HLA-specific memory B cells : the missing link?

Karahan, G.E.

Chapter 3

Detecting the humoral alloimmune response: We need more than serum antibody screening

Gonca E. Karahan[1], Frans H. J. Claas[1] and Sebastiaan Heidt[1]

[1]Department of Immunohematology and Blood Transfusion,
Leiden University Medical Center, Leiden, the Netherlands

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ABSTRACT

Whereas many techniques exist to detect human leukocyte antigen (HLA) antibodies in the sera of immunized individuals, assays to detect and quantify HLA-specific B cells are only just emerging. The need for such assays is becoming clear, as in some patients HLA-specific memory B cells have been shown to be present in the absence of the accompanying serum HLA antibodies. Because HLA-specific B cells in the peripheral blood of immunized individuals are present at only a very low frequency, assays with high sensitivity are required. In this review, we discuss the currently available methods to detect and/or quantify HLA-specific B cells, as well as their promises and limitations. We also discuss scenarios in which quantification of HLA-specific B cells may be of additional value, besides classical serum HLA antibody detection.

INTRODUCTION

The humoral immune system is increasingly recognized as being an important player in the immune response towards transplanted organs. Central in the humoral immune system is the B cell, which finds its origin in the bone marrow. Once mature, antigen recognition can trigger naïve B cells to differentiate into antibody producing plasma cells or memory B cells. In the setting of organ transplantation, the antigens recognized are mostly HLA, although development of non-HLA antibodies has been described as well (1-3). The destructive capacity of alloantibodies was already recognized in the 1960s because of the occurrence of hyperacute rejection in many immunized kidney allograft recipients (4). Although the problem of hyperacute allograft rejection has virtually been eliminated by the introduction of cross-match techniques (5) and detailed pre-transplant antibody analysis (6), *de novo* alloantibody production post-transplantation is likely to be involved in most other types of allograft rejection (7-12).

Importantly, besides differentiating into alloantibody-producing plasma cells, increasing evidence suggests that B cells also play a role as antigen presenting cells during T cell mediated rejection. In a murine cardiac transplantation model, the selective absence of major histocompatibility complex (MHC) class II on B cells resulted in prolonged graft survival (13). Moreover, in clinical transplantation it has been shown that B cells are present in allografts undergoing T cell mediated rejection, in the absence of alloantibodies or complement deposition (14, 15). In addition, B cells with regulatory properties have been described (16, 17). In animal models, regulatory B cells have been shown to be able to induce long-term graft survival (18, 19). Importantly, B cells with regulatory properties may be involved in the induction or maintenance of clinical operational tolerance (20, 21). Whether antigen-specificity plays any role in regulation mediated by B cells remains to be determined.

Novel insights into the impact of the humoral immune response on the outcome of organ transplantation have resulted in a renewed interest in B cell research. However, despite the availability of many tools to study alloantibodies and phenotypical characteristics of B cells, reliable assays for analysis of alloantigen-specific B cells are only just emerging. This review will provide a comprehensive overview of the current techniques to study HLA-specific B cells and discuss their potential clinical application. Because most work have been performed in the field of kidney transplantation, we will focus on the renal transplant setting and will briefly discuss the utility of memory B cell techniques in other transplant settings.

ANTIBODY PROFILE OF *EX VIVO* ACTIVATED B CELLS

Luminex single antigen bead (SAB) assays are used routinely in many laboratories to determine the fine specificity of HLA antibodies in the sera of immunized individuals. This sensitive way of antibody detection is obviously not restricted to serum samples. Perry and colleagues were the first to describe single antigen flow bead analysis of concentrated supernatants from cultures of enriched $CD138^+$ plasma cells that had been isolated from the bone marrow of sensitized individuals (22). In these assays, the specificities found in the culture supernatants greatly overlapped with the specificities found in serum, supporting the notion that serum antibodies are mainly produced by bone marrow-residing plasma cells.

Both antibody-producing plasma cells as well as long-lived memory B cells originate from activated naïve B cells that have undergone class switch and somatic hypermutation in germinal centers upon recognition of cognate antigen (23, 24). Whereas plasma cells are antibody production factories residing in bone marrow, memory B cells circulate through secondary lymphoid organs and peripheral blood. Upon secondary encounter with the same antigen, memory B cells can rapidly respond and become plasma cells, instantly producing high affinity, class-switched antibodies. Whereas it is clear that circulating antibodies are mainly produced by plasma cells, it remains uncertain whether serum antibody levels and frequencies of memory B cells correlate well for most antigens (25). Studies on humoral responses against viral antigens have yielded contradictory results (26-31), indicating the importance of considering the memory B cell compartment alongside serum antibody levels. Memory B cells can be activated *in vitro* to become antibody producing cells, allowing for studying memory B cell specificity and frequency, as has been extensively shown by Lanzavecchia and colleagues (26, 32).

Han *et al*. described a method to determine HLA antibody specificities of *ex vivo* activated B cells (33). After CD40-driven polyclonal B cell activation, supernatants were concentrated and assayed by Luminex SAB assays. In 13 out of 16 transplant recipients tested, they found donor-specific antibodies (DSA) in B cell supernatants. A total of 50 DSA were detected, of which 35 matched those found in serum, and 11 DSA were detected in serum, but not in the supernatant of activated B cells. The latter may be due to limited sampling of B cells from the periphery or low precursor frequencies. Interestingly, 4 specificities were exclusively detected in the supernatant of activated B cells. These may potentially represent antibodies that were absorbed by the graft and thereby led to negative results in serum DSA tests (34, 35). However, other explanations are also possible. A recent study by Snanoudj *et al*. describes HLA antibody levels in supernatants of activated B cells from patients awaiting a kidney transplant, some after rejection of a previous transplant (Snanoudj *et al*., in press). Also here, a proportion of antibodies found in the supernatants were not detected in the serum. Because in the majority of patients no transplant was left *in situ* or no previous transplant had been performed at all, antibody absorption can be ruled out. This leaves the

possibility that memory B cells may be present in the circulation in the absence of antibody production of the same specificity.

We have performed similar experiments in which supernatants from isolated B cells, activated in a CD40-driven fashion, were tested for the presence of HLA antibodies alongside serum samples.

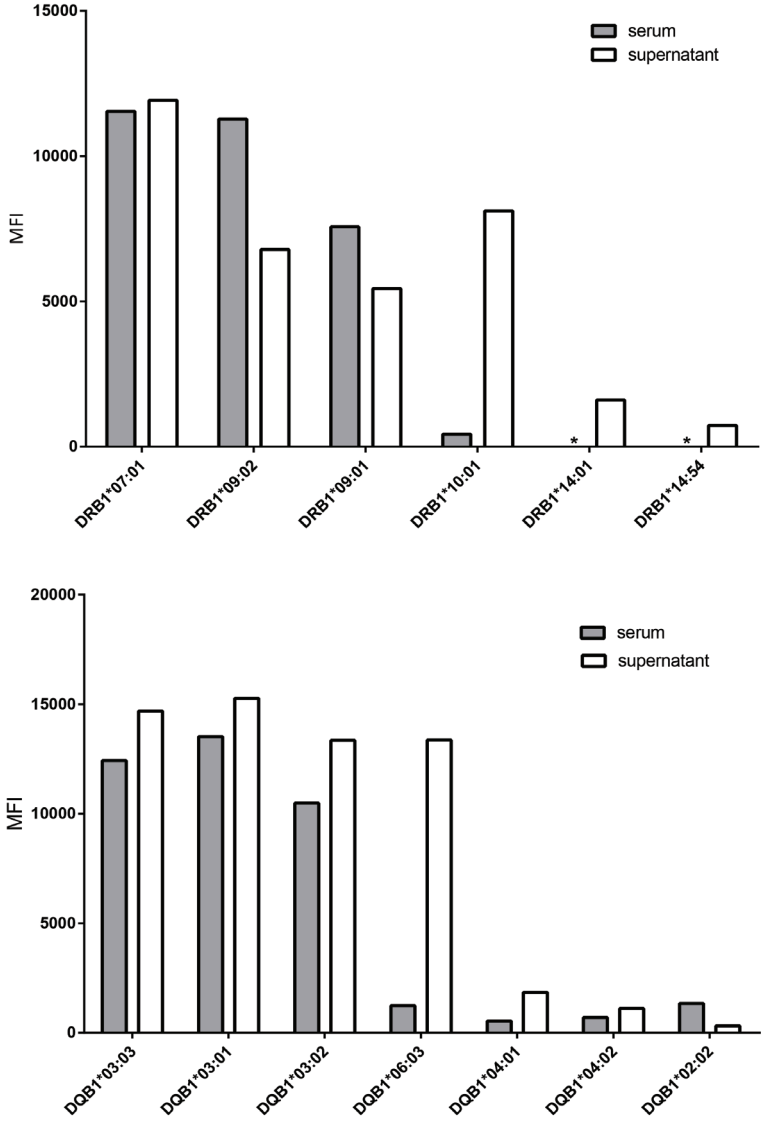

Figure 1. HLA class II antibody specificities in serum and B cell culture supernatants detected by Luminex SAB assay. *B cells isolated from the peripheral blood of an immunized patient awaiting a kidney transplant were polyclonally activated. Ten-fold concentrated supernatants from these cultures were tested for presence of HLA class II antibodies, as well as the sera from the same bleeding date. Among the antibodies detected, HLA-DRB1*10:01 and –DQB1*06:03 were detected in the B cell culture supernatants, whereas these specificities were undetectable in the serum. This patient had no detectable HLA class I antibodies in the serum, nor in the B cell culture supernatant. MFI: mean fluorescence intensity, *: No antibody detected.*

Figure 1 shows an example of an immunized patient on the waiting list for a kidney transplant of whom we tested the serum for HLA antibody specificities, as well as the supernatant of polyclonally activated B cells from the same bleeding date. Several HLA class II antibodies were detected in both the serum and B cell culture supernatant. Most strikingly, despite the absence of HLA-DRB1*10:01 and -DQB1*06:03 antibodies in the serum, the B cell culture supernatants showed strong positive reactions for these HLA specificities as detected by Luminex SAB assay. This clearly shows that memory B cells towards certain HLA antigens can be present, despite undetectable levels of serum antibodies towards these antigens. Potentially, such memory B cells may become activated if a transplant is performed with a donor organ expressing these mismatched antigens. This is illustrated by a study of Zachary and colleagues who described a cohort of kidney transplant recipients (KTR) that had circulating HLA-specific memory B cells in the absence of HLA antibodies pre-transplant (36). In 13 out of 16 patients (81%), they observed HLA antibody formation with specificities corresponding to those pre-existing memory B cells within 2 months after transplantation.

Obviously, alternative techniques exist to determine antibody specificities in both serum and cell culture supernatants, such as the enzyme-linked immunosorbent assay (ELISA) or complement-dependent cytotoxicity (CDC) assay, both of which Mulder and colleagues have used previously to detect HLA-specificity of B cells cultured in limiting dilution (discussed below) (37). However, the high level of sensitivity and specificity makes Luminex SAB analysis particularly useful for comparison of serum and culture supernatant antibody reactivity. Clearly, while using Luminex SAB analysis of B cell culture supernatants is useful to determine the HLA antibody profile of the peripheral memory B cell pool, it does not provide information on the frequency of HLA-specific memory B cells. Therefore, assays that can be used to quantify the HLA-specific B cell compartment would provide an important addition to this test.

FLOW CYTOMETRY BASED ASSAYS FOR HLA-SPECIFIC B CELL QUANTIFICATION

The introduction of HLA tetramer technology has revolutionized the way in which antigen-specific T cells can be quantified. For this purpose, immunodominant peptides are loaded into HLA molecules of interest, allowing for precise determination of antigen-specific $CD8^+$ T cell frequencies by flow cytometry (38). Besides analysis of T cells responsive against, for example, viral or tumor-associated peptides, HLA tetramers can also be used to study alloreactive T cells (39). Because HLA-specific B cells bind epitopes on foreign HLA molecules in their native confirmation by means of their B cell receptor (BCR), HLA tetramers could potentially also be used for quantification of alloreactive B cells.

Mulder and colleagues were the first to show that HLA tetramers could indeed be used for detecting, quantifying and isolating HLA-specific B cells (40). When human B cell hybridomas which specifically produce anti-HLA monoclonal antibodies (mAbs) of defined specificity (41), were incubated with tetrameric HLA complexes, a clear tetramer-positive cell

population could be detected by flow cytometry, showing proof of principle. Moreover, HLA-specific B cells from pregnancy-immunized individuals, isolated by cell sorting after tetramer staining, could be activated to produce HLA-specific antibodies.

Zachary and colleagues followed up on this work by using tetramer staining to quantify HLA-specific B cells in KTR (42, 43). In their initial studies, B cell frequencies directed towards single HLA specificities were found to consist up to 5.5% of $CD19^+$ B cells (42). Importantly, antibody-negative, but tetramer-positive individuals were more prone to produce antibodies of the tetramer specificity early after transplantation compared to tetramer-low or-negative individuals, indicating that the presence of memory B cells in absence of serum HLA antibodies may give rise to rapid antibody formation post-transplant (43). These findings have been extended in a recent report, where HLA-specific B cell frequencies in sensitized kidney transplant recipients who were antibody negative at time of transplantation were determined by tetramer staining. In patients who had not been treated with rituximab induction therapy, the presence of pre-transplant HLA-specific B cells was associated with post-transplant antibody production to the tetramer antigen tested (36). Subsequent B cell reconstitution after rituximab appeared to be accompanied by a delayed return of donor-specific memory B cells compared to third party HLA-specific B cells (44).

A potential caveat of B cell identification by HLA tetramers is binding of B cells to streptavidin-phycoerythrin, leading to non-specific staining of B cells (40, 45). This potentially results in an overestimation of alloreactive B cell frequencies. Because up to a staggering 6% of total $CD19^+$ B cells have been described to bind HLA tetramers of single specificity in patients without the presence of circulating HLA antibodies (36), this possibility should certainly not be ruled out. This technical issue may be overcome by immunomagnetic depletion with streptavidin beads prior to staining (40). Alternatively, one may use identical tetramers conjugated to 2 different fluorochromes as advocated by Chen and colleagues, who used tetramer staining in an experimental animal setting of B cell alloreactivity (46).

ELISPOT ASSAYS FOR QUANTIFYING HLA CLASS I-SPECIFIC B CELLS

Tetramer-based B cell staining is a straightforward means of quantifying HLA-specific B cells. However, for T cells, it has previously been shown that flow cytometric detection of low level responses is less sensitive than enzyme-linked immunosorbent spot (ELISPOT) assays (47). It is likely that for low-level B cell responses the same holds true, although this needs to be formally established. Furthermore, without further sorting and *in vitro* activation of the tetramer stained cells, the isotype and antibody producing capacity of the HLA-specific B cells remains unknown. To overcome these issues, alternative strategies for HLA-specific B cell quantification have been established. One of the first techniques published for estimating the precursor frequency of B cells with HLA specificity was established in our laboratory (37). For this B cell precursor frequency (BCPF) assay, supernatants from polyclonally activated B cells cultured in limiting dilution fashion were tested for the presence of HLA-specific B cells, allowing

for calculation of the HLA-specific B cell precursor frequency. At the time of development of this particular assay, the CD40L-driven B-cell activation protocol applied included a poorly defined supplement of cytokines from phytohaemagglutinin activated T cells, which precluded standardization that would normally be required for a clinical test.

The ELISPOT platform is characterized by its high sensitivity (47) and may therefore be particularly useful for detecting low-frequency (HLA-specific) B cells. The first report on applying ELISPOT assays in the setting of humoral alloimmunity was by Fan *et al*. who used ABO-specific ELISPOT assays to detect blood group tolerance in the setting of infant heart transplantation (48). By using this assay, the authors could show that tolerance in this setting was caused by elimination of blood group-specific B cells.

Subsequently, Perry *et al*. developed an ELISPOT assay for the quantification of bone marrow-residing plasma cells producing HLA antibodies (22). Here, enriched plasma cells from bone marrow aspirates were directly plated onto HLA-coated plates in the presence of PHA and exogenous cytokines. Whereas antibody-secreting cells were detected in bone marrow aspirates from immunized individuals, no antibody secreting plasma cells were found in peripheral blood from the same individuals, confirming that the vast majority of antibody producing cells reside in the bone marrow. Although this approach is very informative with respect to the presence or absence of antibody secreting plasma cells, bone marrow aspirates will not be a regularly available cell source for recipients of solid organ transplants. In contrast, peripheral blood can be routinely analyzed, and harbors memory B cells (25, 49). However, detection of HLA-specific memory B cells from the periphery has different requirements in comparison to plasma cells, most notably because B cells require short-term *in vitro* preactivation to produce antibodies.

By combining the ability of HLA-specific antibodies to bind to synthetic HLA molecules and polyclonal B cell activation in a CD40-driven culture system, we were able to develop the first HLA-specific memory B cell ELISPOT assay (50). For this assay, isolated B cells from peripheral blood are precultured with either L-CD40L cells or recombinant αCD40 mAb in combination with a cytokine cocktail consisting of IL-2, IL-10, IL-21, and CpG DNA (51). This activation protocol solely leads to antigen-specific IgG spot formation in the memory B cell population, and is therefore a suitable tool to quantify pre-existing memory B cells (52). Moreover, by CDR3 fragment analysis, we were able to show that there was no change in B cell polyclonality due to *in vitro* activation (52).

After activation, B cells are transferred to ELISPOT plates that have been precoated with streptavidin, followed by biotinylated synthetic HLA class I molecules (Figure 2). We could demonstrate, by using B cell hybridomas, that these synthetic HLA molecules allowed for the detection of all HLA-antibody producing cells of corresponding specificity, while no spots were detected when testing HLA-antibody producing cells of other specificity. In both pregnancy-immunized individuals, as well as sensitized patients awaiting a kidney transplant, we could detect HLA class I-specific memory B cells in the circulation.

All of the aforementioned techniques use recombinant HLA molecules for detecting either HLA-specific B cells directly or the antibodies they produce. These recombinant HLA molecules are generally stabilized by a single peptide in the peptide-binding groove. Our group has previously shown that peptide selectivity exists for certain HLA-specific monoclonal antibodies to bind to HLA molecules (53), potentially influencing the sensitivity of assays using recombinant HLA molecules. However, data from the group of Zachary, and our own data on the level of polyclonal B cell responses suggest that in a polyclonal response towards foreign HLA, peptide selectivity of single clones is not visible due to the activity of the remaining clones (42, 50).

Regardless, one may still argue that using actual donor HLA molecules for the detection of donor-specific memory B cells will be more clinically relevant. Recently, Lynch *et al*. made the first step towards detecting memory B cells directed at the full spectrum of donor HLA class I molecules (54). For the detection of donor-specific HLA class I-specific memory B cells in ELISPOT format, they isolated donor fibroblasts, which were subsequently cultured as monolayers. Autologous and third party fibroblasts were used as negative controls. Once confluent, polyclonally activated B cells were added to these fibroblast monolayers. By applying this method to a cohort of nine KTR that were HLA antibody negative at time of transplant, the authors showed an increase in donor-specific B cells in all patients. This occurred despite the absence of circulating HLA-specific antibodies after transplantation and provides a novel insight on the possible magnitude of the humoral alloimmune response after clinical transplantation.

Although of interest to get a better understanding of the humoral alloimmune response, the fibroblast-based assay is likely not useful for regular clinical use since it requires an extensive culture period of allogeneic and autologous fibroblasts and appears to suffer from rather high background signals.

ELISPOT ASSAYS FOR QUANTIFYING HLA CLASS II-SPECIFIC B CELLS

All current assays focusing on quantifying the number of HLA-specific B cells have been limited to determining levels of HLA class I-specific B cells. However, the majority of antibodies developing after transplantation appear to be directed against HLA class II molecules (55-57). Therefore, we have recently developed an ELISPOT assay enabling the detection and quantification of HLA class II-specific memory B cells in the peripheral blood of sensitized individuals (Karahan *et al.*, submitted). For this assay, similar to the HLA class I-specific ELISPOT, isolated B cells are pre-cultured with a CD40-driven polyclonal activation cocktail. Activated B cells are then transferred to anti-human IgG mAb-coated ELISPOT plates after which HLA class II-specific antibody secreting memory cells are detected by biotinylated HLA class II molecules (Figure 2).

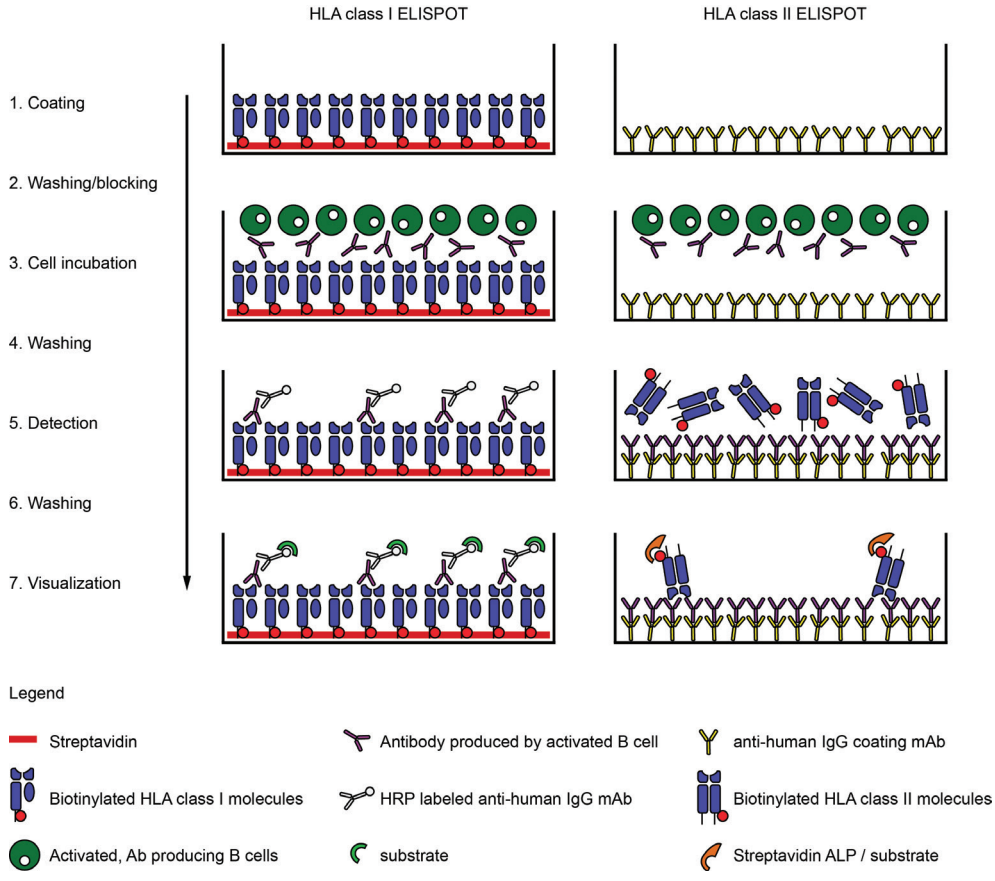

Figure 2. Schematic overview of HLA class I- and HLA class II-specific memory B cell ELISPOT assays adapted from Heidt et al. *(89). (Left panel) ELISPOT plates are coated with streptavidin followed by incubation of biotinylated HLA class I molecules to saturate the plate with the HLA molecules of interest. After blocking of the ELISPOT plate, activated, antibody producing B cells are added. After an overnight incubation, unbound antibodies and the B cells are washed away, leaving bound antibodies that are the footprints of HLA-class I antibody-producing cells. Bound antibodies are detected by an HRP-labeled anti-human IgG mAb. Finally, using a substrate, each HLA class I antibody producing cell can be visualized as a single spot. (Right panel) ELISPOT plates are coated with anti-human IgG mAb. After blocking of the ELISPOT plate, activated, antibody producing B cells are added. After an overnight incubation, cells are washed away and leaving the antibodies produced by the activated B cells bound to the plate. Among these antibodies captured in the plate, the ones that are HLA-specific can be detected by adding biotinylated HLA class II molecules. Finally, using streptavidin-ALP and a proper substrate, each HLA class II antibody-producing cell can be visualized as a single spot. HRP indicates horse radish peroxidase; ALP, alkaline phosphatase; IgG, immunoglobulin G.*

Using this method in peripheral blood samples of pregnancy-immunized women, we found memory B cell frequencies ranging from 25 to 756 per 10^6 B cells, specific for the immunizing paternal HLA class II molecules. We did not detect any spots in non-immunized males, which confirmed that spots detected in pregnancy-immunized women were produced by memory B cells.

Based on the very sensitive and practical ELISPOT platform, HLA class I- and class II-specific ELISPOT assays provide unique tools to monitor and quantify the HLA-specific memory B cell pool in sensitized transplant recipients in a highly specific and reproducible manner.

WHAT WILL BE THE FUTURE OF DONOR-SPECIFIC MEMORY B CELL ASSAYS?

Determining the level of HLA- or donor-specific memory B cells can be performed either before transplantation for risk stratification, or after transplantation for immunological monitoring. In both settings, we foresee that assays to quantify the HLA-specific memory B cell pool will aid clinical decision making in the future.

As discussed above, in the setting of renal transplantation, there are situations in which candidates for transplantation have no detectable DSA at time of transplantation, regardless of the presence of donor-specific memory B cells in the circulation (36). Especially for patients with antibody positivity in historical sera, or husband to wife and child to mother combinations, determining the potential to develop an accelerated humoral rejection is of great importance. In retransplant candidates with the previous allograft still *in situ*, serum HLA antibodies may be undetectable due to antibody absorption by the failed allograft. It has been shown that upon graft nephrectomy, these antibodies become detectable in the serum, while previously undetectable (58). Such humoral immunization may be detected by circulating HLA-specific memory B cells even before the graft is surgically removed.

Another group of patients for which pretransplant donor-specific memory B cell quantification may be of importance are highly sensitized KTR who have been treated with desensitization protocols and B cell depleting therapies (59). Association of increased levels of post-transplant DSA with the incidence and severity of antibody mediated rejection in patients with low levels of pretransplant DSA after desensitization treatment implies a role for the relevance of memory B cell responses in antibody production and eventually on clinical outcome (60). Although desensitization protocols including low/high dose IVIg, plasmapheresis, or immunoadsorption are effective to remove alloantibodies from the serum, little is known about the effects of B cell-depleting therapies on the function of memory B cells particularly in the repopulating B cell compartment after the therapy (44).

As discussed above, HLA antibody formation in the presence of the allograft may remain undetected due to antibody absorption, leaving alloreactive memory B cells as a possible surrogate marker for the humoral alloimmune response. Because many of the HLA antibodies that are detected after transplantation are directed at HLA class II antigens (55-57), it

will be of interest to see the kinetics of both HLA class I and class II-specific B cells after transplantation, and their relation to graft outcome. Interestingly, environmental factors may play a role in amplifying the donor-specific humoral immune response after transplantation. It has been shown for both sensitized patients on the transplant waiting list, as well as for patients transplanted following desensitization that the occurrence of infections or other proinflammatory events result in an increase in the breadth and strength of HLA-specific antibodies (61). This is likely due to a burst of pro-inflammatory cytokines upon recognition of pathogen-associated molecular patterns or damage-associated molecular patterns. In line with this hypothesis, *de novo* HLA antibody production in KTR has been reported after seasonal influenza vaccination (62). These results advocate that monitoring the humoral immune system after transplantation is of great importance.

Longitudinal monitoring of HLA-specific B cell frequencies after transplantation may help to understand not only the effect of pro-inflammatory events, such as described above but also including cellular rejection, on the magnitude and durability of the humoral memory response. Finally, these observations may be transformed into monitoring algorithms with the ultimate aim to tailor immunosuppressive treatments in cases where B-cell memory compartment should be targeted. For clinical validation of HLA-specific ELISPOT assays, we are currently conducting a multi-center trial in the Netherlands. To this aim, blood samples from kidney retransplant patients are taken before transplantation, as well as 3 and 6 months after transplantation to be tested in HLA-specific memory B cell ELISPOT assays. This will provide information on the kinetics of donor-specific memory B cells in the early phase after transplantation in a high-risk renal transplant cohort.

The assays described here have potential use beyond the renal transplant setting. It is now well established that DSA are associated with inferior graft survival and increased acute rejection of thoracic transplants (63-66). Ventricular assist devices, which are a bridge to transplantation in cardiac transplant candidates, are considered as risk factors for HLA immunization (67, 68). Similar to kidney transplantation, plasmapheresis, IVIg and B cell depleting approaches are commonly used to remove circulating antibodies and/or suppress antibody responses in sensitized cardiac patients (68). In case of IVIg treatment, serum HLA antibody screening by Luminex bead assays may result in incorrect interpretation due to the interference of IVIg with the beads (69), a problem circumvented by using memory B cells as source of HLA antibodies *ex vivo* (33). Furthermore, following B cell depletion, assays capable of detecting HLA-specific memory B cells may be informative to determine whether alloreactive B cells reemerge.

The liver has long been considered as being resistant to antibody-mediated rejection possibly due to its capacity to absorb antibodies and secrete soluble HLA class I molecules (70-72). In fact, combined liver-kidney transplants enable transplantation of highly sensitized patients, because the preimplanted liver protects the kidney by antibody absorption (73). However, despite the protective effects of the liver by clearing HLA class I antibodies, there are multiple

reports showing that persisting anti-HLA class II antibodies may be detrimental to the kidney graft (74-76). In addition, there is increasing evidence that DSA could eventually lead to chronic rejection and inferior liver allograft survival (77-79). In case antibody absorption by the liver precludes proper serum antibody identification, assays for quantifying HLA-specific B cells may help detecting the humoral alloimmune response.

Presence of DSA in recipients undergoing unrelated hematopoietic stem cell (HSCT) or cord blood transplantation has been associated with engraftment failure, decreased patient survival, increased occurrence of acute graft *versus* host disease (GvHD), and decreased neutrophil and platelet recovery (80-84). For the latter, many patients need platelet transfusions as a supportive treatment. There are several reports showing that platelet transfusion refractoriness can be a consequence of HLA antibodies produced by donor cells in patients undergoing HSCT from antibody-positive multiparous female donors (85-88). In order to achieve better management of post-transplant platelet refractoriness in patients transplanted from donors with an immunization history such as pregnancy, antibody specificities produced by HLA-specific memory B cells from the donor may be defined in advance by testing the culture supernatants from polyclonally activated donor B cells by Luminex bead assays (33).

In conclusion, several assays for the detection and quantification of HLA-specific memory B cells have been developed over the last few years. The task ahead is to determine their applicability in the various clinical transplantation settings. We envisage that, besides serum HLA antibody detection, HLA-specific memory B cell assays will serve as additional tools to determine risk factors for individual patient management.

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