HLA-specific memory B cells : the missing link?

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Chapter 9

Summary and general discussion

SUMMARY AND GENERAL DISCUSSION

Solid organ transplantation is a life-saving treatment option for patients with end stage organ failure. The effect of HLA matching between the recipient and the organ donor on graft and patient survival has been shown in large scale studies (1, 2). Despite this beneficial effect of HLA matching, it is virtually impossible to find HLA fully-matched organ donors for most of the patients due to the high-level polymorphism in HLA system. As a consequence, patients are transplanted with partially compatible or even fully mismatched organs, made possible by lifelong immunosuppressive treatment. While currently available immunosuppressive agents are mainly targeting T cells, and thus successful in decreasing T cell mediated rejections, the humoral arm of the alloimmune response remains largely uncontrolled leading to antibody mediated recognition of non-self-proteins and carbohydrates on transplanted organs. Among these targets, mismatched HLA most strongly evokes the formation of donor specific antibodies (DSA) (3). Although alloantibodies to other polymorphic targets and several autoantibodies have also been reported in the field of transplantation (4), donor directed HLA antibodies remain the best described correlates of rejection and graft outcome in kidney, heart, lung and to some extent liver transplantation (5-8). Furthermore, the presence of DSA in recipients of allogeneic hematopoietic stem cell transplantation (HSCT) from haploidentical or mismatched unrelated donors has been associated with higher rates of graft failure (9-11).

HLA-specific humoral alloimmune response

HLA antibodies can develop upon exposure to allogeneic HLA via blood transfusions, pregnancies or previous transplantations. As discussed in **chapter 2**, antibody formation to protein antigens such as HLA is a T cell dependent multi-step process taking place in secondary lymphoid organs. In the context of solid organ transplantation, several studies have shown that B cell activation can occur directly in the graft and lead to local antibody production as a result of lymphoid neogenesis and formation of tertiary lymphoid organs (12, 13). Complete activation of naive B cells occurs upon recognition of the alloantigen via the B cell receptor (BCR) and through subsequent interactions with cognate $CD4^+$ T helper cells. Following germinal center reactions, mutated clones with higher affinity are positively selected to differentiate into long-lived memory B cells that continuously circulate between the periphery and secondary lymphoid organs, and into plasma cells majority of which home to bone marrow to maintain serum antibody titers (14).

Pre-existence or *de novo* production of donor HLA specific antibodies produced by plasma cells is associated with the risk of developing hyperacute, acute and chronic antibody mediated rejection (ABMR) in transplant recipients (3). In patients who have a history of alloimmunization, ABMR may develop as a result of rapid DSA formation deriving from an anamnestic response of pre-existing memory B cells (15-17).

Monitoring HLA-specific humoral alloimmune responses

Serum is the only source screened in HLA diagnostic laboratories before and after transplantation for the presence of DSA in patients (18). However, not only plasma cells but also dormant memory B cells can contribute to HLA antibody production (Figure 1).

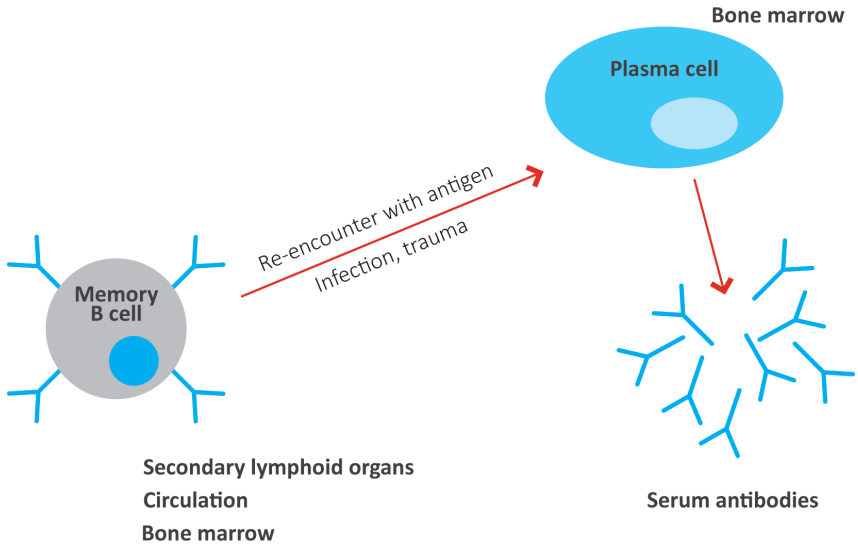

Figure 1. Both long-lived plasma cells and memory B cells contribute to HLA antibody pool. *Serum HLA antibodies are mainly produced by bone marrow residing plasma cells and serum samples are periodically screened in HLA diagnostic laboratories for the presence of HLA antibodies. However, bone marrow residing plasma cells are not the only source of HLA antibodies since silent memory B cells can as well become antibody secreting cells upon antigen-rechallenge. Memory B cells continuously circulate between the secondary lymphoid organs, bone marrow and peripheral blood and need to be polyclonally activated in order to be detected in vitro.*

As exemplified in **chapter 3**, some patients with a history of alloimmunization may harbor HLA-specific memory B cells in the absence of serum antibodies (15). For detection purposes, these dormant HLA-specific memory B cells need to be polyclonally activated *in vitro* to be able to differentiate into antibody secreting cells. Several protocols exist to activate B cells *in vitro*. However, it is critical that the polyclonal activation protocol in use does not induce isotype switching in the naïve B cell population during the culturing process and thereby enables the accurate detection of preexisting memory B cells. Data presented in **chapter 4** characterizes an anti-CD40 driven polyclonal activation protocol that can reliably be used to detect HLA-specific memory B cells in peripheral blood samples since the IgG type of tetanus toxoid (model antigen) specific spots were only found in the memory B cell fraction and not within the naïve B cells. Likewise, the polyclonal activation cocktail comprising of TLR7/8 agonist (R848) in combination with IL-2 used in chapters 6, 7 and 8 does not induce any isotype switching in the naïve B cell population and gives rise to even higher levels of IgG

production compared to the anti-CD40 driven protocol (data not shown) (19).

Current knowledge on the fate of B cells after germinal center suggests that following germinal center reactions, B cells can pursue a pathway where they simultaneously become plasma cells and memory B cells or alternatively, they may leave the germinal center as memory B cells and then gradually differentiate into plasmablasts and plasma cells (20). Nevertheless, in both cases, memory B cells can serve as potential precursors of terminally differentiated plasma cells. Therefore, quantification of these precursors, i.e., HLA specific memory B cells, may extend the understanding of potential HLA specific humoral immune responses that can be elicited upon antigen re-challenge. This line of information may particularly be useful in risk assessment for patients who have antibodies in the historical serum but do not have detectable DSA at time of transplantation as well as those undergoing desensitization therapies. In this respect, HLA-ELISPOT assays allow for quantification of circulating memory B cells directed at mismatched HLA class I and/or class II. Previously, using recombinant HLA class I monomers as the HLA targets (21) and as described in **chapter 5**, using synthetic HLA class II molecules as the detection matrix, we were able to determine HLA specific memory B cell frequencies in peripheral blood samples from women with a history of pregnancy. These assays are of value in selected cases whereby the presence or level of B cell memory against specific HLA antigens needs to be determined. However, these HLA monomer-based assays are limited by the availability of commercial HLA molecules and even if all combinations are used, the HLA repertoire of an individual can never be completely represented. To overcome this restriction, we have developed a donor-specific HLA-ELISPOT assay using cell lysates instead of synthetic HLA monomers as a natural source of both HLA class I and class II, which is described in **chapter 6**. Using this highly sensitive donor-specific HLA-ELISPOT assay, we found a median frequency of 31 HLA class I and 89 HLA class II-specific memory B cells per million IgG producing cells directed at paternal HLA in peripheral blood samples of women with a history of pregnancy using cell lysates from their spouses. The possibility to include autologous cell lysates covering the complete self HLA repertoire allowed for the interpretation of spouse-specific positive responses relative to self and served as an excellent intra-assay negative control. This assay can readily be translated to HLA diagnostic laboratories as a "memory B cell crossmatch assay'' since it allows for donor-specific memory B cell detection in peripheral blood samples of patients both in the setting of solid organ transplantation and HSCT. However, it should be noted that peripheral blood memory B cells comprise only a fraction of the memory B cell pool in an individual. Bone marrow, spleen and lymph nodes in addition to intragraft tertiary lymph nodes can as well harbor memory B cells (22).

Long lived plasma cells and memory B cells are the two mediators of serological memory (23). Whereas it has long been accepted that long-lived plasma cells preferentially reside in niches in bone marrow (24), there are only a few reports on the presence and function of memory B cells in bone marrow (25, 26). Data presented in **chapter 7** clearly presents that bone marrow samples contain both memory B cells and plasma cells specific for the same antigen. Interestingly, while memory B cells residing in or circulating through bone marrow

have similar immunoglobulin isotypes compared to peripheral blood B cells, plasma cells enriched from the same bone marrow samples display a distinct isotype distribution.

Conclusions and future prospects

Transplant recipients with a history of alloimmunization comprise a high-risk patient group for developing ABMR. Pre-transplant immunological risk assessment in these patients is solely based on the presence of serum HLA antibodies. However, currently available methods to detect HLA antibodies in serum do not provide any information on the size of HLA-specific memory B cell pool or memory B cell-derived HLA antibody specificities. Considering that the absence of serum DSA does not reflect the absence of B cell immunity against the graft, repeat transplantation candidates, women receiving grafts from their spouses as well as patients undergoing desensitization treatments may benefit from the assays described in this thesis. In this context, our lysate based HLA-ELISPOT assay can be used to estimate the HLA-specific memory B cell load in high-risk patients prior to transplantation of an organ from a living donor. In addition, utilization of monomer-based HLA-ELISPOT assays in samples from patients on the waiting list for an organ from a deceased donor can aid in quantification of memory B cells directed at a certain HLA antigen. Both assays can be performed in conjunction with B cell supernatant analysis.

Antibody mediated rejection remains to be the major cause of graft loss in the field of kidney transplantation (27-29). Therefore, it is very important to make an inventory of the HLA antibodies that are present in a patient before transplantation in order to define the safest donor candidates. Currently available single antigen bead technology enables extensive determination of HLA antibodies present in the serum of transplant recipients. However, particularly for highly sensitized patients, the broad range of HLA antibodies present in the serum makes it virtually impossible to find an acceptable donor. Nonetheless, it is still a matter of debate whether all HLA antibodies detected by single antigen bead assays are clinically relevant. As described in **chapter 8**, our current focus is towards detection of HLA antibodies using single antigen bead assays in culture supernatants which commonly present with a more restricted HLA antibody profile compared to serum samples. Defining discrepancies in HLA antibody specificities found in serum samples and those detected in B cell culture supernatants in a standard and validated way might be useful in excluding some HLA antibodies from highly immunized patients` antibody profiles. This approach needs further validation but can be supplementary in increasing the possibility of transplantation for highly immunized patients. Defining whether a patient with a history of alloimmunization has generated HLA-specific memory in the presence or absence of plasma cells, i.e. serum antibodies, may be useful in determining clinically relevant HLA antibodies since not all patients with pre-transplant DSA develop antibody mediated rejections (16, 30). This line of information may enable better risk stratification by providing a more complete picture of the humoral alloimmune response in patients undergoing desensitization treatments.

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