HLA-specific memory B cells : the missing link?

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Chapter 1

General introduction

THE IMMUNE SYSTEM

The immune system has evolved to discriminate between self and pathogenic non-self in order to enable elimination of the foreign intruder. This defense system is devised by innate and adaptive immune responses with an extensive cross-talk between the two. In addition to the defense provided by physical barriers (skin and mucosal epithelium) and soluble mediators such as the complement system, the cellular effectors of the innate immune system (granulocytes, mast cells, macrophages, dendritic cells and NK cells) provide the first line of defense against pathogens through germ-line encoded pattern recognition receptors that sense conserved molecular structures in and on microorganisms (1). Innate immune cells rapidly exert their functions in a generic way against pathogens, whereas cells of the adaptive immune system (T and B lymphocytes) provide a relatively slower but more specific response through their somatically rearranged receptors. A feature unique for the adaptive immune system is the capacity to develop immune memory (2-4). Upon re-encountering the same antigen, the adaptive immune system manifests a much more rapid and stronger response compared to the primary response. Although innate immune cells are known to lack the ability to learn from primary antigenic exposure, recent reports suggest memory cell-like functions also for activated NK cells (5, 6).

An effective immune response depends on the interplay between innate and adaptive immune system components. While B cells can directly recognize epitopes on intact antigens with their B cell receptors, T cells recognize processed peptides of the antigen presented through major histocompatibility complex (MHC) molecules. $CD4^+$ T cells recognize peptides in the context of MHC class II on antigen presenting cells (APCs), such as dendritic cells, macrophages and B cells. $CD8^+$ T cells (cytotoxic T cells) can directly kill target cells upon binding of their T cell receptor (TCR) to MHC class I/peptide complexes, whereas the majority of $CD4^+$ T cells (helper T cells) binding to MHC class II/peptide complex are responsible for providing help to cytotoxic T cells to become fully activated, and B cells for antibody production and class switching. Not all $CD4^+$ T cells are driving immune responses as inhibiting $CD4^+$ T cells, or regulatory T cells have also been described (7).

HLA and ALLOIMMUNE RESPONSES

MHC proteins in humans are encoded by the highly polymorphic human leukocyte antigen (HLA) genes located on chromosome 6p23 region (8). There are 2 classes of HLA molecules, class I and class II, which are heterodimeric transmembrane glycoproteins both functional in presenting peptides to T cells. Extracellular domains of the HLA molecules responsible for peptide binding and interaction with variable regions of the TCRs are highly polymorphic, facilitating the binding of a wide variety of peptides. This extensive polymorphism in HLA alleles ensures that populations will not succumb to new pathogens since a wide range of processed foreign peptides can be presented to the TCR to generate an immune response (9). While contributing to effective adaptive immune responses against a variety of pathogens, the polymorphism of HLA represents a hurdle in allogeneic transplantation. The enormous polymorphism in HLA as evident with currently more than 16000 HLA class I and class II alleles, makes it almost impossible to find a fully HLA-matched unrelated donor for a recipient (10). Disparities in HLA alleles between recipient and donor enhance the recognition of the graft as non-self by the host and may initiate the graft destruction process. The classical way of pathogen encoded antigen recognition by $CD4^+$ T cells also applies to the recognition of alloantigens, namely the indirect allorecognition. Through this pathway, T cells recognize graft MHC alloantigens that are processed into peptides and presented by APCs. A second way of allorecognition, which is unique to transplantation, is the direct recognition of the intact MHC alloantigen on the surface of the donor APCs. While indirect recognition plays an essential role in chronic allograft failure due to the involvement of $CD4^+$ T helper cells in alloantibody generation, the direct pathway of allorecognition is considered to be principal contributor of cytotoxic T cell responses mediating early rejection episodes (11, 12).

In the setting of solid organ transplantation, HLA mismatches between patient and donor evoke both the cellular and the humoral arms of the adaptive immune response in a recipient (13, 14). To prolong the survival of the HLA mismatched grafts, the vast majority of recipients rely on lifelong immunosuppressive treatment. While T cell alloimmunity is effectively kept under control with current immunosuppressive drugs, donor-specific alloantibody (DSA)-driven humoral immune responses are still considered as a risk factor for long term solid organ allograft survival (15-17).

Today, pretransplant risk assessment regarding the donor-specific humoral alloimmune response in a transplant recipient is based on detection of serum antibodies directed to mismatched donor HLA class I (HLA-A,-B,-C) and HLA class II (HLA-DR,-DQ,-DP) antigens (18). Serum HLA antibodies are mainly produced by bone-marrow residing plasma cells. However, dormant memory B cells may also contribute to HLA antibody repertoire of an individual with a history of alloimmunization upon re-encountering the same HLA or as a result of bystander activation (19, 20). Although there are several methods (complement dependent cytotoxicity (CDC), ELISA, bead-based assays) that are commonly used to detect these serum antibodies, none of the currently available assays provide any information on the magnitude of HLA-specific memory B cell responses.

AIM OF THE THESIS

The aim of this thesis was to develop tools to detect and quantify HLA-specific memory B cells in peripheral blood of HLA-immunized individuals and to assess the applicability of the newly developed assays in the setting of clinical transplantation. B cells are mainly known for their merit of antibody production; however, they have a broad range of functions other than antibody production. **Chapter 2** provides an overview on diverse functions of B cells in solid organ transplantation and highlights their roles in generation of humoral immune responses. Humoral alloimmune responses directed at mismatched donor HLA are usually detected as serum HLA antibodies of IgG isotype. Despite the abundant number of ways to detect serum HLA antibodies, methods to detect HLA-specific memory B cells are limited due to the technical challenges associated with the detection of these dormant cells. Various methods to detect HLA-specific memory B cells which are all limited in their capacity to quantify only HLA class I-specific memory B cell responses are described in **chapter 3**. Since memory B cells are dormant cells, polyclonal activation is required for them to be detected *in vitro*. However, it is critical that the polyclonal activation cocktail used does not induce isotype switching in naïve B cells and enables detection of IgG type of antibodies deriving solely from memory B cells. **Chapter 4** describes a polyclonal activation cocktail that can be used to activate isolated B cells in order to detect pre-existing antigen-specific memory B cells without inducing antibody isotype switching. Using this polyclonal activation protocol, an ELISPOT method was developed in which synthetic HLA class II molecules serve as the HLA target as described in **chapter 5**. Methods to detect memory B cells mainly utilize synthetic monomeric/tetrameric HLA molecules which generally do not represent the complete HLA repertoire of an individual. A donor-specific HLA-ELISPOT assay enabling the screening for HLA-specific memory B cells in peripheral blood of immunized individuals using cell lysates as a natural source of both HLA class I and II is described in **chapter 6**. While peripheral blood samples can be used to detect memory B cells *in vitro*, secondary lymphoid organs and bone marrow also contain memory B cells. As described in **chapter 7**, the bone marrow harbours not only plasma cells but also memory B cells specific for the same antigen whereas the two cell types display a different immunoglobulin isotype distribution. While serum HLA antibodies are produced by bone marrow residing plasma cells, memory B cells can contribute to HLA antibody formation upon activation, and these antibodies may not necessarily mirror the antibody specificities produced by bone marrow residing plasma cells. Therefore, determination of HLA antibodies in B cell culture supernatants supplementary to serum HLA antibody detection may provide a more complete picture of the potential humoral alloimmune response in an alloantigen immunized individual. **Chapter 8** elaborates on accurately discriminating HLA antibody specificities found in serum and B cell culture supernatants from kidney transplant recipients.

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