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New Insights on Innate B cell immunity in transplantation

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Abstract

Innate B cells and natural antibodies (Nabs) have been extensively studied in normal physiological conditions as well as several diseases. However, their significance in the context of ABO compatible solid organ transplantation is only emerging. The present review summarizes recent studies exploring these often neglected innate immune elements in situations related to sensitization and clinical graft rejection. A focus is placed on class-switched IgG Nabs that develop amidst inflammation, rather than IgM Nabs abundant at the steady state, as new evidence point to their implication in serum reactivity to HLA and kidney graft failure. The involvement of innate B cells in the pathophysiology of CAV is also presented. Lastly, we discuss key questions that need answering to understand whether and innate B cell immunity contributes to the outcome of solid organ transplantation.

1. Introduction

Humoral immunity to allografts is traditionally divided in two main arms. **The adaptive B cell immunity** involves the production of high-affinity antibodies reactive to antigens displayed on donor cells through a process requiring T cell help. These are mostly antibodies specific to donor MHC class I and class II molecules, also called donor-specific antibodies (DSA), considered to be the major pathogenic elements in antibody-mediated rejection(1, 2). Autoantibodies to self-antigens such as vimentin, cardiac myosin and angiotensin 1 type II receptor are also presumed to originate from T cell-dependent class-switched follicular B cell responses (3–8). The second arm corresponds to **innate B cell immunity** and encompasses pre-existing antibodies to ABO blood group antigens as well as xenoantibodies such as IgM reactive to Gal α-1, 3 Gal(9). Xenoantibodies share a number of characteristics with “natural antibodies” (Nabs) a category of immunoglobulins described in animals and humans in both health and disease(10). Here, we will focus on Nabs and summarize recent observations supporting a contribution of these antibodies in the outcome of kidney and heart transplantation. We will also discuss the implication of Nabs in the assessment of serum reactivity in transplant recipients.

Nabs were described more than 50 years ago(11), yet for the larger community of immunologists, these antibodies are still ill-defined and their function unclear. Perhaps the

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main hurdle comes from their lack of specificity, which is also their primary characteristic. Most Nabs are polyreactive in that they react to multiple, seemingly unrelated, antigenic structures, including determinants on apoptotic cells, oxidation–specific epitopes, tumor-associated carbohydrates (mucins), components of senescent red blood cells and various self-proteins(10). The reactivity of a monoclonal Nab, M2.3, generated from a healthy donor is shown in Figure 1 as an example. This monoclonal Nab reacts to LPS, insulin, double-stranded DNA, single–stranded DNA as well as permeabilized Hep-2 cells (figure 1A, B). The polyreactivity of M2.3, however, is best illustrated when used to probe proteins separated from an epithelial cell lysate by electrophoresis on a polyacrylamide gel. As presented in Figure 1C, a great number of proteins are recognized by this single monoclonal Nab, highlighting its broadly cross-reactive nature. M2.3 also reacts to late apoptotic cells, a hallmark of serum Nabs (Figure 1D). While Nabs are primarily IgM they can also be detected as IgG in the serum of healthy individuals, albeit at a much lower concentration. In certain pathological conditions, however, the level of IgG Nabs can markedly increase(12–14). It is assumed that this increase results from class-switch recombination (CSR) of a pre-existing pool of innate B cells towards IgG-secreting plasmablasts or plasma cells as illustrated in Figure 2. The remainder of this review will focus on IgG Nabs and their implication in the immunology of solid organ transplantation.

2. Nabs and serum reactivity to HLA

In recent years, Luminex-based assays have become the test of choice to detect anti-HLA antibodies in patient serum(15). The sensitivity of these assays surpasses that of previous methods. Moreover, the use of beads coated with single HLA molecules allows the precise determination of the serum reactivity to specific antigens. The flip side of such sensitivity is that Luminex tests can also detect non-specific antibodies, resulting in false-positive readings(16). Distinguishing signal from noise can often be challenging. For lack of a better strategy, most centers rely on mean fluorescence intensity (MFI) as a way to evaluate the significance of the measurements. Using this technique it is not exceptional to detect antibodies reactive to HLA other than that of the donor in the serum of transplant recipients. These antibodies are called non-donor-specific HLA antibodies (NDSA)(17, 18) and are usually assumed to be cross-reactive to several HLA. In itself, a certain level of cross-reactivity is expected due to the high sequence homology and the presence of immunogenic “public” epitopes shared among HLA (19, 20). Gao et al. proposed an alternate mechanism that may substantially contribute to serum reactivity to HLA observed with Luminex assays(21). The authors derived Nabs-producing B cell clones from the peripheral blood and graft infiltrates of kidney transplant recipients. Assessing the reactivity of the monoclonal antibodies secreted by these cells, they observed that a small percentage also reacted to SAB used in Luminex(21). These monoclonal antibodies display the characteristic polyreactive profile of Nabs, i.e. reactivity to LPS, apoptotic cells, dsDNA and other antigenic structures, and also reacted to numerous HLA class I coated on SAB. Each clone’s reactivity pattern was unique and covered up to 45 distinct molecules(21). Remarkably, the complex reactivity profiles could not be explained by the presence of epitopes shared among the different antigens. Thus far, no polyreactive monoclonal Nab binding to class II molecules was found, suggesting that they may be less frequent than HLA class I-reactive clones. The study also

revealed that serum adsorption on apoptotic cells reduced the overall serum reactivity to class I molecules, MICA and, to a lesser extent, HLA class II molecules for some kidney transplant recipients. This report provides supportive evidence that serum Nabs cross-reactive to HLA on SAB, are detectable by Luminex and can significantly contribute to the observed anti-HLA reactivity. For example, the presence of Nabs could explain the detection of NDSA in the absence of prior immunizing events(22, 23). Based on this observation, Nabs should be taken into account when interpreting complex results from Luminex assays or other methods used to assess serum anti-HLA antibodies.

3. Nabs, antibody-mediated rejection and kidney graft loss

Until recently, Nabs had only been implicated in graft rejection in the context of xenotransplantation or ABO incompatible transplantation. In both situations, preformed Nabs in the host react vigorously to xeno- or allogeneic carbohydrate determinants on the donor endothelium, triggering a hyperacute form of rejection. In 2013, a cross-sectional case control study by Porcheray et al., using retrospective samples from two small cohorts, reported higher serum levels of IgG Nabs in kidney transplant recipients with ABMR(24). In this study, Nabs were detected using flow cytometry by their capacity to bind apoptotic cells. Moreover, these Nabs could activate complement, leading to C4d deposition on target cells in vitro, suggesting a similar function in vivo. This was arguably the first report of Nabs having potentially contributed to ABMR. A subsequent single-center study by the same group showed that higher levels of IgG Nabs pre-transplant were associated with reduced graft survival after kidney transplantation in non-sensitized recipients(25). Even though measurements were carried out using pre-transplant serum, the effect on graft loss was only apparent after one year post-transplant, implying a mechanism distinct from that seen in cases of hyperacute rejection. In this latter report, graft loss was also associated with ABMR. Remarkably, IgG Nabs were almost exclusively of the IgG1 and IgG3 subclasses and could activate complement in vitro, corroborating previous observations(24, 25).

A follow-up study was conducted by the same team at Columbia University in collaboration with Necker Hospital in Paris, France, to investigate Nabs IgG in a larger cohort of over 600 kidney transplant recipients. In this most recent study, retrospective samples collected pre-transplant and either at 1 year post-transplant or during the first year post-transplant in cases with complications, were tested blindly for the presence of Nabs reactive to the generic oxidized antigen malondialdehyde (MDA)(26). Results revealed a highly significant association between the increase in Nabs between pre- and post-transplant and graft loss. All patients included in this cohort had either protocol biopsy at 1 year or clinically-indicated biopsy during the first year, allowing the analysis of histological signs of transplant rejection. Increase in Nabs during the first year was accompanied with graft deterioration and histological lesions reminiscent of ABMR. A significant association was found between Nabs and higher BANFF scores for 5 out of the 6 most relevant histological markers of rejection: microvascular inflammation (glomerular and peritubular capillaritis), C4d deposition, transplant glomerulopathy, interstitial inflammation and tubulitis as well as arteriosclerosis(26). The significance of Nabs in graft loss was further highlighted by the multivariable analysis that isolated DSA and Nabs as two independent risk factors associated with graft loss in this series. The effect of Nabs and DSA was then evaluated separately.

Nabs increase alone, i.e. in the absence of DSA, was significantly associated with reduced graft survival. However, the highest incidence of graft loss was observed in patients with both DSA and Nabs, suggesting an additive effect. Remarkably, the development of Nabs in patients without DSA correlated with higher BANFF scores for 4 out of 6 histological markers of graft rejection, including C4d deposition on the graft endothelium. Taken as a whole, the three studies described above strongly support an important role of Nabs in the pathophysiology of ABMR(24–26). Most specifically, it is likely that Nabs IgG1 and IgG3 activate complement in vivo and contribute to C4d deposition alongside DSA. Based on these findings, it would be particularly interesting to determine the predictive value of these antibodies for ABMR and their possible usefulness in risk stratification. Determining the kinetics of Nabs development following kidney transplantation in relation to DSA would greatly facilitate this task.

4. Nabs sensitization following ventricular-assist device implantation

Ventricular–assist devices (VAD) are now routinely used to correct cardiac circulation in patients with advanced heart failure. VAD can serve as bridge to transplantation or destination therapy. However, long-term VAD use increases the risk for multiple complications such as gastrointestinal bleeding, stroke or pump thrombosis(27). VAD also triggers the production of anti-HLA antibodies by an unknown mechanism(28–30). Investigating this immune sensitization phenomenon, See et al. observed a sharp increase in serum IgG Nabs levels following VAD implantation and before allotransplantation(31). Virtually all patients included in this study reached high-level IgG Nabs within 6 to 12 months post-VAD. Such profound effect likely results from a broad activation of innate-like B cells and their differentiation into IgG-producing plasma cells as depicted in Figure 2. The retrospective study by See et al. could not determine whether Nabs development resulted in sensitization to HLA. Nevertheless, an association between Nabs levels pre-transplant and primary graft dysfunction was found in this cohort, suggesting that Nabs, or the inflammatory reaction associated with their generation, may have predisposed transplant recipients to this life-threatening complication. Since Nabs have been involved in atherosclerosis(32, 33), it is also very plausible that these antibodies play a part in VAD complications. Additional studies are needed to clarify this potential role.

5. Innate-like B cells in cardiac allograft vasculopathy

Composite immune infiltrates are frequent in rejected kidney and cardiac allografts during rejection(34–44). A large B cell component can almost always be detected in these infiltrates, even in the context of acute T cell-mediated rejection(45–47). As determined by immunochemistry, B cell clusters are invariably adjacent to T cells and frequently intertwined with macrophages. In these infiltrates, B cells display either a memory phenotype or a class-switched IgG-secreting plasma cell phenotype(37, 43). In heart transplant recipients, these infiltrates have been described in the context of cardiac allograft vasculopathy (CAV) by independent research groups, including ours(37, 42, 48). Recently, we reported on the reactivity profile of such graft-infiltrating B cells. More than 100 B cell clones were isolated from 3 freshly explanted cardiac grafts with CAV and immortalized in vitro by EBV transformation(48). In all 3 cases, between 46 to 67% of B cell clones derived

from the graft tissue secreted monoclonal Nabs characterized by their reactivity to a combination of LPS, DNA, MDA, apoptotic cells or cardiolipin(48). This study was the first to uncover the reactivity of cardiac allograft-infiltrating B cells. The main observation contradicts the widely accepted notion that most infiltrating lymphocytes during rejection are allospecific. A predominance of polyreactive B cells presents a paradoxical picture where the innate immunity component appears to outweigh the adaptive, more specific element of the response. Innate immunity had already been known to play a part in all graft rejection processes but its contribution may have largely been underestimated. Whether this pattern is found in all types of rejection or only specific to chronic, slow progressing forms like CAV remains to be determined. B cells are also documented in kidney and liver rejection cases. While the environment may arguably vary for different organs, it is plausible that the immunological contexts may still be similar to that of heart transplants during CAV. Graft-infiltrating B cells in these situations may therefore also be part of the innate immunity. It is important to note that, to date, the function these B cells exert in situ is still unknown.

6. Unanswered questions

Collectively, the studies described in the previous sections and recapped in Figure 2 (blue boxes) portray innate B cells and IgG Nabs in an unusual light, as important immune elements in host interactions with solid organ grafts. Notwithstanding, several critical questions, also included in Figure 2 (red boxes), still await answers. These questions are discussed below:

6.1 Mechanisms of generation of IgG Nabs

The origin of serum IgG Nabs is still undefined. A likely scenario is that innate B cells, constitutively secreting IgM Nabs at the steady state, would undergo CSR in response to the inflammatory milieu (Figure 2). This reaction would generate plasma cells producing IgG Nabs either locally or systemically, with the same reactivity profile as conventional IgM Nabs. The kinetics of IgG Nabs and especially their fluctuation in the serum in a matter of months rather than years, suggests that plasma cells or even plasmablasts producing these antibodies are short-lived compared to conventional bone-marrow-resident long-lived plasma cells. As evidenced in two separate studies, CSR in innate B cells appear to be primarily if not exclusively directed towards IgG1 and IgG3, the two complement-activating subclasses of human IgG(25, 31). The orientation of CSR towards specific IgG subclasses is known to be influenced by both the B cell stimulus and the cytokine milieu. It will be particularly important to identify the precise stimulatory combination triggering innate B cell differentiation into Nabs-secreting cells.

6.2 Nabs reactivity spectrum

As illustrated in Figure 1C, a single monoclonal Nab can recognize a large number of proteins present in a cell lysate. Further, Nabs often recognize apparently unrelated antigenic structures such as nucleic acids, lipids and carbohydrates. The molecular principle underlying such polyreactivity is still a matter of debate. Dr. Notkins proposed that the antigen-binding sites of Nabs benefit from a higher degree of flexibility, allowing a wider

range of conformation than that of monospecific antibodies(49). An alternative view contends that Nabs recognize the same determinants (glycan, other adducts) decorating different proteins or molecules. Additional studies are needed to elucidate this point. Another important point: because of their polyreactivity, it is also virtually impossible to determine with confidence what Nabs bind to in vivo. For instance, the fact that Nabs react to insulin by ELISA does not imply that they can also bind this hormone in the blood. The same is true for Nabs reacting to apoptotic cells. There is little, if any, hard evidence that Nabs actually bind apoptotic cells in vivo. However challenging, new studies are now needed to identify the true targets of Nabs in graft rejection. Answering this question would also provide crucial pointers as to what the function of these antibodies may be (see below).

6.3 Function of IgG Nabs

A large body of literature describes the function of IgM Nabs. These antibodies form an important “first line of defense” against microbial infections. They can neutralize viruses, facilitate their uptake by phagocytes, kill bacteria through complement activation and mediate destruction of pathogen by antibody-dependent cellular cytotoxicity(10, 50). IgM Nabs also have essential immunoregulatory properties (review in(51)). In contrast, the function of IgG Nabs is less established, likely due to their low abundance in the serum of healthy individuals. A recent study revealed how IgG Nabs also contribute to the natural protection against bacteria with the help of lectins(50). Remarkably, this function does not appear to depend on the reactivity of the IgG Nabs as they primarily form a bridge between ficolin-opsonized bacteria and phagocytes(50, 52). In systemic lupus erythematosus, IgG Nabs produced at higher levels than in normal physiological conditions, are associated with neuronal damage(14). Additionally, polyreactive IgG Nabs binding to citrullinated proteins produced by B cells present in the synovial fluid, have been implicated in the pathophysiology of rheumatoid arthritis (RA)(53). While these instances highlight the pathogenic potential of IgG Nabs, they do not easily point to their role in graft rejection. Identifying the mechanisms whereby IgG Nabs directly contribute to graft injury is critical to fully demonstrate their pathogenicity.

6.4 Antibody-independent function of innate B cells in graft rejection

B cells have many biological activities aside from antibody secretion (see review in(54)). In light of their abundance in situ amidst rejection, it is highly probable that CD20+ innate B cells exert functions distinct from that of neighboring Nabs-secreting CD20-CD138+plasma cells. These functions, however, remain largely unknown. Three main hypotheses are considered: 1) Innate B cells activated through the recognition of danger-associated molecular patterns (DAMPs), produce pro-inflammatory cytokines and chemokines enhancing the local immune reaction. 2) These B cells uptake and present antigens to infiltrating T cells. Because of their polyreactive BCR, innate B cells could potentially uptake a large spectrum of antigens. 3) Innate B cells could also have immunomodulatory capacities identical to or aligned with that of IL-10-producing Bregs(55). Future studies will uncover the exact biological properties of these cells and indicate whether they can be considered as therapeutic targets for the prevention or treatment of rejection.

7. Conclusion

In conclusion, Nabs and innate B cells are an integrative part of B cell immunity that has long been neglected when studying rejection of ABO compatible allotransplants. Yet, evidence is accumulating that this component is far more significant than originally thought. How these elements interact with other immune cells and contribute to the alloresponse awaits further investigation.

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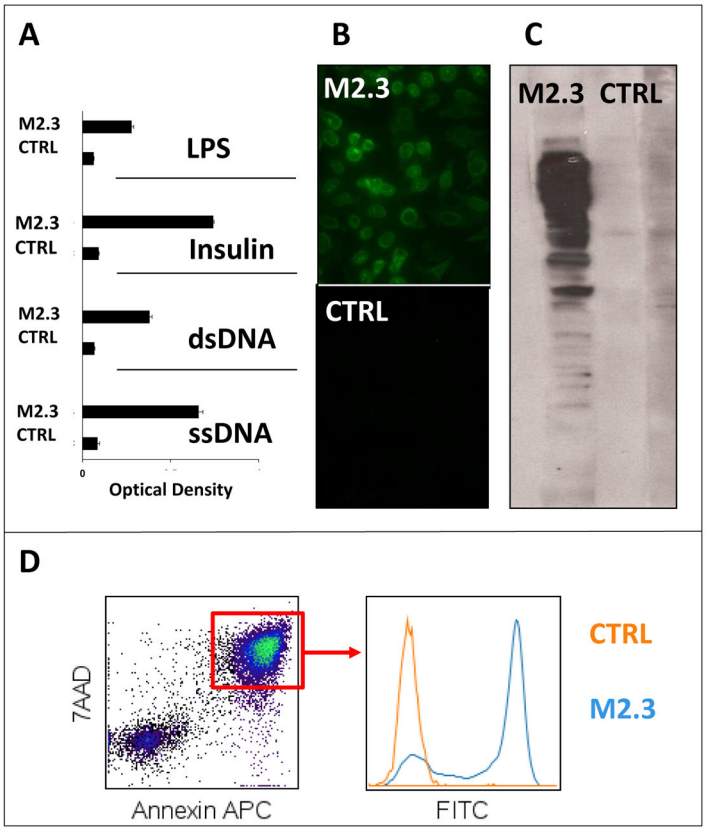

Figure 1. Reactivity profile of a monoclonal IgM Nab derived from a healthy donor

A) Reactivity of a IgM Nab M2.3 and control IgM to LPS, insulin, dsDNA and ssDNA was assessed by ELISA. **B)** M2.3 reactivity to Help-2 cells by indirect immunofluorescence. **C)** M2.3 and control IgM were used to probe HEK293 Lysates in Western Blotting assay. **D)** Reactivity of IgM Nab M2.3 and control IgM to apoptotic Jurkat cells assessed by flow cytometry.

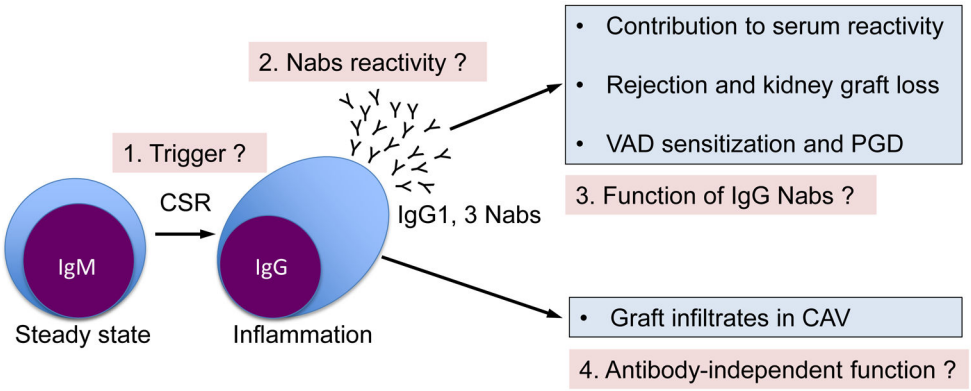

Figure 2. Contribution of Nabs to transplantation and unanswered questions

Innate polyreactive B cells undergo CSR in inflammatory conditions and produce IgG Nabs contributing to serum reactivity, kidney graft loss and sensitization post-VAD (blue boxes). Innate B cells also infiltrate cardiac allografts during CAV (blue boxes). Key unanswered questions about innate B cells and IgG Nabs are indicated in red boxes.