75.3%; 95% CI, 69.9–79.8) alone. Data from experimental transplant models that are manipulated either by passive transfer of autoimmune serum (34, 75, 204, 211) or pre-transplant immunisation with target autoantigen (27, 28, 30, 33, 212) have also suggested an independent role for autoantibody in graft rejection.

With regards encounter of foreign antigen, the innate B cell response is considered a primordial first line of defence against foreign antigen, rapidly supplanted by the adaptive humoral response. Thus it is perhaps surprising that such an immediately responsive system influences, and appears to continue to impact upon, graft survival many years after transplantation (53, 71). This possibly reflects early triggering or augmentation of other mechanisms that continue to effect rejection at late time points – most obviously adaptive alloimmunity - but whether IgG NAb can continue to directly mediate graft damage remains unclear. Evidently, some aspect of the transplant process (not necessarily antigen-specific BCR ligation) triggers differentiation and IgG NAb production from the innate B1 pool, but the subsequent phenotype and survival of these antibody secreting cells is unclear.

5.2 The contribution of adaptive B cell humoral autoimmunity to allograft rejection

The complexities of the more evolutionary advanced adaptive B2 B cell system make it difficult to establish where breakdown in B cell self-tolerance occurs, and, as discussed in Section 3, experimental and clinical studies of autoimmune disease have suggested that the dysregulation that ultimately triggers autoimmunity may occur at various stages in the B cell cycle, from central development, through to peripheral maturation, and thence to GC activation.

Breakdown in central or peripheral exclusion of autoreactive B cells following transplantation

Although B cell-intrinsic defects may break central tolerance and result in a greater proportion of autoreactive bone marrow emigrants (87), it is doubtful that similar congenital defects contribute to TAA, because, intuitively, it's an acquired process that's triggered by transplantation; in support, evidence linking prior autoimmunity to *de novo* TAA is lacking. One caveat to consider, however, is that the pro-inflammatory milieu associated with transplantation results in a loss of regulatory T cell suppression that normally prevents early breakdown in self-tolerance as immature B cells egress the bone marrow (213).

Although originating as a consequence of BCR ligation to self-antigen at the immature, transitional stage, evidence that the anergic B cell population contributes to autoimmune disease is, as discussed above, limited, and the role of anergic B cells in TAA is as yet unknown. However, one scenario with possible particular relevance is the use of B cell depleting agents (such as alemtuzumab or rituximab) as part of the induction regime at transplantation. The excess levels of BAFF that ensue (BAFF levels are partly controlled by B cell absorption (214)) would, upon B cell recovery, facilitate follicular entry and maturation of autoreactive transitional B cells that would otherwise be destined to become short-lived anergic B cells. Whether B cell depletion at induction is associated with rebound recipient autoantibody responses has not been assessed, but notably, late development of DSA has been reported (215, 216) in association with increased levels of circulating BAFF (216).

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In any event, because of reduced induction of Ebi2 and Irf4 due to suboptimal BCR signalling, autoreactive B cells that are 'rescued' from an anergic state are thought to be biased towards the GC pathway rather than to plasma cell differentiation – see below (102).

Breakdown in follicular B cell self-tolerance - Extrafollicular vs. Germinal Centre foci

With regards activation of follicular B2 B cells, the recent demonstration that non-mutated IgD^{+ve} CD27^{-ve} activated naïve B cells may contribute to SLE disease (113) raises the possibility that autoantibody generated predominantly within extrafollicular foci may contribute to transplant rejection. This is untested, and it is not immediately apparent how such characteristically transient responses (acknowledging that this tenet is not inviolate ((121, 217)) would impact upon long-term transplant outcomes. The activated naïve B cell subset that has been described in SLE is associated with acute flares in disease, and this cycle of remittance and relapse is not typically observed in clinical transplantation, with graft failure more resembling a culmination of chronic and insidious immune insult.

Instead, from the central role that GC humoral autoimmunity is thought to play in development of autoimmune disease (117), it seems more likely that the GC reaction is critical for effecting allograft damage. Our recent murine studies have examined the potential contribution of GC-generated autoantibody to chronic graft rejection (218-221). In the MHC class II mismatched 'bm12' to C57BL/6 strain combination – one of the most commonly studied models of chronic allograft rejection - we reported that bm12 cardiac allografts showed features of humoral vascular rejection, and that peptide-degenerate, graft-versus-host recognition of MHC class II on recipient C57BL/6 B cells by donor bm12 CD4 T cells that were passengers within the heart graft triggered widespread B cell activation (222), but that terminal plasma cell differentiation also required simultaneous BCR ligation (220). Thus cognate interaction between donor CD4 T cells and recipient B cells could provide help for, firstly and somewhat counterintuitively; generation of alloantibody directed against foreign determinants expressed on the surface of the donor T cell, and secondly; production of class-switched autoantibody directed against a wide panoply of self-antigen (as demonstrated by the heterogeneous staining patterns observed on assay with nuclear-antigen expressing HEp-2 cells). We hypothesised that the autoantibody was generated principally through rescue of anergic autoreactive B cells.

The impact of donor CD4 T cell interaction with host B cells on the tempo of chronic rejection was, at least in several murine models, profound, resulting in markedly accelerated graft rejection (220, 223). Whether passenger donor CD4 T cells can similarly influence clinical transplant outcomes is unknown, and notably, their effect in the murine systems was dependent upon avoidance of rapid killing by the recipient NK cell – hence graft rejection was not accelerated in completely MHC-mismatched strain combinations (220). However, because organ allocations systems are generally designed to maximise MHC compatibility, matching for killer cell immunoglobulin-like receptor (KIR) recognition is achieved in approximately 50% of deceased-donor human kidney transplants (224). Furthermore, increasing evidence, particularly from the field of *ex vivo* organ perfusion (223, 225, 226), suggests that substantial numbers of donor lymphocytes persist within the donor organ and are transferred to the recipient after implantation. In addition to the interaction with conventional B2 B cells, donor CD4 T cell transfer could plausibly contribute to host innate B cell activation, because although IgM NAb production is considered a T-independent phenomenon, the helper T cell requirements for Ig class-switching have not been defined. Of note, anti-nuclear autoantibody was assessed in our models by binding test sera to a HEp-2 human carcinoma cell line, and the same assay was used, with very similar binding patterns observed, in the initial Zorn studies that detailed development of polyreactive antibody responses in human kidney transplant recipients (64, 68).

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In our experiments, it is clear that GVH cognate recognition by donor bm12 CD4 T cells is the key initiator in breaking autoreactive B cell tolerance in the C57Bl/6 recipient, but our recent studies have highlighted that this by itself does not result in heart graft rejection, and that the recipient CD4 T cell population is also required, because this provides essential T_{FH} cell function for generating long-lasting GC autoimmunity (218). Most notably, GC activity did not develop in C57BL/6 recipients genetically deficient in SLAM-associated protein [(SAP) – a key ligand for T_{FH} cell differentiation (227)] and bm12 heart grafts survived indefinitely. Presumably, the initial interaction with the donor T cell primes, or licences (228), the autoreactive B cells for prolonged, SAP-dependent engagement with the recipient CD4 T cell that results in T_{FH} cell differentiation of the latter and seeding of the GC reaction (Figure 2).

Given the robust nature of the extrafollicular autoantibody response triggered by the bm12 allograft, it is perhaps surprising that a recipient GC reaction is so critical for graft rejection, and why other effector mechanisms, such as the CD8 T cell alloresponse, appear to make a minimal contribution. Nor is this observation confined to the bm12 model; a consistent finding in our recent studies of various murine models of chronic rejection is the presence at late time points of active GC allo- and auto- immunity (218, 220, 221, 223, 229, 230), indicating ongoing T_{FH} cell differentiation (231). There are several possible explanations for why GC humoral autoimmunity becomes such a prominent feature in chronic graft rejection, related to either direct effects of autoantibody on the graft or to indirect effects on ancillary mechanisms of rejection. With regards a direct impact, identified target autoantigens in TAA are often expressed specifically within the transplanted organ (e.g. cardiac myosin) and even if located intracellularly, are likely to be expressed on the endothelial surface in response to stress. Circulating autoantibody binding to exposed autoantigen may therefore mimic mechanisms that are thought to underpin the damage triggered by alloantibody recognition of endothelial MHC alloantigen. Fc-receptor ligation may provoke NK cell degranulation and an antibody-dependent cellular cytotoxicity (ADCC)-like response, thereby replicating the NK cell signature observed in the transcriptome of human renal transplant recipients with AMR (232). Autoantibody binding to the surface of the graft endothelial cell may alternatively trigger, as detailed for alloantibody ligation of surface MHC molecules (233-236), a number of intracellular signalling cascades, with the injury-repair cycles that are initiated culminating in allograft vasculopathy. As mentioned above, activation of complement, with formation of the C5-9b MAC may further contribute to endothelial damage and progression of allograft vasculopathy. Autoantibody generated from the GC reaction is likely to exacerbate all these mechanisms, both because somatically mutated antibody would bind target autoantigen with greater affinity, and because of the potential for the plasma cell product to be deposited in the bone marrow and produce autoantibody for many years. In support, our recent studies have reported strong complement C4d deposition within the chronically rejecting bm12 heart grafts, and an important role for NK cells in mediating progression of allograft vasculopathy (218).

GC autoimmunity may further contribute to chronic allograft rejection through another of its key attributes – epitope diversification. Rigorous control mechanisms generally ensure that autoreactive clones that spontaneously develop as a consequence of SHM within the dark zone undergo apoptosis from lack of selection in the light zone. However, stochasticity within the GC does enable rare, low-affinity variants to occasionally eventually dominate (237, 238), and once a GC response has been subverted to autoimmunity, the standard control mechanisms appear ineffective, and inter molecular epitope diversification can spread the response to encompass new target autoantigens (157). Interesting, C57Bl/6 recipients developed *de novo* IgG autoreactivity against vimentin autoantigen at late time points (~2 months) after challenge with a bm12 heart (218), possibly in

response to heightened expression of vimentin within the rejecting graft (201); this late diversification did not occur in recipients manipulated to prevent GC development.

The most likely means by which humoral autoimmunity indirectly influences allograft rejection is through augmentation of adaptive alloimmunity. Such augmentation may occur preferentially within the allograft. Recent evidence suggests that although adaptive T alloresponses are initiated by cognate encounter of graft alloantigen within secondary lymphoid tissue, further re-encounter with target alloantigen presented within the graft by professional antigen presenting cells (APCs) is required for full effector differentiation and graft rejection (239, 240). Clinical and experimental studies have highlighted that islands of ectopic or tertiary lymphoid tissue (TLT) are often detectable within chronically rejecting allografts (reviewed in (241, 242)), and we have previously described that the TLT found in bm12 heart allografts are predominantly composed of B cells and depict GC like characteristics (243). In the context of the effector mechanisms operating in this model, these TLTs are presumably autoreactive, and notwithstanding their precise specificity, the general pro-inflammatory milieu that the TLT islands create would be expected to favour effector differentiation of alloreactive T cells that have migrated into the allograft (239). T cell activation would be further supported by intra-graft complement activation (244-246) from binding of locally-produced autoantibody; Jane-Wit and colleagues have, for example, shown that deposition of MAC enhances the capacity of allograft endothelium to activate alloreactive CD4 T cells (244). Furthermore, TLTs may function autonomously from responses occurring simultaneously within conventional secondary lymphoid organs, and ectopic AID expression (243) and SHM may tailor the plasma cell output to reflect local conditions within the allograft, such that high affinity mutations for locally expressed autoantigen are favoured (247, 248). This may explain why B cells obtained from human heart transplants have acquired greater levels of somatic mutation than those in blood (249). Binding of exposed graft autoantigen by such high affinity variants would likely further enhance local T cell reactivation (Figure 3).

In addition to high affinity autoreactive plasma cells, GC reactions within intra-allograft TLT will generate autoreactive memory B cells that then reside within the graft and may also contribute to alloreactive T cell priming by acting as APCs (250). Although poor stimulators in a naïve state, once activated and upon Ig isotype switching, B cells can become the dominant antigen presenting cell for naïve T cell activation (251-255), and notably in a model of experimental allergic encephalomyelitis, are critical for presenting encephalitogenic peptide to pathogenic T cells within the CNS (256). B cell antigen presentation is limited to those antigens acquired through BCR internalisation – B cells have generally poor phagocytotic capability – but it is possible that in the complex environs of the allograft, the autoreactive B cell acquires alloantigen that is physically associated with its target autoantigen (257, 258).

Can humoral autoimmunity independently effect graft damage?

Thus the perennial question resurfaces whether autoantibody can independently mediate allograft rejection, or whether its impact is principally via augmenting conventional recipient alloimmune responses. To address this, we used parental to F1 hybrid or bone marrow chimeric models of chronic heart allograft rejection, in which the recipient was incapable of mounting conventional T and B cell alloresponses against the donor, but GVH recognition by donor passenger CD4 T cells within the allograft triggered autoantibody production. Such recipients rejected heart grafts with a similar tempo to that observed in wild-type recipients that harboured a functional alloimmune response, but with rejection again dependent upon the development of long-lasting GC humoral autoimmunity that was maintained by recipient T_{FH} cell differentiation (218). These experiments therefore suggest that humoral autoimmunity can autonomously effect graft rejection.

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Why in that case does humoral autoimmunity appear to damage the allograft selectively? This most plausibly reflects that, even in the absence of conventional alloimmunity directed against the donor, the allograft is subject to various injuries stemming from: the catecholamine storm and inflammatory cytokine upregulation related to donor brain death; prolonged cold storage and subsequent ischaemia reperfusion injury; and surgical trauma from organ implantation. Exposure of previously sequestered autoantigen, possibly as denatured neoantigen (182), allied to increased DAMP expression would create an environment whereby circulating autoantibody preferentially bound to allograft endothelium and triggered a variety of downstream effector responses. These injury responses may be compounded by concurrent 'innate allorecognition'; a recently described phenomenon in which monocytes responding to mismatched polymorphisms in signal regulatory protein alpha (SIRPα) undergo maturation to an inflammatory DC within the allograft (196, 259). SIRPα polymorphisms are however, unlikely to contribute to rejection of bm12 allografts by C57BL/6 recipients, because the bm12 donor is a naturally occurring mutant strain that differs from the C57BL/6 parent strain by 3 amino acids only in the classical MHC class II I-A locus, and yet GC humoral autoimmunity is the main effector mechanism of rejection.

6 Summary and Future Directions

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Recent clinical research has demonstrated that natural humoral immunity may have a deleterious impact on long-term transplant outcome. With regards adaptive B2 B cell responses, the detection of circulating T_{FH} cells is increasingly associated with development of anti-HLA antibody and poorer graft survival (260-263), suggesting that GC responses are important mediators of chronic rejection; a finding mirrored in experimental transplant studies by several groups (229, 264-266). Our recent murine findings highlight that the GC reaction may be similarly critical for autoantibody-mediated graft rejection. Exactly how autoantibody responses cause graft damage remains unclear, and while it is possible that innate and adaptive autoantibody responses effect graft rejection through different mechanisms, the possibility that both arms converge on the GC as a final pathway that leads to long-term production of high-affinity autoantibody is compelling, and would provide an explanation as to how relatively short-lived innate responses can influence long-term transplant outcomes. It is not known, however, if the IgG Nab response observed following transplantation can integrate into a GC response, and doing so could theoretically risk generating long-lasting autoantibody responses with high affinity for self. Against that, natural anti-phosphorylcholine antibodies have been reported to be affinity matured (267), suggestive of a GC reaction. Furthermore, following on from their initial transcriptomic analysis of human renal transplant biopsies that suggested that early development of chronic kidney disease was associated with an intrarenal innate B cell signature, Cippa et al. have demonstrated that murine renal IRI initially triggered a broadly reactive, innate B cell response, but that at late time points, an antigen-driven autoreactive GC-like response was evident within the kidney (268). Perhaps most convincingly, Hebert and colleagues have shown that injection of syngeneic 'apoptotic exosomes' initially triggered what was thought to be an innate IgG autoantibody response directed against LG3 perlecan (176), but which later spread to encompass anti-nuclear antigen autoreactivity, with evidence of a splenic GC response (76).

In summary, the increasing focus on humoral autoimmunity in graft rejection, and in particular, the long term follow-up of large numbers of human transplant patients, has provided strong evidence that humoral autoimmunity contributes to allograft rejection, but in doing so, has raised a further set of unanswered questions. The precise triggers for autoantibody production, and the extent to which these are influenced by either innate B cell responses to IRI or by GVH recognition by donor passenger lymphocytes, remain unclear. Most importantly, little is known about how humoral

autoimmunity contributes to the progression of chronic rejection and long-term transplant outcomes. This should be a key area of further study, with particular focus on how innate and adaptive humoral autoimmunity integrate in generating an effector response, and whether damage is mediated by systemically produced autoantibody or by modification of the transplant milieu from autoantibody produced locally within the graft. Answering these questions, likely by a combination of transcriptomic and proteomic analysis of circulating blood and transplant biopsy will provide a focus for translational research aimed at inhibiting effector autoantibody responses and improving transplant outcomes.

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Figure Legends

Figure 1: T-dependent B cell differentiation pathways

Upon antigen encounter, the B cell migrates to the T:B cell border (1), and upon 'cognate' interaction between the MHC class II peptide-complex of the B cell and the T cell receptor of the helper T cell (that has similarly migrated to the T:B cell border (2)), the B cell either forms short-lived extrafollicular foci within the red pulp (3), or migrates back to the B cell follicle to seed the germinal centre response (4). Composed classically of a dark zone, containing proliferating centroblasts, and a light zone in which centrocytes encounter target antigen presented by follicular dendritic cells (FDCs) and compete for help for specialised T follicular helper (T_{FH}) cells dark zones, the germinal centre produces long-lived plasma cells (LLPCs) and memory B cells.

Figure 2: Graft-Host-Recognition by passenger donor CD4 T cells triggers long-lived recipient Germinal centre responses.

'Peptide-degenerate' graft-versus-host recognition of MHC class II on recipient B cells by passenger donor CD4 T cells that migrate from the allograft triggers widespread B cell activation (1). Without help from recipient CD4 T cells, this results in formation of short-lived extrafollicular foci and production of anti-nuclear autoantibody (2). Activated B cells can alternatively 'license' recipient CD4 T cells, possibly at the T-B cell border as depicted (3), but this has not been confirmed experimentally. Licenced recipient T cells migrate with the B cell to form long-lasting autoreactive germinal centres, which support late epitope diversification to encompass, amongst other target antigens, anti-vimentin autoantibody responses. These late germinal centre responses can mediate the progression of allograft vasculopathy independently of other effector mechanisms.