HLA epitopes in kidney transplantation: from basic science to clinical application

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CHAPTER
1

General Introduction

KIDNEY TRANSPLANTATION

Chronic kidney disease is one of the leading causes of death and morbidity, affecting over 800 million individuals worldwide[1]. A proportion of patients suffering from chronic kidney disease will progress to end-stage kidney disease and require renal replacement therapy, either with dialysis or transplantation. Kidney transplantation has significant survival advantage as compared to dialysis and is the treatment of choice for most patients suffering from end-stage kidney disease[2]. Since the first successful kidney transplantation in 1954, improvement in patient and donor selection, advancement of surgical techniques and development of more efficient immunosuppressive therapies have led to better patient and graft survival[3]. Although significant progress has been made in graft survival rates over the past decades, this improvement has decelerated[4]. The leading cause of late graft loss, excluding death with a functioning graft, is chronic allograft rejection[5, 6].

The immune system

The immune system is responsible for the body's defense against infections with bacteria, viruses, parasites and fungi and comprises the innate and the adaptive immune system[7]. Besides anatomical barriers and soluble mediators, the innate immune systems consist of various cell types such as macrophages, granulocytes, mast cells and dendritic cells which are activated upon inflammatory signals. The innate immune response occurs rapidly, within a few minutes to hours, as the body's first line defense against pathogens[8]. The adaptive immune system comes into play when the innate immune system is overwhelmed by pathogens. Although the adaptive immune response takes much longer to develop; several days, it is more specific than the innate response, because it consists of lymphocytes that express highly specialized antigen receptors. These antigen-specific lymphocytes can be divided into T cells and B cells that express T cell- and B cell receptors, respectively. While T cells are responsible for cellular immunity, B cells account for humoral immunity through the production of antibodies, which both play a role in allograft rejection[7].

The major histocompatibility complex

While the immunoglobulins that serve as the antigen receptor on B cells can recognize extra-cellular antigens in its native conformation, the T cell receptor can only recognize antigenic peptides that are presented by the major histocompatibility complex (MHC) on the cell surface[7]. In humans, the MHC is known as the Human Leukocyte Antigen (HLA). The HLA system consists of several HLA class I (HLA-A, -B and -C) and HLA class II (HLA-DR, -DQ and -DP) genes. HLA molecules are glycoproteins, of which the building blocks are amino acids. Whereas HLA is inherited as haplotypes and therefore all individuals have maximum two different alleles per locus, the polymorphism at the population level is much bigger. For each HLA locus, thousands of different HLA proteins have been described[9]. The HLA class I molecule is present on all nucleated cells and platelets, and consists of an α-chain which accounts for three polymorphic α-domains, and β2-microglobulin which is not polymorphic[10, 11]. The polymorphism of HLA class I is mainly due to amino acid differences in the α1 and α2 domains, which together from the

peptide-binding groove[12]. The HLA class I molecule can present peptides which are generated by proteases within the cell which are then loaded onto the HLA molecule in the endoplasmic reticulum, after which the peptide-HLA complex is transported to the cell membrane to be expressed at the cell surface[13, 14]. Subsequently, receptors of cytotoxic $CD8^+$ T cells bind to the peptide-HLA complex to recognize the peptide. In case of an infection, the cytotoxic T cell will recognize the peptide as foreign, which will result in killing of the infected cell[15].

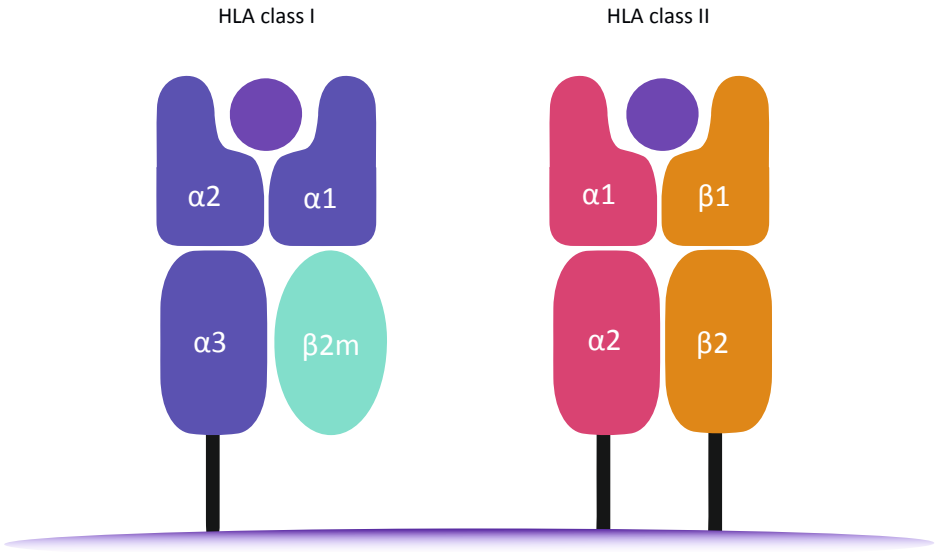

Figure 1. The structure of HLA class I and HLA class II molecules.

The HLA class II molecule is formed by an alpha and beta chain which are both divided into two domains. For HLA-DR, only the beta-chain is polymorphic, while for HLA-DQ and -DP, both the alpha and beta-chain are polymorphic (Figure 1)[16, 17]. HLA class II molecules are primarily present on professional antigen-presenting cells such as dendritic cells, macrophages and B cells. These cells can capture and take up extracellular proteins, which are processed into peptides in the lysosomal compartments, and are then presented by HLA class II molecules[18]. $CD4^+$ T cells can subsequently interact with the peptide-HLA complex which results in the differentiation to effector T cells, activation of macrophages to kill the pathogen, or the recruitment of T cell help for antibody production[7].

The alloimmune response in transplantation

While the presence of multiple different HLA genes ensures that cells from an individual can present a wide range of peptides to their immune system, the high polymorphism of HLA alleles within a locus ensures diversity in the HLA gene expression on a population level. This mechanism is important from an evolutionary perspective, as it increases the chance that an individual in a population can present a peptide of an endemic pathogen to clear the infection.

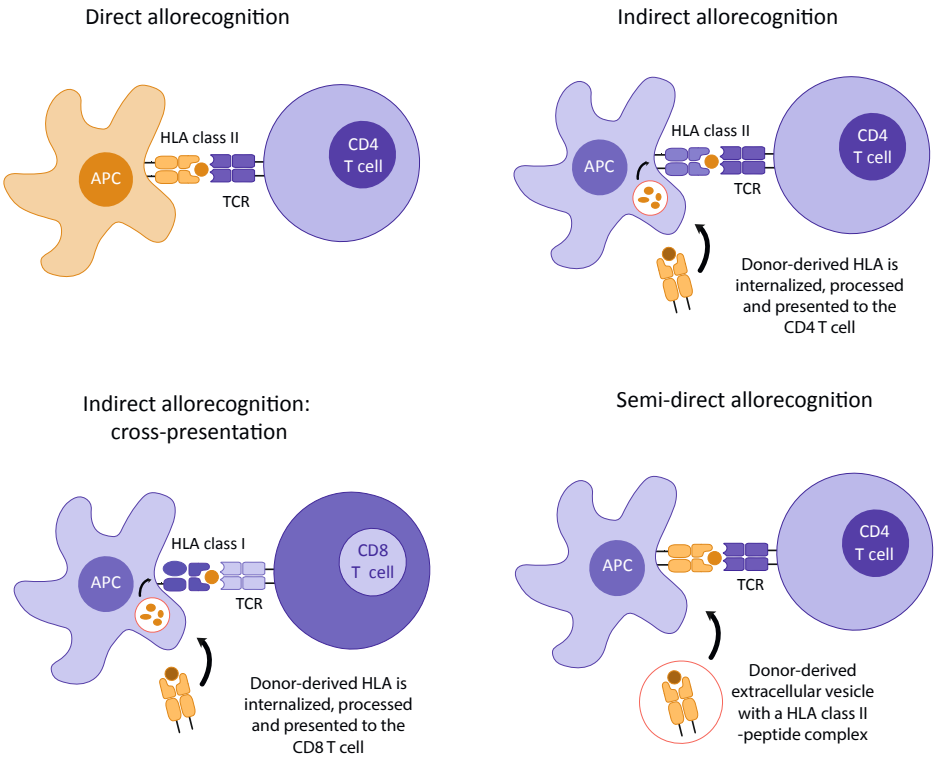

Figure 2. The pathways of allorecognition. Donor-derived cells and molecules are depicted in orange and recipient cells and molecules are depicted in blue. APC, antigen presenting cell; HLA, human leukocyte antigen; TCR, T cell receptor.

However, it is a major hurdle in transplantation. Upon transplantation, MHC molecules of the organ donor can be recognized by the immune system as foreign[19]. This process, known as allorecognition, contributes to graft rejection via three pathways: the direct, indirect and semi-direct pathway (Figure 2)[20].

The direct allorecognition pathway occurs early after transplantation and is thought to be primarily responsible for acute rejection. As antigen-presenting cells of donor origin migrate to the secondary lymphoid organs of the recipient, recipient T cells are activated. These activated alloreactive effector T cells can then circulate to the graft for a direct cytotoxic response[19].

At a later stage after transplantation, when no donor-derived APCs are present anymore, donor-derived HLA peptides are primarily presented in the context of self-HLA to $CD4^+$ T cells by APCs from the recipient, or to CD8+ T cells through cross-presentation[21]. This pathway, referred to as indirect allorecognition, is associated with chronic rejection. The T cells that are activated by the indirect pathway are likely to be important in the development of antibodies directed against the graft[22].

Although for a long time only the direct and indirect pathways were described in transplantation, more recent evidence has suggested that there is a third pathway: semi-direct allorecognition. In this pathway, the intact HLA molecule of the donor is acquired by the recipient's APC and subsequently presented as an intact donor-derived HLA-peptide complex to the T cell[20, 23]. This process, also known as trogocytosis or ''cross-dressing'', is thought to be mediated through extra-cellular vesicles[21].

Donor-specific antibody formation

As acute graft rejection is primarily mediated by T cells, the majority of immunosuppressive therapies in transplantation has traditionally been aimed at suppression of T cell activation[24]. However, the role of B cell activation and donor-specific antibody (DSA) production, especially in chronic allograft rejection should not be underestimated. B cells can recognize intact HLA molecules from the donor that will be internalized after which donor-HLA derived peptides will be presented to $CD4^+$ follicular helper T cells. The activation of these helper T cells results in class switching which allows B cells to produce antibodies with a variety of effector functions, and stimulates B cell differentiation into antibody-producing plasma cells and memory B cells (Figure 3)[25, 26]. The induction of HLA-specific antibodies is determined by the binding region of the B cell receptor, the paratope, that interacts with the binding region on the HLA molecule; the epitope. The specificity of the subsequently induced antibody is determined by the interaction of the complementarity-determining region 3 of the heavy chain (CDR-H3) of the antibody that interacts with a part of the epitope[27-29].

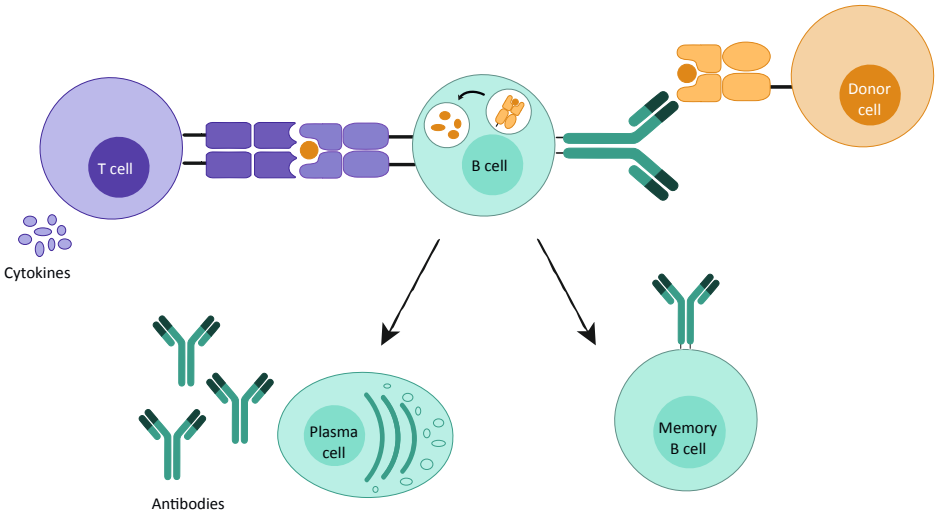

Figure 3. Donor-specific antibody production. An HLA-specific B cell recognizes a mismatched HLA molecule on the donor cell, which is subsequently internalized. After processing, donor HLA-derived peptides are presented to CD4+ helper T cells in recipient HLA class II molecules. Upon recognition by follicular helper T cells with the correct T cell receptor, T cell activation is induced, leading to upregulation of costimulatory molecules on the cell surface and production of B cell activating cytokines which will stimulate memory B cell formation and differentiation into plasma cells that produce affinity matured, class-switched antibodies.

The first anti-HLA antibodies, induced by blood transfusion and pregnancy, were described in the 1950s by Jean Dausset, Rose Payne and Jon van Rood[30-32]. The finding of deleterious effects of pre-existing donor-reactive antibodies before transplantation[33] meant the start of testing for preexistent antibodies in prospective kidney transplant recipients. To this purpose, a micro-cytotoxicity assay was developed by Paul Terasaki in which cells of the potential donor were mixed with the patient's serum[34]. This allowed for the identification of complement-fixing DSA in the serum of the prospective transplant recipient. By performing this crossmatch, hyperacute rejection caused by preexistent DSA has become extremely rare. However, it also became clear that transplantation with an HLA mismatched organ may lead to *de novo* DSA formation, which is associated with decreased graft survival[35]. In fact, more that 50% of long-term kidney failure can be attributed to chronic allograft rejection mediated by DSA[36].

HLA epitopes

HLA matching in kidney allocation algorithms aims to minimize the chance of rejection and the development of *de novo* DSA. In Eurotransplant, current HLA matching occurs on HLA-A, -B, and -DR only, with priority for full-house matches, and a point system for all instances. However, due to the high polymorphism of HLA and the scarcity of donor organs, the majority of kidney transplant recipients receive a graft with a certain degree of HLA antigen mismatch[37]. Refinement in HLA typing techniques has demonstrated that the high level of polymorphism of HLA can be explained by a few hundred polymorphic amino acid configurations, which are often referred to as epitopes[38, 39]. These epitopes can be shared between different HLA molecules, but every HLA molecule is comprised of a unique epitope set (Figure 4).

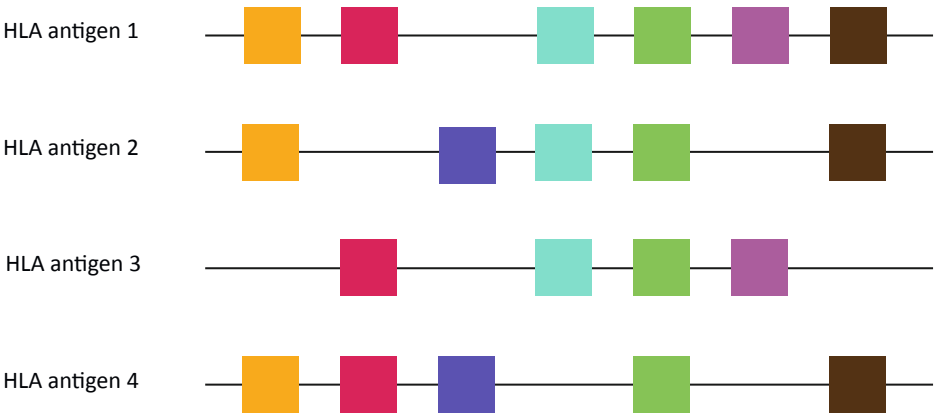

Figure 4. The HLA epitope principle. The colored squares depict the different epitopes on the HLA molecule. While each HLA antigen expresses a unique set of epitopes, the individual epitopes are shared between different the HLA molecules.

In theory, this means that an HLA antigen mismatched graft could be actually fully matched on the HLA epitope level (Figure 5). In 2006, Rene Duquesnoy introduced the term HLA eplet to describe a configuration of polymorphic amino acids within a 3.5 Angstrom radius on the HLA

molecule, that can be recognized by the B cell receptor through interaction with the CDR-H3 region[40]. Since then, eplet matching has been proposed as a more feasible method than HLA matching on the antigen level[39, 41, 42], and many studies have shown the association between eplet mismatches and *de novo* DSA formation, rejection and graft loss[43-47]. Despite these associations, eplet matching has not been adopted in transplantation programs yet, due to the lack of empirical evidence for clinically relevant eplets.

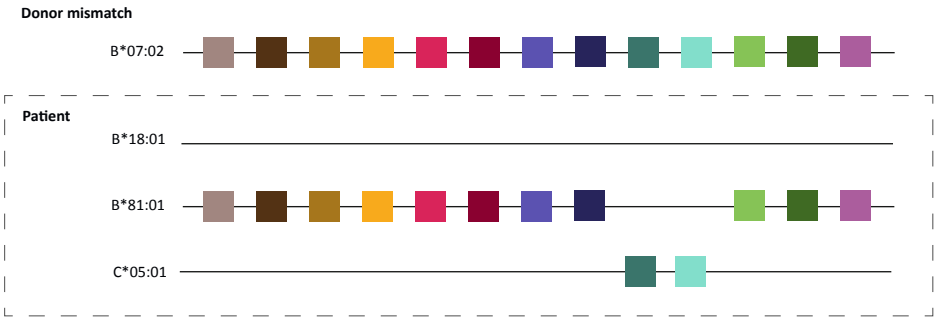

Figure 5. HLA epitope matching principle. Although there is one HLA antigen mismatch between donor and recipient, there are no epitope mismatches, due to shared epitopes between the donor allele and the recipient's HLA B*81:01 and C*05:01 alleles.

OUTLINE OF THIS THESIS

This thesis is composed of a two sections: the basic science of HLA epitopes and the clinical application of HLA epitopes in transplantation. In **Chapter 2**, we review the determinants of HLA immunogenicity and the different algorithms that have been developed for HLA epitope analysis, including amino acid mismatch analysis and eplet analysis. Because eplets have been theoretically defined based on the differences in amino acid sequences of HLA alleles, eplets need to be antibody verified to validate that they can be bound by antibodies. We describe the studies that investigated immunogenicity of individual eplets and explain the requirement of antibody verification to identify clinically relevant eplets for transplantation. In **Chapter 3**, we present the generation of HLA-DQ-specific human monoclonal antibodies (mAbs) from pregnancy immunized women using single cell B cell isolation and recombinant technology, and use these mAbs to verify theoretically defined HLA-DQ eplets. In **Chapter 4**, we review all previously generated evidence for antibody verification of eplets that has been listed in the HLA Eplet Registry and introduce a classification system for eplet antibody verification. In **Chapter 5,** we describe site-specific mutagenesis of single amino acids on the HLA-A1 molecule to decipher which amino acids are crucial for binding of an HLA-A1-induced human monoclonal antibody and use this method to redefine a previously defined eplet.

The second part of the thesis is dedicated to the clinical application of HLA epitopes in transplantation. Although many studies have investigated HLA eplets in kidney transplantation,

they are difficult to compare because of the many different versions of HLA eplet analysis software that have been used. Since eplets are based on amino acid polymorphism and the amino acid sequence of HLA alleles is a fixed entity, amino acid mismatch assessment may be a good alternative for HLA mismatch analysis. In **Chapter 6** we describe amino acid mismatches analysis in a large cohort of high-resolution HLA typed kidney transplant recipients. The association of HLA eplet and amino acid mismatches with DSA formation and graft failure allows for the use of these parameters as a tool for immunological risk assessment of transplant patients, possibly allowing for tailor-made immunosuppressive regimes. Since immunosuppressive therapy post-transplantation causes severe adverse effects including increased risk for infections, malignancies and cardiovascular disease [48-50], there is a need for a personalized approach which allows for minimization of immunosuppression in low-risk kidney transplant recipients[51]. A promising approach for reducing immunosuppression and improving patient and graft survival is cellular therapy[52]. By the administration of immunomodulatory cells, such as mesenchymal stromal cells (MSC) or regulatory T cells, the immune system is modulated to a more tolerogenic environment, so that less immunosuppression is required. In **Chapter 7**, we performed eplet analysis of the Triton study cohort, in which kidney transplant recipients were treated with autologous MSC therapy and subsequent tacrolimus withdrawal[53]. We investigated whether eplet mismatch loads could have identified patients that were eligible for MSC therapy and tacrolimus withdrawal without an increased risk of de novo DSA formation.

While most studies have investigate autologous MSC therapy in transplantation, allogeneic MSC therapy would be more feasible as an off-the-shelf product[54]. In **Chapter 8**, we describe two cohorts of kidney transplant recipients that have been treated with allogeneic MSC therapy[54, 55]. As administration of allogeneic HLA mismatched MSC might be an additional risk factor for *de novo* DSA formation, we investigated whether MSC selection to avoid repeated mismatches between the kidney and MSC donor on the HLA antigen level leads to a lower number of repeated mismatches on the amino acid level, and we analyzed whether repeated amino acid mismatches were associated with *de novo* DSA formation.

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