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Beyond Adaptive Alloreactivity: Contribution of Innate B Cells to Allograft Inflammation and Rejection

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Abstract

Innate B cells are a heterogeneous group of cells that function in maintaining homeostatic levels of circulating natural antibodies and as the first line of defense against infections. Innate B-1 cells and marginal zone (MZ) B cells may relocate to lymphoid follicles, and differentiate into cytokine and antibody-secreting cells in T-independent and T-dependent manners. While MZ B cells are widely described in humans, the presence of B-1 cells is more controversial. Here, we review the basic features of the innate B cell subsets identified in mice and their equivalent in humans, as well as their potential roles in transplantation. We summarize the findings of Cascalho and colleagues on the unexpected protective role of TNF receptor superfamily member 13B (TNFRSF13B) in regulating circulating levels of protective natural IgM, and the studies by Zorn and colleagues on the potential pathogenic role for polyreactive innate B cells infiltrating allograft explants. Finally, we discuss our studies that took a transcriptomic approach to identify innate B cells infiltrating kidney allografts with antibody-mediated rejection (AMR) and to demonstrate that local antigens within the allograft together with inflammation may induce a loss of B cell tolerance.

INTRODUCTION

The mammalian immune system comprises the innate and adaptive arms; innate immunity senses pathogens through germline coded receptors to generate protective responses whereas the adaptive immune system uses clonal antigen receptors that are generated by gene rearrangements, to sense antigens. Overall, the two systems seem to be well compartmentalized in terms of cell subsets, with innate immunity driving adaptive immunity mediated by classical T and B cells, and innate T and B cells playing an important role that precedes, or is complementary to, adaptive immune responses[1–3]. Three major subsets of peripheral mature B cells have been identified, based primarily on data from mice: B2 follicular, marginal zone (MZ) B cells, and B-1 B cells (CD5+ B-1a and CD5- B-1b cells)[4](Table 1). Innate B1 and MZB produce germline encoded natural antibodies that spontaneous arise without known antigen exposure, and typically bind with low affinity to non-protein antigens such as phospholipids or carbohydrates expressed by pathogens and self-antigens[5,6]. In contrast, B2 cells are responsible for T-dependent class-switched and

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affinity-matured antibody responses, and they may also be costimulated by innate immune receptors[7].

While the most recognized role of B cells is the secretion of antibodies, it is not their only function. Activated B cells upregulate MHC Class II antigens and function as professional antigen-presenting cells (APCs) following their efficient capture and uptake of low concentrations of antigen with their B cell receptors (BCRs)[8]. B cells can also produce anti- and pro-inflammatory cytokines depending on their activation states[9]. Regulatory B cells secrete anti-inflammatory cytokines IL-10 or TGFβ–1, while effector B cell populations produce cytokines such as IL-2, IL-4, TNFα, IFNγ, and IL-12, and their roles in shaping T cell effector function and transplant rejection has been suggested[9,10]. This review will discuss the characteristics and functions of innate B cells in mice and humans and on their potential roles in allograft rejection.

Innate B cells

Two major innate B cell subsets have been identified in mice, namely the MZ and B-1 B cells. MZ B cells have classically been considered as exclusively residing in the marginal sinus of the spleen, but more recently have been identified in the subcapsular sinuses of mouse lymph nodes[11]. B-1 B cells are divided into $CD5^+$ B-1a, and $CD5^-$ B-1b, and they primarily reside in the peritoneal and pleural cavity, and are present in low numbers in the lymph nodes and spleen[12]. Both MZ B cells and B1 B cells typically produce low-affinity, broadly cross-reactive antibodies that provide early protection to particulate bacterial antigens[13]. MZ B cells are well-described in the human spleen, while the existence of human B-1 cells remains controversial[14,15].

MZ B cells.—MZ B cells ($IgM^{hi}IgD^{low}CD21^{hi}CD23^{-}CD1d^{hi}$) are fully mature innate B cells and account for 5% of splenic B cells in mice[16]. Like follicular B cells, murine MZ B cells continuously develop from transitional T2 B cells emerging from the bone marrow, and upon reaching the spleen MZ and specifically receiving Notch signals, the T2 B cells complete their differentiation into MZ B cells[17]. Compared to follicular B cells, surface IgM levels are higher and IgD levels are lower in MZ B cells. In addition, MZ B cells express high levels of CD21, CD1, and CD9, but low levels of CD23, CD5, and CD11b, consistent with their pre-activated state that requires lower activation thresholds, and their propensity to produce IgG and IgA upon class switch recombination[18–23]. Finally, human MZ B cells can undergo somatic hypermutation in the absence of immunization or infection at very early developmental phases, although the process by which this is achieved is not well understood[24].

Unlike follicular B cells which express monoreactive BCRs, MZ B cells express polyreactive BCRs that bind to multiple antigenic patterns, and high levels of Toll-like receptors (TLRs)[25]. The engagement of BCR and TLR with conserved pathogen-associated molecular patterns such as lipopolysaccharide (LPS) or peptidoglycan stimulates MZ B cells to initiate low-affinity antibody responses earlier than the high-affinity antibody production by follicular B cells. Belperron et al. reported that MZ B cells started secreting *Borrelia hermsii* specific IgM as early as 24h post-infection[26], as such, MZ B cells function as

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an important bridge between innate and adaptive immune responses. More recently, it has become appreciated that gut commensals can induce innate-like IgM memory B cells in both mice and humans, and that MZ B cells can enter germinal centers where they may acquire somatic mutations and emerge as IgM memory B cells[27–29].

In addition to their role as early antibody-producers, Attanavich and Kearney showed that MZ B cells are good antigen-presenting cells, and are able to induce the clonal expansion of antigen-specific T cells in vivo and in vitro[30]. When MZ B cells are depleted, the infection burden of *Borrelia burgdorferi* increases drastically due to decreased levels of *Borrelia burgdorferi* specific IgM and IgG. In addition, MZ B cells were responsible for *B. burgdorferi* specific $CD4^{+}$ T cell priming and early $CD4^{+}$-dependent IgG response [31]. Additionally, MZ B cells act as sensors for TLR ligands, and the in vivo stimulation of MZ B cells with TLR agonists leads to MZ B cell activation and accelerated antigen-specific IgM responses[32].

Human MZ B cells are phenotypically characterized as $IgM^{+}IgD^{+}CD21^{+}CD23^{-}CD1c^{+}CD27^{+}$ and comprise around 15–20% of splenic B cells and around 15% of B cells in peripheral blood. In addition, MZ B cells also inhabit the inner wall of the subcapsular sinus of lymph nodes, the epithelium of tonsillar crypts, and the subepithelial dome of intestinal Peyer's patches[24]. Their expression of CD21 and CD1c is indicative of MZ B cells responding to the complement fragment C3b and interacting with NK-T cells[33]. Furthermore, the expression of costimulatory and memory B cell marker; CD27, provides insight into the signals driving their differentiation into plasma cells [34,35]. The majority of human MZ B cells are somatically mutated even in infants suggesting that they may undergo pre-immune hypermutation[19,36]. Upon encounter with antigen, MZ B cells can undergo both T-independent pathways to generate antibodies specific for microbial polysaccharides, as well as T-dependent antibodies. Compared to follicular B2 cells, MZ B cells have a distinct mechanism of IgV gene repertoire diversification during ontogeny, a different pattern of IgV gene usage, fewer IgV gene mutations, a slower rate of accumulation of IgV gene mutations, and a lesser dependence on germinal centers and CD40L-expressing $CD4^{+}$ T cells[24]. Notably, a subpopulation of human cells with a transitional-to-MZ B cell phenotype is enriched for IL-10 production, a feature of regulatory, IL-10–producing B regulatory (Breg) cells[37].

B1 B cells—B-1 cells comprise a very small portion of total B cells in mice. They have high surface IgM, CD19 expression, low to no surface IgD, CD23, and B220, and can be either $CD5^{+}$ (B-1a) or $CD5^{-}$ (B-1b)[38,39]. B-1 cells are localized in the peritoneal cavity and pleural cavity as $CD11b^{+}$ B-1a cells, in the spleen and bone marrow as $CD11b^{-}$ B-1a, but they are barely detectable in the blood and lymph nodes[39–41]. Montecino-Rodriguez et al. reported that B-1a cells are derived from a unique $CD19^{+}$, $B220^{-}$ and Lin^{-} progenitor lineage found in fetal liver and fetal bone marrow[42], and their development largely depends on IL-7Rα and Flt-3 ligand[43]. B-1a B cells maintain their number by self-renewal in adulthood[44], and while B-1 progenitor cells can be found in the adult bone marrow, their contribution to the maintenance of B-1a cell numbers in the periphery is still an enigma[42,45].

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The main function of B-1a cells in the innate immune system is the spontaneous secretion of natural IgM, thereby maintaining resting IgM levels in the body[46]. These natural IgM form the first line of humoral defense against infection, and it is estimated that 80 % of serum IgM is derived from B-1a B cells[47]. Antibodies secreted by B-1a B cells contain little or no somatic hypermutation and minimal N-region addition[48], and their repertoire is skewed towards low affinity and polyreactive, and target bacterial antigens, apoptotic cells, and oxidized lipids [47]. B-1a B cells have also been shown to be autoreactive, so they can participate in the clearance of cell debris and thus preventing uncontrolled immune activation and further tissue damage[49]. In addition, Zimecki et al. reported that B-1a B cells can present antigens to $CD4^+$ T cells[50], while Zhong et al. showed that B-1a B cells induced T cells to express IL-17 and IFN-γ more effectively than follicular B cells[51]. Finally, B-1a B cells can produce IL-10, GM-CSF, and IL-3 and when stimulated with LPS and may also have an immunoregulatory role[52].

B-1b cells, on the other hand, have been studied far less than B1a B cells. B1b cells are thought to derive from adult bone marrow precursors, in contrast to $CD5^+$ B-1a cells that are largely fetal and neonatal derived[53]. Two non-mutually exclusive models may explain the presence of B1a and B1b B cells: a “division of labor” model where each subset preferentially responds to different infection, or degree of self-antigen-mediated stimulation of the BCR and/or additional costimulatory signals that determines the responding B1 subset. Finally, despite early reports, the existence, significance, and phenotypic identifiers of human B1 cells, especially in the blood or secondary lymphoid organs, remain in dispute[14,54]. Nevertheless, Rodriguez-Zhurbenko et al. reported that approximately 2% of circulating $CD19^+$ B cells are $CD19^+CD20^+CD27^+CD38^{low/int}CD43^+$ B-1 B cells[55]. Another a recent study by Cordero et al. reported on the presence of B cells in the thymus of human neonates (<7 days after birth) that display a unique innate-like B cell gene signature[56]. Furthermore, some of these B cells differentiate into $CD138^+$ plasma cells that secreted antibodies with a reactivity profile consistent with natural antibodies, prompting the authors to speculate that these intrathymic B cells and plasma cells develop without exposure to a foreign antigen, and are responsible for generating the repertoire of protective natural antibodies in newborn humans. Whether these innate-like B cells derived from the B1 lineage or are MZ B cells remains to be clarified.

Innate B cells in transplant rejection

We will review three main lines of investigations whereby the role of innate B cells and the antibodies they produced might play a role in clinical transplant rejection: the findings of Cascalho and colleagues on the protective role of TNF receptor superfamily member 13B (TNFRSF13B), the studies by Zorn and colleagues on polyreactive innate B cells infiltrating allograft explants, and our studies that took a transcriptomic approach to demonstrate innate B cells infiltrating kidney allografts with antibody-mediated rejection (AMR).

TNFRSF13B polymorphisms control T-independent antibody responses and graft outcomes.—TNF receptor superfamily member 13B (TNFRSF13B) encodes the transmembrane activator and CAML interactor (TACI) expressed by B cells, and it binds to three ligands: a proliferation induced ligand (APRIL), B cell activation factor (BAFF),

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and calcium modulating ligand (CAML). In addition, TACI binds heparan sulfate chains associated with syndecan-2 and–4 cores and potentiates signaling by Toll-Like receptors (TLRs)[57]. Initial insights into the role of TACI in regulating humoral immunity came from the observations that TACI-deficient mice have fewer plasma cells in secondary lymphoid organs and the bone marrow, and lower concentrations of IgM, IgA, and IgG in serum[58]. Furthermore, mutations in TNFRSF13B are associated with common variable immunodeficiency in humans[59,60]. It is well characterized that TACI signaling drives the expression of the transcription factor, BLIMP-1, which is essential for the development of plasma cells. Paradoxically, TACI knockout mice mount proficient antibody responses and antibody-mediated defenses against pathogenic bacteria, which we now understand is due to the necessity of TACI inducing BLIMP-1 only for T-independent B cell responses, whereas in T-dependent antibody responses, T cell help generates CD40 and IL21/STAT3 signals that bypass the need for TACI to induce BLIMP-1 expression[57].

TNFRSF13B is one of the most polymorphic genes in humans, leading to Cascalho and colleagues to test whether TNFRSF13B alleles might determine the magnitude of innate B cell responses and graft outcome[61]. Their study showed that human kidney transplant recipients with missense mutations *in TNFRSF13B* comprised 33% of those with antibody-mediated rejection (AMR) but < 6% of those with stable graft function had *TNFRSF13B* missense mutations. These observations raised the possibility that WT levels of TACI were protective, and conversely, reduced TACI resulted in more aggressive alloreactivity. To define the mechanisms for these unexpected observations, de Mattos Barbosa et al. used a mouse cardiac transplant model to show that allografts in *Tnfrsf13b*-mutant recipients underwent early and severe AMR[61]. The increased propensity for developing AMR in *Tnfrsf13b*-deficient mice was not caused by increased alloantibodies but rather, was associated with decreased “natural” IgM production.

Natural IgM was postulated by de Mattos Barbaso et al. to be protective because of its polyreactivity results in their binding to circulating C3b, thereby preventing C3b from become activated/fixed on the membrane of eukaryotic cellular targets[61]. In *Tnfrsf13b*-deficient recipients, compromised complement regulation as a result of low levels of circulating “natural” IgM resulted in increased complement deposition in heart allografts as well as in the recipient’s kidneys. Thus, WT TACI regulated innate B cell functions by limiting complement-associated inflammation, contrary to some common variants of *Tnfrsf13b* genes that intensified inflammatory responses. From an evolutionary perspective, these variants may be maintained as low levels of “natural” antibodies help clear microbial infections, but allow inadvertent tissue injury to ensue as in the case of transplant rejection. Indeed, de Mattos Barbosa et al. showed that transplant recipients with *TNFRSF13B* missense mutations had significantly lower concentrations of IgM natural antibodies and C3 in serum compared to transplant recipients with WT alleles, and an increased risk of AMR[61]. These elegant studies point to a novel and unexpectedly protective role for natural IgM produced by T-independent B cells in transplantation, and the control that TACI exerts on the levels of circulating protective natural IgM.

Polyreactive B cells and Antibodies promote transplant rejection.—Natural antibodies have been classically defined as antibodies encoded by germline immunoglobulin

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genes and produced in individuals without overt antigen sensitization[6]. Recognized examples of natural antibodies are those that are highly specific to ABO blood group antigens or carbohydrate xenoantigens, such as α-(1,3)-galactose (α-Gal) and N-glycolylneuraminic acid (Neu5Gc). These antibodies are driven, at least in part, by gut microbiota and are responsible for precipitating hyperacute rejection in the context of ABO incompatibility or xenotransplantation, respectively[62–64]. Another class of natural antibodies is characterized by polyreactivity, which is defined as the ability of a single antibody to bind to multiple and apparently unrelated antigenic structures[65]. In the laboratory, polyreactive antibodies are defined by their ability to bind to a panel of antigens that typically include bacterial antigens such as phosphorylcholine and lipopolysaccharide (LPS), viral proteins such as hemagglutinin, proteins that are targets of autoimmune diseases such as insulin and double or single-stranded DNA, and products generated by oxidative stress, such as malondialdehyde (MDA)[66]. It should be noted that polyreactivity can only be definitively demonstrated using monoclonal antibodies (mAbs); in contrast, serum with broad reactivity may be explained by the presence of an extended repertoire of antigen-specific antibodies or a limited repertoire of polyreactive antibodies.

Seminal observations by Zorn and colleagues raised the hypothesis of a potential role of polyreactive B cells, and the antibodies they produce, in human transplant rejection [67]. Because of the lack of definitive markers for innate human B cells, the approach they took was to isolate B cells infiltrating rejected allografts and interrogate the specificity of those B cells. In their earliest studies, Porcheray et al. investigated B cells isolated from a kidney explanted because of suspected pyelonephritis, and diagnosed with acute cellular rejection superimposed on chronic rejection[68]. Graft infiltrating B cells were immortalized by EBV transformation, and 102 clones were examined for HLA-reactivity as well as for polyreactivity to double-stranded DNA (dsDNA), whole protein extract from human embryonic kidney cell line (HEK-293), and insulin. One clone was found to be reactive to multiple HLA Class I alleles, and 7 clones were polyreactive, including the clone that was reactive to HLA. Thus, ~7% of the B cells examined were polyreactive, a frequency that is not significantly enriched over 16.7%–26.3% of circulating polyreactive B cells in healthy individuals[69]. Indeed, Porcheray et al. went on to show that one of the clones with polyreactivity was highly expanded in the blood, and likely contributed to the polyreactive antibodies detected in the serum[68]. Thus, despite numerous caveats, this study established proof of principle for the expansion of polyreactive B cells in a rejected kidney explant.

Supporting data on the accumulation of polyreactive B cells in rejecting allografts came from studies of heart allograft explants with cardiac allograft vasculopathy (CAV), which is characterized by intimal thickening and lumen narrowing of the main coronary arteries. CAV is a major cause of heart graft loss, and the majority of these grafts present with immune infiltrates of T and B cell clusters together with plasma cells and macrophages. Chatterjee et al. generated 102 EBV-immortalized B-cell clones from three explanted heart grafts with CAV and reported that while none were HLA-reactive, approximately half of the clones were polyreactive, namely reactive to apoptotic cells, MDA, insulin, dsDNA, LPS, cardiolipin, and/or apoptotic cells[70]. Overall, the rates of polyreactivity were considerably higher than observed in the blood of healthy individuals and thus provided convincing evidence of an accumulation of polyreactive B cells around the coronary arteries

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of allografts with CAV. Approximately half of the clones were IgM and the rest were IgG, and the percentage of mutated Ig sequences was significantly higher in B cells from the graft compared to the blood. The authors characterized these as "natural" antibodies, although it is unclear if those polyreactive IgM and IgG antibodies were in fact produced by innate B1 cells infiltrating the graft or by B-2 cells. This uncertainty is due to the lack of consensus on definitive phenotypes of B1 B cells in humans, and the inability to lineage trace in a way that is possible in mice.

Because of the technical challenges involved in studying innate B cells in human transplant recipients and linking their presence to graft outcomes, clinical studies have focused on investigating the presence of polyreactive antibodies and correlating them with graft outcomes[71,72]. The caveat of these studies is that serum polyreactivity may be the result of a wide repertoire of antigen-specific antibodies or a limited repertoire of broadly reactive antibodies. Nevertheless, judicious selection of antigenic targets, by using apoptotic cells or the oxidized epitope, malondialdehyde, allowed See et al. to conclude increased levels of natural polyreactive antibodies in the serum from patients on ventricular assist devices (VAD) compared to those who were not[73]. More recently, Zorn and colleagues assessed natural antibodies from a retrospective cohort of 635 patients who received a kidney transplant[72]. Defining natural antibodies as a 50% increase in reactivity to malondialdehyde, they showed that the presence of anti-malondialdehyde antibodies is a significant risk factor for graft loss (hazard ratio, 2.68; 95% confidence interval, 1.49 to 4.82; P=0.001). While the authors label these as natural antibodies based on reactivity, whether anti-malondialdehyde IgG detected in transplant recipients are the product of innate B cells, or whether B2 cells can also produce antibodies with these reactivities, remains to be definitively demonstrated.

Innate-like B cells driving immunity in kidney allografts undergoing AMR—The role of innate B cells and autoantibodies in allograft rejection was recently assessed by Asano et al. by applying single-cell transcriptomics to B cells isolated from fresh kidney biopsies diagnosed with active or chronic AMR, and their BCRs were expressed as human IgG1 mAbs and specificity assessed [74]. In their single-cell transcriptomics dataset, they compared intrarenal B cells with activated tonsillar B cells from tonsillectomy samples. Overall, intrarenal and tonsillar B cells had similar ratios of class-switched (IgA, IgE, IgG) and unswitched (IgD and IgM) B cells. Unswitched IgM^+ B cells in rejected renal allograft and tonsil tissue were transcriptionally similar, whereas, class-switched intrarenal B cells were transcriptionally distinct from class-switched tonsillar B cells.

Pathway enrichment analysis of the differentially expressed genes by intrarenal compared to tonsillar B cells was related to innate receptors and signaling pathways which included the pattern recognition receptors, NLRP1, NOD1, TLR2, and TLR7, the interferon (IFN)-related pathways, and several cytokine ligands and receptors, including *IL15*, *TNFRSF1B*, and *TNFRSF13B*[74]. Interestingly, the transcriptional repressors, *BCL6 and BACH2*, that are critical to the differentiation into GC B cells were preferentially expressed in class-switched tonsillar B cells but downregulated in intrarenal B cells. One gene that was notably upregulated in intrarenal B cells was *AHNAK*, a scaffolding protein that is upregulated in murine peritoneal B1a and B1b cells [75]. By conducting a more detailed analysis of the human counterparts of murine AHNAK covariate genes, the authors concluded that AHNAK

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covariate genes were enriched in intrarenal B cells. Furthermore, by analyzing 2,855 human genes that were orthologs to murine genes enriched in peritoneal B1 cells, they additionally demonstrated an enrichment of this gene set in intrarenal B cells. Thus the majority of intrarenal B cells accumulating in the context of human kidney transplant rejection have a transcriptome that is enriched for mouse orthologs of innate B cells from mouse, and were classified as a unique subset of human innate “Bin” cells.

Consistent with the innate-like transcriptional phenotype, class-switched intrarenal B cells preferentially expressed the innate cytokine IL-15. Immunofluorescence microscopy confirmed that infiltrating B and other immune cells in rejected renal allografts expressed IL-15, while IL-15RA was moderately expressed in both tonsil and renal graft tissue. These data suggested that IL-15 secreted by B cells might be captured by tubular cells for presentation to immune cells; indeed, IL-15 has been reported to upregulate activation molecules and the costimulatory molecule CD80 on B-1a cells, and to prompt an anti-inflammatory to pro-inflammatory shift in the B-1a cells[76]. Furthermore, the abundance of IL-15 in rejected renal allografts and improved graft survival following the antagonization of IL-15 have been recognized in previous studies [77,78].

The specificity of the innate-like B cells was also examined by cloning the BCRs from intrarenal and tonsillar B cells and expressing them HEK293 as human IgG1 mAbs (Fig 1A). A total of 105 BCRs expressed as recombinant mAbs was initially assessed for HLA reactivity. Although 15% of mAbs showed multiple HLA reactivity, predominantly towards HLA-C, they were not donor-specific even in patients with circulating donor-specific antibodies. Furthermore, epitope sharing could not explain the broad HLA reactivity, and instead the mAb binding to HLA was shown to be due to low-affinity polyreactivity, as addition of serum completely abrogated HLA-binding. Thus, mAb binding to HLA was likely a technical artifact, and their low affinity binding unlikely to have physiological relevance.

Asano et al. then focused on assessing the clonal relationships among the sequenced BCRs where they noted a limited number of shared clonal families in Bin cells from most patients, and that many of the plasma cells were clonally related to each other and to intrarenal B cells[74]. These findings raised the possibility that local self-antigens were driving in situ selection and differentiation of intrarenal B cells into antibody-secreting cells and plasma cells, instead of low-affinity polyreactivity[74,79–81]. Consistent with this notion, 76% of mAbs from clonally expanded plasma cells had HEp-2 reactivity. Because the differentiation into plasma cells requires high-affinity interactions between the BCR and their ligand, Asano et al. went on to identify their potential antigenic targets. Three mAbs with antinuclear reactivity and from different clonal families were used in immunoprecipitation assays with HEp-2 cell lysates. Tandem mass spectrometry of the immunoprecipitates identified that the nucleolar antigens, Ki67 and HEATR as the top hits targeted by the innate-like B cells. Collectively those observations showed that a breach of Bin cell self-tolerance and strong selection for self-antigens can occur in the kidney of renal allografts during rejection.

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In a subsequent analysis of 28 highly mutated mAbs expressed from Bin cells isolated from 6 patients, 21% (from 5 different patients) showed reactivity to inflamed kidney tissue. Importantly, these mAbs expressed with a FLAG tag to reduce non-specific detection showed specific nuclear or perinuclear binding, and the majority of mAbs bound to limited cell types, often tubules[74]. Taken together, the extensive analysis by Asano et al. showed that kidney infiltrating B cells have innate B cell phenotypes, secrete the pro-inflammatory cytokine, IL-15, and exhibit a Type 1 interferon signature[74]. Importantly, a small fraction of these cells respond to local antigens and undergo clonal expansion and differentiation into plasma cells, whereas the majority of graft-infiltrating B cells were likely to have been trapped in bystander B cells (Fig 1B). Future studies are needed to clarify how autoreactive B cells and antibodies contribute to rejection.

CONCLUSION

There are substantial gaps in our understanding of the immunobiology biology of innate B cells in solid organ transplantation, including what drives their loss of self-tolerance and whether their roles extend beyond the antibodies they produce. The function of the antibodies in transplantation produced by these innate B cells requires clarification, but are likely to differ from donor-specific antibodies since the former tends to recognize intracellular targets whereas the latter recognizes cell surface HLA molecules. Finally, while innate B cells have long been considered as a bridge that integrate innate and adaptive immunity, new data suggest that they may play a role in sustaining pro-inflammatory states and thus serve as a biomarker for tissue injury and chronic rejection. While clinical studies may provide associative insights into potential role, ultimately, answers to the fundamental questions of the roles innate B cells and the antibodies they produce in allograft rejection will require ex vivo experimentation, as well as in vivo investigations in pre-clinical models where innate B cell subsets are better defined.

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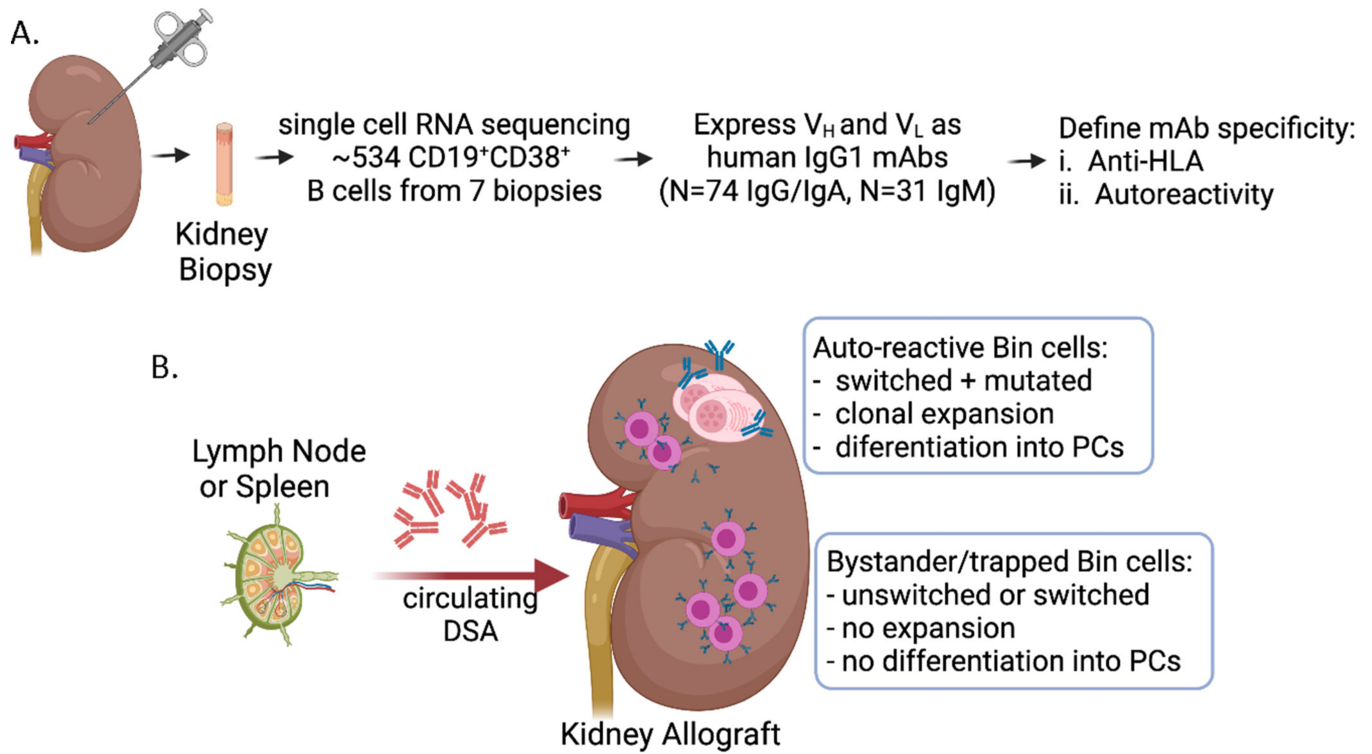

Fig 1.

(A) Experimental approach taken by Asano et al. to isolate intrarenal B cells from biopsies taken from kidneys diagnosed with active or chronic AMR. (B) Cartoon depicting the two major groups of Bin cells infiltrating kidney allografts, while donor-specific antibodies are likely to be generated in the lymph nodes and spleen.

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Table 1.

Features of B cell subsets.

B cell type	T cell-dependent response	T cell-independent response	Somatic hypermutation	Class switching
Follicular	High[82,83]	Low[83]	+[82]	Yes[82,84]
B1	Low[83]	High[83]	+[85]	Yes[84,85]
Marginal Zone	Low[82,83]	High[83]	+[82]	Yes[82,84,85]