SMGroup

Human Leucocyte Antigen (HLA) System in Solid Organ Transplantation and Few Novel Concepts on HLA Matching

Francisco Salcido-Ochoa*

Department of Renal Medicine, Singapore General Hospital, Singapore

***Corresponding author:** Francisco Salcido-Ochoa, Department of Renal Medicine, Singapore General Hospital, Academia Level 3, 20 College Road, Singapore, Telephone +65 63266165; Fax +6562202308; Email: francisco.salcido.ochoa@sgh.com.sg; Website: www.tregsandhla-researchforce.com

Published Date: May 10, 2015

ABSTRACT

The molecules of the Human Leukocyte Antigen system play a central role in immunity, serving as the antigen presentation molecules recognized by T cell receptors. The large degree of polymorphism within the molecules of the Human Leukocyte Antigen system, and their counterparts in other species, provide individuals with a large responsive repertoire to signal any potential invading agent or altered self-molecule to T cells. However, in transplantation, having different variants of these molecules within the population is counterproductive. The molecules of the Human Leukocyte Antigen system are the triggers of alloreactivity leading to the establishment of transplant rejection and the potential destruction of the transplant. In solid organ transplantation, recipient individuals tend to respond immunologically to differences in the antigenic composition of the donor HLA molecules; and the assessment of the degree of antigenic mismatch between donor and recipient can serve as a surrogate measurement of the risk for transplant rejection and eventual transplant loss. While waiting for the development of newer more efficacious and safer immunotherapies, achieving the best possible match or the less deleterious mismatch between donors and recipients appear to be a sensible strategy. Here we discuss the role of the Human Leukocyte Antigen system in solid organ transplantation,

and explain few interesting concepts of Human Leukocyte Antigen matching and how it affects transplant outcomes.

Keywords: Allorecognition; Human Leukocyte Antigen; HLA Allele; HLA Matching; HLAMatchmaker; Major Histocompatibility Complex; MHC Class I Chain-Related Protein; Minor Histocompatibility Antigens; Non-Inherited Maternal Antigens; Transplantation.

ABBREVIATIONS

APC-Antigen Presenting Cell; bp-Base Pair; DC-Dendritic Cell; HLA-Human Leukocyte Antigen; IMA-Inherited Maternal Antigens; IPA-Inherited Paternal Antigens; MHC-Major Histocompatibility Complex; MICA-Major Histocompatibility Complex Class I Chain-Related Protein A; MICB-Major Histocompatibility Complex Class I Chain-Related Protein B; NIMA-Non-Inherited Maternal Antigens; NIPA-Non-Inherited Paternal Antigens; TCR-T Cell Receptor; Treg Cell-Regulatory T cell.

INTRODUCTION

Organ transplantation is regarded as the best therapy for end-stage organ failure. However, transplantation is not a naturally occurring phenomenon, and the immune system of transplanted individuals identifies transplanted tissues as foreign and potentially dangerous elements. A series of effector mechanisms are then deployed by the immune system to eliminate them. The transplanted organ is hence infiltrated by a myriad of immune cells, leading to an immune-mediated inflammatory process with consequent injury to the transplant, referred to as transplant rejection. Transplant rejection is the most powerful and diverse immune response known; therefore, therapeutic manipulation to allow the prolonged engraftment of transplant organs has been extremely challenging.

Transplant patients have to receive immunosuppressive drugs to subjugate the immune system so as to avoid transplant rejection. However, immunosuppressive drugs, although to a certain extent are effective in reducing rejection rates and in prolonging transplant survival [1], come with a constellation of toxic effects and have not actually been proven to induce immune tolerance. It appears that a detailed dissection of the immunogenicity of transplant organs is a crucial step for the improvement of current immunodiagnostic tools and the design of a more targeted and effective armamentarium to prevent and combat transplant rejection. Nonetheless, we have not been dismayed in our efforts to unveil the mysteries of transplantation tolerance and to fight transplant rejection. On a positive note, few promising tolerogenic protocols are already in the pipeline [2,3].

Certainly, a better understanding of the molecules of the human leukocyte antigen (HLA) system and their role triggering transplant rejection would help the researchers, as well as the clinicians alike, in deciphering the complex immunology behind the transplant process, i.e. the effector mechanisms deployed to destroy the transplant, as well as the regulatory, homeostatic

and potentially tolerogenic pathways to minimise or prevent excessive injury to the transplant. Here we present the current understanding of the role of the HLA system in solid organ transplantation, and discuss the concept of HLA matching and how it affects transplant outcomes.

IMMUNOGENICITY OF ORGAN TRANSPLANTS IS CONFERRED BY HISTOCOMPATIBILITY GENES

Transplanted foreign organs are inherently immunogenic to genetically different recipient individuals and inexorably succumb to rejection. These transplants are referred to as allogeneic, while transplants performed between individuals genetically identical, so-called syngeneic (e.g. identical twins), can be accepted indefinitely in the absence of immunosuppression. Both the relative immunogenicity of the transplant tissue from the donor (being either foreign or self), and the discriminatory capacity and response to it by the recipient immune system (rejection vs acceptance), are in great part conferred genetically within a set of loci referred to as the major histocompatibility complex (MHC). This genetic loci received its name after being identified as the crucial determinant for rejection of transplanted tumours in mice [4], though it was later identified to be responsible for conferring the capacity to respond immunologically to environmental antigens [5]. In fact, the actual role of the MHC genes and molecules is to signal the presence of foreign invading elements or altered self-components to the immune system and to stimulate the activation of T cells to orchestrate the elimination of these infective organisms or the abnormal, potentially cancerous, cells. MHC molecules are expressed on the surface of cells, where they serve as the antigen-presentation cards to T cells. MHC molecules possess a binding groove in their apical portion that can bind different distinct sets and sizes of antigenic peptides, and only bound peptides to MHC molecules are recognisable by the immune receptors of T cells (Figure 1). Thus, the set of inherited MHC genes by an individual determines his/her potential to respond to environmental antigens and its capacity to stimulate T cells.

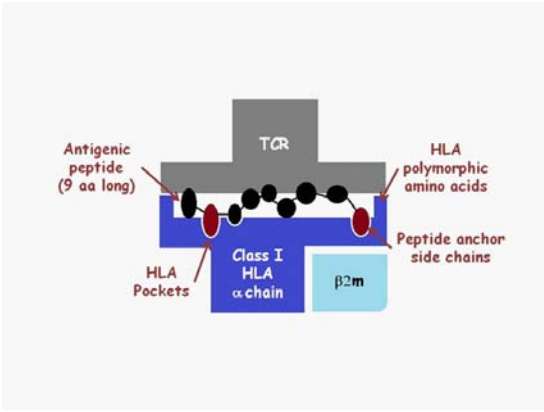

Figure 1: Depiction of the interaction between TCR and HLA:peptide complexes. Polymorphic residues on HLA molecules are crucial for the interaction with the TCR. Specific pockets in the peptide binding groove on HLA molecules interact with anchor side chains on antigenic peptides, and they determine the different sets of antigenic peptides to be bound.

In the case of transplantation, MHC genes and molecules from the donor serve as the triggers of rejection, and the MHC and genes in the recipient determine his/her capacity to respond to allogeneic antigens, in the same way they do it for conventional immune responses. In other words, it appears that it is the combination and the 'interplay' between the donor and the recipient MHC genes, not only the donor MHC antigens, what determines immunologically the outcome of the transplant in the recipient. The MHC genes are the most polymorphic genes in the genome and each genetic variant, or allele, in the donor provides an array of antigenic possibilities to recipients. But the immunogenicity of these alleles for recipient T and B cells depends on the particular alleles present in the recipient [6]. Thus, certain recipient individuals will produce alloantibodies to certain donor alleles but not others. This is due to the fact that certain conformational antigenic determinants on allogeneic MHC molecules are present also on certain MHC alleles in the recipient (shared determinants), not eliciting an immune response; while others are not shared, becoming immunogenic. See Figure 2 for a diagrammatic representation.

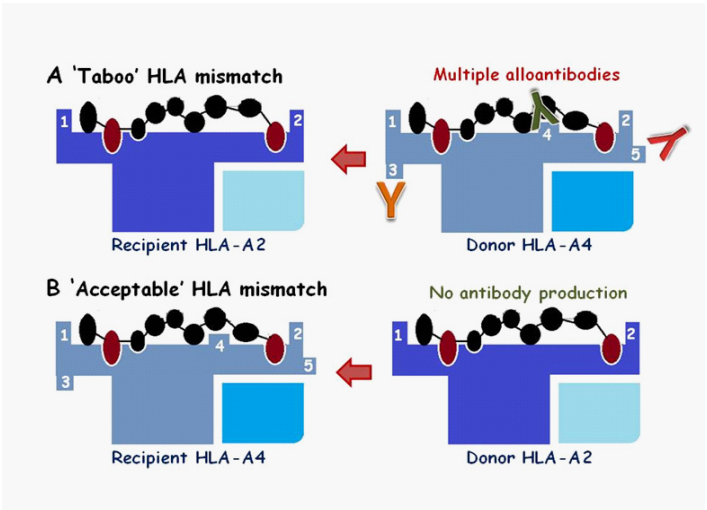

Figure 2: Diagrammatic depiction of the relative alloimmunogenicity of HLA alleles. Recipient's alloreactivity to donor HLA molecules is dependent on the actual antigenic determinants present or absent on the recipient's own HLA molecules. A recipient sharing HLA alleles with the donor will not produce alloantibodies (not shown). However, recipients transplanted across HLA mismatches with many antigenic (or very immunogenic) differences at the conformational molecular level between them and donors will produce alloantibodies (A); therefore those mismatches have been dubbed as 'taboo' HLA mismatches. On the contrary, recipients transplanted across HLA mismatches with few antigenic (or poorly immunogenic) differences at the conformational molecular level between them and donors tend not to produce alloantibodies (B); regarded as 'acceptable' HLA mismatches. The red vectors indicate the directionality of the mismatches. This is a fictional pictorial representation, not reflecting the actual differences between alleles of the HLA-A2 and HLA-A4 families or their relative alloimmunogenicity.

T CELL RECEPTORS ARE THE SENSING MOLECULES FOR ALLOANTIGENS

The immune receptors for T cells, or T cell receptors (TCR), possess the inherent capacity to bind to MHC molecules bearing peptide antigens, and they are the sensing elements to discriminate self from non-self. TCR genes have the capacity to undergo recombination and other molecular events for the generation of a vast TCR repertoire, able to bind and respond to virtually any given antigen in the Universe. However, the infinity of TCR specificities generated must undergo a series of selective processes in the thymus to ensure exported T cells would carry TCR able to bind foreign peptide antigens presented on self-MHC molecules on the surface of peripheral antigen-presenting cells (APC) [positive selection], while unable to react autoreactively to self-antigens born by self-MHC molecules (negative selection) [7,8]. The set of MHC alleles of a given individual shapes the TCR specificities to be selected through these processes, creating an immunoreactive T cell repertoire to foreign antigens presented by APC in the periphery, which is both self-MHC-restricted and self-tolerant.

BIOLOGICAL ROLE OF MHC MOLECULES

Broadly speaking, the role of class I MHC molecules in immune defence is to sample and present to T cells endogenous antigens present in the intracytoplasmic compartment [9]. These antigens are typically derived from cells infected with intracellular microorganisms or from cancerous cells expressing mutated proteins. Thus, class I MHC molecules serve as a sensing mechanism of cellular health that, drastically and necessarily, lead to the elimination of unhealthy cells by $CD8^+$ cytotoxic T cells. In transplantation, alloreactive cytotoxic T cells get activated by allogeneic donor cells expressing allogeneic class I MHC molecules. On the other hand, the defensive role of class II MHC molecules appears to be the sampling and presentation of exogenous antigens, reaching the lysosomal compartment, derived from extracellular microorganisms [9]. Since class II MHC molecules are expressed preferentially on APC, their main role is to stimulate $CD4^+$ T cells to orchestrate different arms of the immune response. The restriction of class I MHC molecules to activate $CD8^+$ T cells and of class II MHC molecules to activate $CD4^+$ T cells is conferred at the molecular level by the presence of a binding motif for CD8 in class I MHC molecules and for CD4 in class II MHC molecules, respectively. However, exogenous antigens can also be presented by class I MHC molecules to activate $CD8^+$ cytotoxic T cells through the phenomenon of cross-presentation [10].

ALLOSTIMULATION DURING THE PROCESS OF SOLID ORGAN TRANSPLANTATION

In the immune response against transplants, the activation of effector T cells and B cells, as well as the activation of the innate defence system, are crucial for the establishment of transplant rejection. T helper cells are activated upon binding foreign MHC molecules or their processed

peptides bound to recipient MHC molecules, and they orchestrate both the alloreactive immune response and the innate response against the transplant. Likewise, cytotoxic T cells, becoming activated in similar fashion, are the main effectors of lysis of allogeneic parenchymal cells, in the way they eliminate virally-infected or neoplastic cells. On the other hand, B cells are activated to produce alloantibodies upon recognising conformational epitopes on foreign MHC molecules directly through their B cell receptor; usually requiring T cell help for proper activation, generation of memory B cells, immunoglobulin isotype switching and maturation of the affinity of their alloantibodies. But not all the immune response deployed in response to the transplant organ is destructive. Regulatory T (Treg) cells, the master immune moderators, are also activated during an immune alloreaction. Treg cells may play a role in re-establishing immune homeostasis, and perhaps also in promoting the long-term operational acceptance of the transplanted organ [11-14]. It has been hypothesised that the balance or interplay between the effector and the regulatory T cells can determine the ultimate outcome of the alloresponse and the fate of the transplant [15], which could be potentially be modified by the strength of the alloactivation caused by the number, antigen density and relative immunogenicity of foreign MHC molecules. In addition, the innate defence system has also been shown to play an important role in this respect. The innate defence system is primed to full force in the process of organ transplantation, owing to the cytokine release accompanying the injury to the transplanted organ related to organ harvesting, storage damage and ischaemia-reperfusion injury; which can be more pronounced when using organs from brain-death donors (associated with a cytokine storm) and after prolonged cold ischaemia times following donation after cardiac death. The activation of the innate system leads to the release of powerful inflammatory cytokines and other inflammatory mediators like oxygen reactive species and complement molecules, as well as the expression of damage-associated molecular patterns; all of them important co-adjuvants for the activation of dendritic cells (DC) and other APC, and eventually T and B cells.

PATHWAYS OF ALLORECOGNITION OF THE ALLOANTIGENS

As mentioned previously, APC play an important role in activation of alloreactive T cells. APC provide the three necessary signals for T cell activation: 1) MHC:peptide complexes, 2) costimulatory molecules and 3) inflammatory cytokines. They also find different ways to allostimulate T cells depending on the origin of the APC and the source of the MHC molecules, the so-called pathways of allorecognition [16]: (a) direct, (b) indirect, and (c) semi-direct (Figure 3). Following transplantation, the alloreactive T cells from the recipient can be activated directly by the donor APC, bearing intact surface allogeneic MHC molecules, that migrated from the graft into the lymphoid tissue of the recipient (direct pathway of allorecognition). Alternatively, alloreactive T cells can be stimulated indirectly by recipient APC taking up alloantigens derived from the donor tissue, which can be MHC molecules or other polymorphic molecules, digesting them into peptides and conjugating them to their own MHC molecules and present them to other alloreactive T cells (indirect pathway of allorecognition). The direct pathway of allorecognition is the initial pathway

of T cell allostimulation and it is very powerful. It was initially thought that this pathway started to fade away once the short-lived donor-derived APC started to perish in the recipient, and then the indirect pathway of allorecognition was the dominant pathway driving T cell activation throughout the life of the transplant. However, it has been demonstrated that recipient APC are able to uptake entire allogeneic MHC molecules from donor-derived cells in the transplant [17] and present them successfully to alloreactive T cells generated through the direct pathway of allorecognition, allowing the persistence of directly-activated T cells throughout the life of the transplant. This pathway has been referred to as the semi-direct pathway of allorecognition.

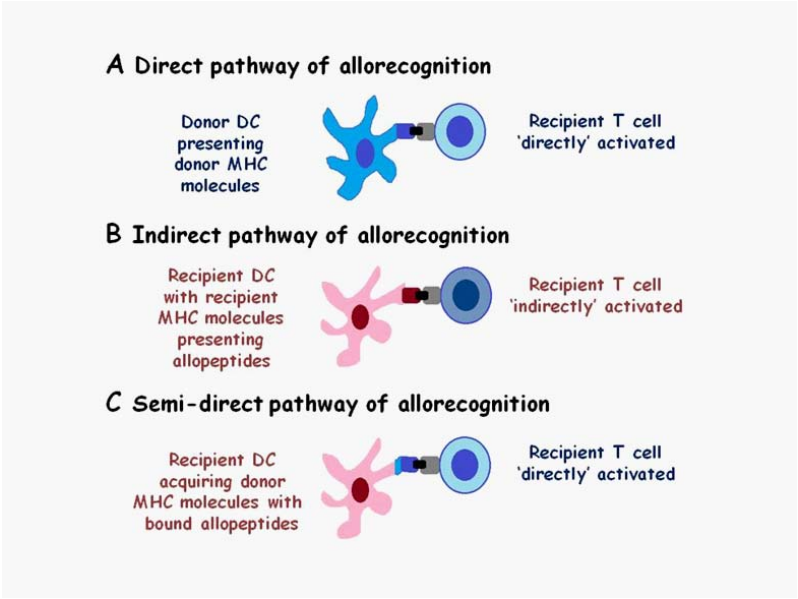

Figure 3: Pathways of allorecognition. The direct pathway of allorecogniton (A) involves the activation of recipient alloreactive T cells directly by the donor APC bearing intact surface allogeneic MHC molecules. The indirect pathway of allorecogniton (B) refers to the activation of recipient alloreactive T cells by recipient APC presenting allopeptides derived from the donor HLA molecules and other polymorphic molecules bound to recipient HLA molecules. The semi-direct pathway of allorecognition (C) occurs when alloreactive T cells are activated by the presentation of donor HLA molecules presented intact by recipient APC that acquired them from donor APC or other cells.

As alluded to before, transplant rejection is the most powerful immune response with peculiar features different from conventional immune responses. Even in the absence of prior alloimmunisations, through the phenomenon of cross-reaction, circulating alloreactive memory T cells are present and detectable. The TCR has the inherent capacity to bind MHC molecules and easily cross-react with allogeneic MHC:peptide complexes, whose structure resembles that of the complexes formed by self MHC molecules and any given environmental antigen [18-19] (Figure 4). Thus, memory T cells originally specific for different infectious organisms can attack rapidly

and efficiently the transplant tissue as if the patient would have been transplanted with those alloantigens before. Added to this issue, memory T cells specific for alloantigens can be formed and boosted in recipients having history of blood transfusions, multiple pregnancies and prior transplants.

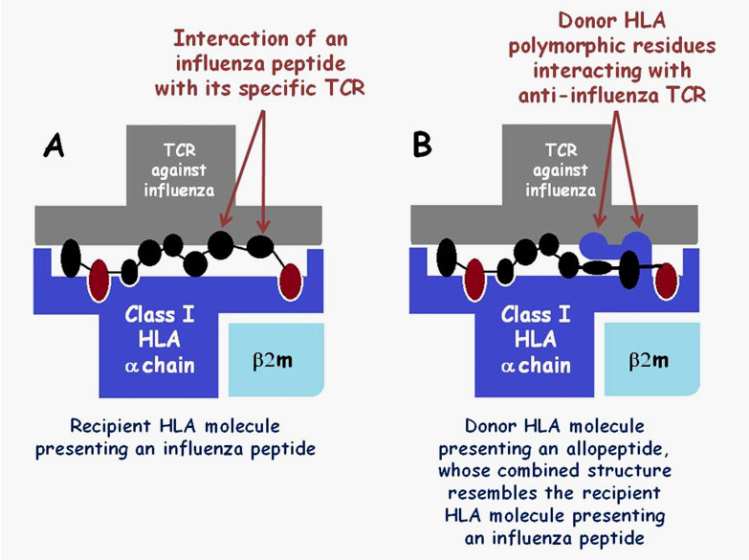

Figure 4: Cross-reaction and the HLA system. TCR have the inherent capacity to interact with HLA molecules, therefore they can easily cross-react with other non-tolerated HLA:peptide complexes molecules, such as allogeneic HLA:peptide complexes. Cross-reaction occurs due to conformational antigenic similarities between the HLA:peptide complex for which a given TCR was specific for and a different allogeneic HLA:peptide complexes. In this pictorial representation, TCR specific for a given influenza peptide (A) can interact with an allogeneic HLA:peptide complex resembling the structure of the original activating HLA:influenza peptide complex (B). In such way, cross-reaction triggers memory-like responses to HLA antigens never encountered by the host.

THE HUMAN MAJOR HISTOCOMPATIBILITY COMPLEX AND ITS GENES

In the human, the MHC loci are located within a 4 million nucleotide base-pair (bp) stretch on the short arm of the chromosome 6 in the region 6p21.3 and it is referred to as the HLA system, because their protein products were first identified on the surface of human leukocytes [20]. There are two main classes of proper MHC/HLA genes, working as antigen-presenting molecules, class I and class II, which are also the main determinants of histocompatibility and the focus of discussion of this chapter (Figure 5). However, a series of genes with different roles in the immune system, including cytokines, heat-shock proteins, enzymes for steroid metabolism and proteins involved in the complement cascade and antigen-processing are located within the MHC loci in

between these two classes. They are referred to as class III MHC molecules by some authors, but others believe this is a misnomer as they are genetically different and have different functions from proper MHC molecules. The latter molecules will not be discussed in the rest of the text.

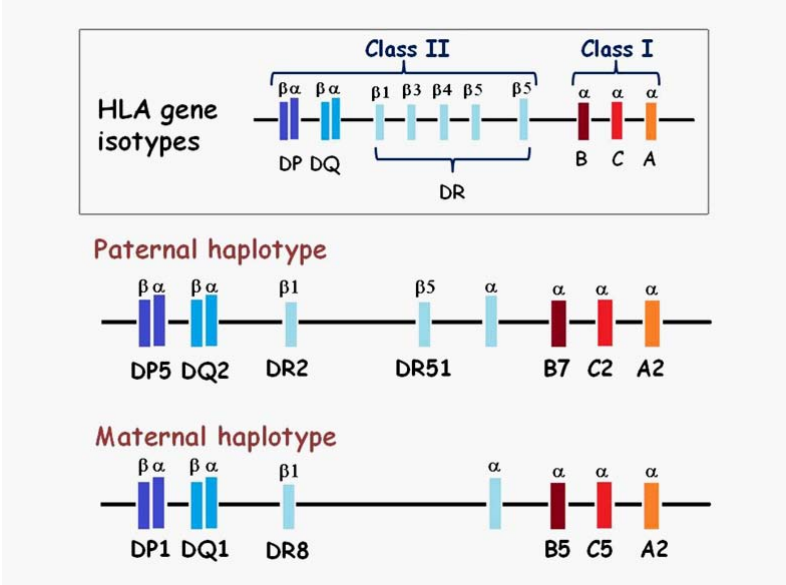

Figure 5: Schematic depiction of the composition of the classical class I and class II genes and their isotypes within the HLA loci on chromosome 6.

The genes encoding class I HLA molecules extend by approximately 2 million nucleotide bp, consisting of three main isotypes HLA-A, -B and -C, which participate in the presentation of endogenous antigens. But other genes encoding distinct non-classical or class I-like molecules (HLA-E, -F, and -G) with roles in natural killer cell function or foeto-maternal immunity; as well as some pseudogenes (HLA-H, -J, -K and -L) and other genes fragments (HLA-N, -P, -S, -T, -U, -V, -W, -X, -Y and -Z), can also be found within this region [21]. The most likely role of these pseudogenes and gene fragments is to serve as genetic reserve to provide genetic material for gene conversion to increase further the diversity of HLA alleles. In contrast, the class II HLA molecules extend for around 1 million nucleotide bp and their main representatives are the HLA-DR, -DP and -DQ molecules.

Expressed class I HLA molecules are composed of two different chains non-covalently bound. Their alpha chain (45 kDa), containing the peptide binding groove, is encoded by the class I MHC genes, while their non-covalently bound beta chain or beta2microglobulin (12 kDa) is encoded on the chromosome 15. Beta2microglobulin binding is necessary for class I MHC molecules expression and for their proper folding for the right conformation of the peptide-binding groove. Their peptide binding groove is closed at the ends and accommodates short peptides ranging from 8-11 amino acids long (Figure 1). This groove has certain pockets (labelled A to F) that are

the actual regions binding the anchor side chains of antigenic peptides. Specific pockets within the groove determine the sets of different peptides able to bind by class I MHC molecules [9,22]. The stoichiometry necessary for proper signalling for $CD8^+$ T cell activation is one class I HLA molecule to one TCR molecule.

Class II HLA molecules, both the alpha and the beta chains, are encoded by the respective genes on the chromosome 6. They are both bound non-covalently and both contribute to the conformational organisation of the peptide binding groove. The peptide binding groove in class II HLA molecules is open at the ends and accommodates larger peptides than for class I HLA molecules, ranging from 15-30 amino acids long. Similar to class I HLA molecules, pockets in the groove of class II HLA molecules for anchor side chains of the antigenic peptides also stabilise the interaction, but their binding to specific peptide side chains is less stringent than for class I HLA molecules. The stoichiometry necessary for proper signalling for $CD4^+$ T cell activation consists of dimers of class II MHC molecules interacting with dimers of TCR molecules.

For both class I and II HLA molecules, most of the polymorphic residues reside in the regions forming the peptide-binding groove. The great polymorphism of HLA genes has been maintained through evolution due to their central role in initiating and regulating immune responses rather than due to their role determining histocompatibility or the risk for rejection. For conventional immune responses, e.g. against infections, the greater the polymorphism within the MHC genes, the greater the advantage a species will have to survive through epidemics, since this will provide that species with a wider responsive repertoire and the capacity to bind different foreign peptides, with a consequent greater capacity to counterattack immunologically. In other words, without the diversity of the MHC molecules, if a given agent mutated in the way that their peptide antigens could not longer sit on the binding groove of the MHC molecules of a given species, the responding species would definitively succumb due to incapacity to defend itself against the threat.

Having two different classes of HLA molecules with different gene representatives or isotypes (isotypic diversity), and each gene isotype having different alleles (allelic variability) is greatly responsible for the diversity of the HLA system at the population level. At the individual level, being most humans heterozygous for HLA alleles and having their HLA alleles on each parental chromosomes expressed co-dominantly on the cell surface, increases further their antigen-presenting repertoire. Furthermore, HLA haplotypes contain different numbers and combinations of distinct expressed HLA-DR beta genes (HLA-DRbeta1, HLA-DRbeta3, HLA-DRbeta4 and HLA-DRbeta5), which contribute to further increase the diversity of the peptides to be presented (Figure 5 and Table 1). Finally, the alpha and beta chains of some of the class II HLA isotypes (in particular HLA-DP and -DQ) have the capacity to dimerise between each other. This process is referred to as cis-dimerisation when occurs between expressed products from the same chromosome, and as trans-dimerisation when occurs between different chromosomes. The total number of HLA alleles, null and expressed as protein products, are reported and updated periodically on the

IMGT/HLA Database [23]. But the total number of existing expressed HLA forms in the entire population and in a given individual might differ depending on how well or not some of the HLA molecules dimerise with each other.

Table 1: Genetic linkage within the HLA-DRB loci. In some HLA haplotypes within the population, some HLA-DRB1 alleles are in linkage disequilibrium with three additional expressed HLA-DRB isotypes found with the HLA-DR loci: HLA-DRB3, HLA-DRB4 and HLA-DRB5; which confer respectively the serological specificities for HLA-DR52, HLA-DR53 and HLA-DR51. Having extra HLA-DRB molecules increases the repertoire of antigenic peptides to be presented to the immune system, but, in transplantation, mismatches within the HLA-DRB isotypes will enhance the alloimmunogenicity of organ transplants.

HLA-DRB1 alleles by serological specificity	Genetically-linked extra HLA-DRB3,4,5 isotypes	HLA-DRB3,4,5 serological specificity
DR1, DR103, DR8, DR10	None	NA
DR3, DR5, DR6, DR11(5), DR12(5), DR13(6), DR14(6), DR1403, DR1404, DR17(3), DR18(3)	DRB3	DR52
DR4, DR7, DR9	DRB4	DR53
DR2, DR15(2), DR16(2)	DRB5	DR51

Unfortunately, in transplantation, these properties are counterproductive as the greater the polymorphic variants expressed by a given donor and the greater the alloantigen density on the foreign cells, the stronger the alloresponse and the potential effector rejection mechanisms deployed by the immune system of the recipient. Therefore, it is of pivotal importance to discuss how histocompatibility is defined operationally in the clinical setting for solid organ transplantation.

HLA MATCHING AND GENETICALLY-RELATED DONATION

In transplantation medicine, HLA matching refers to the degree of similarity of the HLA alleles between a donor and a recipient and is preferentially applicable to genetically related donation, i.e. family members. But for a better understanding of the risk of rejection conferred by different degrees of histocompatibility between related donors and recipients, a brief explanation on the inheritance rules for the HLA genes is pertinent. The set of HLA genes, from both class I and class II loci, in a given chromosome have the peculiar characteristic of being inherited together in block to further generations, and this complete set of closely related HLA alleles is referred to as a haplotype. Thus, any individual would receive, following Mendelian rules, one maternally-derived and one paternally-derived haplotype, each consisting of an entire set of alleles of class I HLA (HLA-A, -B, -C) and class II HLA (HLA-DR, -DP and –DQ) genes (Figure 6). These HLA alleles are inherited as haplotypes because the recombination rate within the HLA genes during meiosis is lower than expected to most genes [24], so the HLA genes are said to be in linkage disequilibrium, as the genetic equilibrium would be the mixing of parental alleles before segregating them to other generations, rather than being linked together. As a consequence, every child would share

a haplotype with each parent, which has been used for paternity assignment. In addition, there is a 25% probability of two siblings sharing both parental haplotypes (HLA identical/HLA identical by descent); a 50% probability of sharing at least one parental haplotype (HLA haploidentical); and 25% probability of inheriting totally different parental haplotypes (HLA disparate or non-identical). A useful clinical consequence of all this is that HLA matching can be achieved more easily among family members than among unrelated individuals, or even among individuals from the same ethnic group or with similar population migratory history than among those from distant ethnical or geographical backgrounds, depending on the allele and haplotype frequencies in the population.

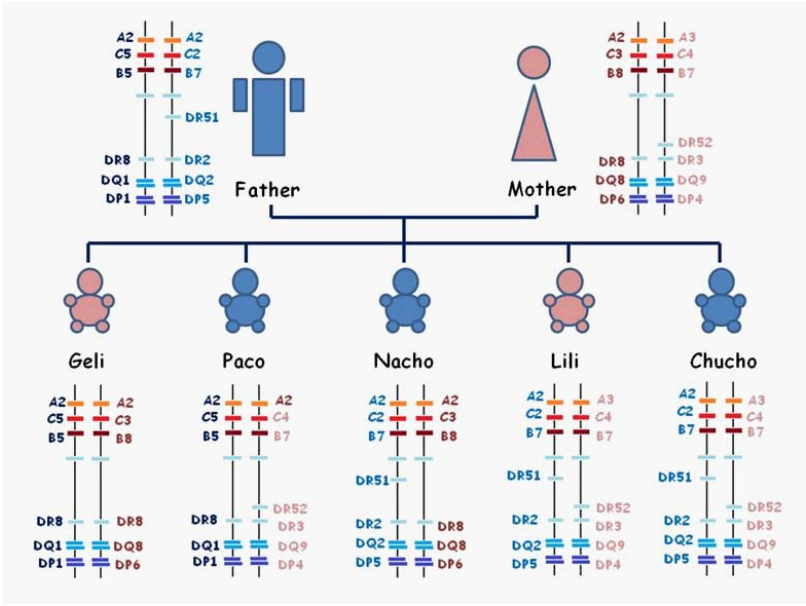

Figure 6: Inheritance rules in the HLA system. Depiction of a family tree demonstrating the Mendelian segregation of paternal HLA haplotypes in linkage disequilibrium. One quarter of the progeny share both parental haplotypes (HLA identical); half of them shares at least one parental haplotype (HLA haploidentical); and one quarter of the children have inherited totally different parental haplotypes (HLA disparate or non-identical). Despite the high linkage disequilibrium within the HLA system, some children show recombination, like Paco, showing recombination in the maternally-derived HLA-A locus. When organ donation is pursued, HLA typing of all family members allows a better selection of potential donors. As an example, in the hypothetic case that Chucho would require an organ transplant, Geli will provide the worst HLA match. Paco and Nacho would be fair candidates because they are haploidentical to Chucho. Lili is HLA identical to Chucho and will provide the best possible HLA match. In none of these transplant situations there would be a mismatch of the H-Y system as the recipient, Chucho, is a male. However, mismatches at other polymorphic loci are possible among members of this family.

In genetically-related donation the concept of non-inherited HLA antigens is worthy of a brief description. We have above described the role of the inherited maternal and paternal HLA haplotypes, which can also be referred to as the inherited maternal antigens (IMA) and the inherited paternal antigens (IPA). But the effect of the so-called non-inherited maternal antigens (NIMA) is of academic interest and clinical relevance in certain scenarios. NIMA consist of the HLA alleles present in the maternal haplotype non-inherited to a given child but to which the immune system of that child was exposed during foetal development and potentially developed tolerance. Because of this, transplants derived from mothers are less immunogenic than from fathers, and transplants performed between siblings when the mismatched haplotype is part of the NIMA do better than when the mismatched antigens derive from the non-inherited paternal antigens (NIPA) [25]. Figure 7 depicts the role of NIMA in solid organ transplantation.

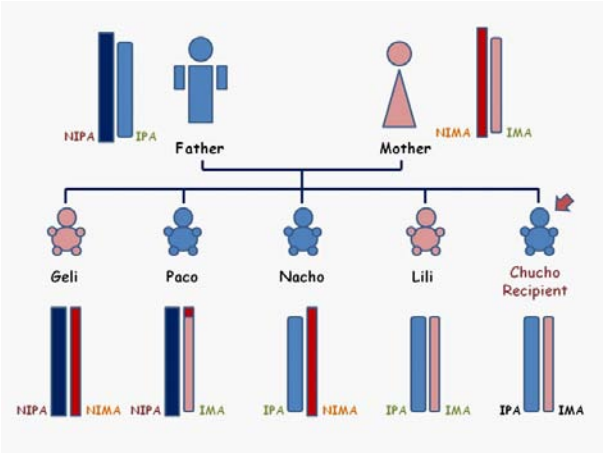

Figure 7: Mismatch at the level of the non-inherited maternal HLA antigens (NIMA). NIMA consist of the HLA alleles maternally-derived non-inherited to a given member of her progeny but to which the immune system of her child was exposed to them during foetal development and potentially developed immune tolerance. As a consequence, transplants derived from mothers are less immunogenic than from fathers, and transplants performed between siblings when the mismatched haplotype is part of the NIMA do better than when the mismatched antigens are part of the NIPA. For example, in the hypothetic case that Chucho required an organ transplant, Lili would provide the best possible match, being HLA identical to him, and Geli would provide the worst HLA match. Nacho would be better donor candidate in comparison to Paco (both being haploidentical), because the non-inherited HLA haplotype in Paco is part of the NIPA, having Chucho no exposure to them during foetal life. The haplotype mismatch with Nacho is part of the NIMA, and Chucho potentially is tolerant to them. Likewise, if any of the parents would be the selected donor, the mother would be better candidate over the father. Finally, among haploidentical combinations, the mother would be always better candidate over Nacho, because of the exposure to other alloantigens from the mother during foetal development, which did not take place if Nacho were to be the donor.

However, most of the transplants performed worldwide are between genetically-unrelated individuals and the degree of HLA mismatch rather than HLA match is a more adequate measurement of histocompatibility, or rather, lack of histocompatibility and risk for an alloreactive response.

HLA MISMATCHING AND GENETICALLY-UNRELATED DONATION

HLA mismatching refers to the degree of differences among the HLA alleles from the donor and a recipient, and this concept has more clinical relevance for genetically-unrelated donation, i.e. non-family members or deceased donors. However, what matters immunologically is the directionality of the mismatches, i.e. not just the nominal differences in the HLA alleles between the donor and the recipient, but the differences in the HLA alleles of the donor that are absent in the recipient and thus able to elicit an immune response by the recipient (Figure 2, note the vector denoting the directionality of the mismatch). These differences are quantified and referred to as the number of HLA mismatches. As mentioned before, it is advantageous to have different sets of HLA alleles for a broader immune responsiveness, but in transplantation this is counterproductive as those differences or mismatches in the HLA phenotypes between donors and recipients are the main stimulators of alloresponses. It has been demonstrated that the number of HLA mismatches in clinical transplantation is associated with the risk of acute rejection and worse transplant outcomes [26-27], and organ allocation policies in many international centres give preference to donor:recipient pairs with the best possible HLA match or the minimal HLA mismatch. In fact, the negative effect of HLA mismatch appears to be more apparent in deceased donor transplantation, perhaps because the HLA mismatches become more immunogenic after the massive activation of the innate system and consequent immunostimulation due to the ischaemia-reperfusion injury. It is important also to remember that the effect of HLA mismatches, allostimulating the immune system of the recipient, will persist throughout the entire life of the transplant; and this could become more relevant in organs with lower tissue quality or parenchymal reserve, characteristic of many deceased donor-derived organs, when compared to those derived from live donors.

With modern immunosuppression, the big negative influence that HLA mismatches had for transplant outcomes in the past has become less apparent, especially in short term outcomes [26-27]. However, long term outcomes still remain suboptimal despite safer and more effective immunosuppressants, and in spite of improvements in transplantation logistics and patient care protocols [26-27]. Cold-ischaemia and its duration are important sources of injury and activation of the innate system in deceased donor transplantation, but it should never forgotten that cold-ischaemia injury and other peri-transplant insults are transient events, while the HLA mismatches are there to remain, in particular for class I HLA molecules, continuously serving as source for allostimulation. Furthermore, the immune response against those HLA mismatches will tend naturally to escalate due to chronic alloantigen persistence and perennial stimulation of the immune system, with the consequent development of the indolent process of chronic rejection.

Thus, efforts to offer the best possible matched organ pay out in the long run, though this is not possible in many occasions in clinical transplantation.

HLA MISMATCHES AND THE PRODUCTION OF ALLOANTIBODIES

HLA mismatches are not only the trigger for alloreactive T cells to destroy the transplant parenchyma, they also lead to the formation of alloreactive anti-HLA antibodies; and together they contribute to acute and chronic rejection, and the eventual immunologically-mediated transplant loss [28]. But it is not the number of mismatched antigens what stimulate the immune system, but rather the antigenic differences, even of one single aminoacid, between the HLA molecules of the donor and the recipient. Thus, the traditional model of HLA matching, counting the absolute number of HLA mismatches, fails to identify mismatches that are more immunogenic from those less immunogenic. For instance, HLA-DR mismatches appear to confer poorer prognosis than mismatches in class I HLA antigens [29]. Therefore, two mismatches in HLA I class molecules will not have the same immunological impact than two mismatches in class II HLA molecules. Thus, achieving the best possible antigenic match, or the most acceptable antigenic mismatch (and likely least immunogenic), according to the HLAMatchmaker principle [6] shall instead reflect more realistically the impact of HLA mismatch in solid organ transplantation.

The HLAMatchmaker is an interesting computer algorithm assessing the degree of HLA matching or mismatching according to the number of antigenic epitopes shared or not between donor and recipient. These antigenic epitopes are referred to as eplets, which represent the conformational epitopes formed by the polymorphic aminoacid residues able to elicit an immune alloresponse, more specifically an antibody response [6]. The number of mismatched HLA eplets has been shown to associate with transplant outcomes and anti-HLA antibody production in kidney transplant patients [30]. Furthermore, the HLA matching based on this algorithm has proven to increase the chances to find suitable donors in highly sensitised patients [31] through the principle of 'acceptable vs taboo mismatches' (Figure 2). Thus, an individual can be mismatched according to the traditional HLA matching model, but at the eplet level the patient might not have an unacceptable mismatch, because donor and recipient might share the same eplets between their respective HLA molecules, or because the dose of eplets given by the donor HLA molecules is lower than in other donor-recipient HLA allele combinations. Therefore, HLAMatchmaker appears to be the most reliably and intelligent method up to date to relate HLA matching/mismatching with transplant outcomes and to quantify the degree of mismatch by counting the number of mismatched epitopes or eplets, which can be regarded as the epitope or eplet load. However, the HLAMatchmaker can only be applied if four-digit split HLA typing resolution has been achieved or inferred through a four-digit convertor provided by the HLAMatchmaker founders.

The detection of anti-HLA antibodies and donor-specific anti-HLA antibodies, is an important part of the immune risk assessment, donor-recipient serological histocompatibility evaluation (cross-match) and deceased donor organ allocation. Alloantibodies can be pre-formed, that is,

present before transplantation as a consequence of previous alloimmunisations or through the phenomenon of cross-reaction. Alternatively, these alloantibodies can be formed de novo as a consequence of allostimulation by the HLA mismatches as discussed above. Both pre-formed and de novo anti-HLA antibodies, especially if directed to the donor, and worse if able to fix complement, associate with the risk of antibody-mediated rejection and reduced transplant survival [28]. A detailed discussion of the methods for serological histocompatibility testing in the clinical setting and the means to de-sensitise patients (remove the alloantibodies or reduce the synthesis of alloantibodies through immunomodulation) are important but beyond the scope of this review chapter.

TCR EPITOPE MATCHING

As mentioned previously, the peptide binding groove of MHC molecules has pockets defining the types of peptides bound to their surface, and the peptides have anchor side-chains able to bind those pockets. Therefore, from the theoretical stand-point, it is possible to infer from the known amino acid sequence of HLA alleles from the donor and the recipient, whether certain peptides from the donor HLA molecules will be generated in certain recipients and whether they would be able to bind stably to their recipient's HLA molecules, and to contribute to the allostimulation via the indirect pathway of allorecognition. Being able to predict such interactions would be of clinical relevance as the indirect pathway of allorecognition appears to perpetuate a state of chronic rejection, which is very difficult to manage and eventually leads to transplant loss. Available prediction tools like the Immune Epitope Data Base and Analysis Resource [22] could be used to facilitate this enterprise by experienced labs. Furthermore, HLA matching at the T cell epitope or cryptotope level would be also expected to be affected by recipient's polymorphic profile in molecules involved in antigenic peptide generation and perhaps by the TCR repertoire of each recipient (shaped according to their own HLA alleles). It might or not be the right 'match' for the HLAMatchmaker but could provide a fair 'deal' in the assessment of compatibility between the donor and the recipient (potentially a new tool dubbed as HLAMatchdealer).

HLA ALLELE IDENTIFICATION AND HLA NOMENCLATURE

In the clinical setting, the molecular identification of the set of HLA alleles and molecules via low two-digit resolution is performed by serological typing, typically using complement-based or flow cytometric techniques, while the four-digit high-resolution and the allelic resolution involves molecular typing using polymerase chain reaction, DNA sequencing and/or DNA primer hybridisation methods. Molecular typing has been able to identify a large number of HLA alleles and pseudogenes and provides the basis for the current detailed HLA nomenclature, which is suggested by the World Health Organisation HLA Nomenclature Committee [21]. Every HLA allele is first named according to its HLA isotype (eg HLA-A or HLA–DR and so on) followed by an asterisk. Then, a series of digits separated by colons are used to designate different alleles. The first two digits indicate the allele family, corresponding in most occasions to the serological

equivalent; the next two to three digits represent the allele number according to its nucleotide sequence, within that allele family, and each allele consisting of a HLA protein differing at least in 1 to 2 amino acids; the next two digits indicate silent synonymous nucleotide differences; and the last two digits indicate differences in the introns or in the 3' or 5' non-coding regions. After the numerical designation, further letters are used as follows to designate other characteristics of the HLA alleles: (N) to indicate non-coding sequences or null alleles; (L) low expression; (S) secreted, soluble but not surface expression; (C) cytoplasmic expression only; (Q) questionable expression; (A) aberrant or doubtful expression. For example, a given allele could be denoted as HLA-A*01:01:111:01A. A complete list of currently identified HLA alleles is published in the HLA dictionary and updated regularly [23].

MINOR HISTOCOMPATIBILITY ANTIGENS

Minor histocompatibility antigens are alloantigens with apparently lesser contribution in the allostimulation of recipients by the transplanted organ. They constitute the set of all polymorphic genes (or antigens) different among genetically-different individuals, able to elicit an alloreactive immune response. A classical example of this category of alloantigens is the H-Y antigen system, present in the Y chromosome, therefore only in males. A H-Y mismatch is therefore able to elicit an immune response when female individuals receive organs from male individuals. Although they have lesser immunogenicity than HLA antigens, their contribution is not clinically insignificant as minor histocompatibility mismatches trigger rejection episodes in animal models fully MHC-matched, but at slower pace and strength. Minor histocompatibility antigens are presented to T cells in a similar fashion to conventional environmental antigens, on the surface of recipient-derived APC, as foreign peptides bound to self-MHC molecules.

MAJOR HISTOCOMPATIBILITY COMPLEX CLASS I CHAIN-RELATED PROTEINS

Major histocompatibility complex class I chain-related protein A (MICA) and B (MICB) are surface polymorphic proteins involved in innate immunity. They appear not to present antigenic peptides to T cells but this is controversial. However, they are believed to be stress molecules recognised by the activating receptor NKG2D on natural killer cell [32] and the TCR on gamma-delta T cells [33]. MICA and MICB genes are located within the HLA loci, but their protein products do not form dimers with beta2microglobulin. Mismatches in the MICA and MICB antigens can induce the production of alloantibodies, which have been associated with antibody-mediated transplant rejection.

CONCLUDING REMARKS

Transplantation was regarded as the most significant breakthrough in Immunology and Medicine in the last century. With no doubt, deciphering transplantation tolerance and achieving it reliably in the clinical setting would be of a greater impact to humankind. The differences

between the HLA molecules of donors and recipients are the initial trigger to allostimulate the immune system of the recipient, and those differences are to persist for the entire life of the transplant, simply escalating the alloresponse and aggravating tissue injury. Until the advent of targeted specific immunotherapies or tolerogenic protocols, we shall aim to ensure the best possible histocompatibility between the donor and the recipient, and to conceptualise and utilise HLA matching from a less conventional perspective. Our returns will be the optimisation of solid organ allocation, the prolongation of the life of transplanted organs and the reduction of the side-effects and costs of enhanced immunosuppression and anti-rejection therapies.

DISCLAIMER

The opinions expressed in this chapter are those of the author and might or not reflect those of his scientific affiliations. The scientific content of this chapter is of educational purpose and not aimed to serve as a clinical guidance, but to inspire academic reflections. Its content is also intended to be comprehensive and accurate to the best knowledge of the author, but not exhaustive. The author can be contacted for discussion, suggestions or corrections.

ACKNOWLEDGEMENTS

The author would like to express gratitude to his wife Susan for her love and support, and to his daughter Ayra for being his delightful inspiration. Finally, a big thank you to El Mesie for sponsoring the submission fees, and to The Editor, Dr. Shabir Ahmad Mir, for inviting him to contribute to this e-book.

References

1. Cohen DJ, St Martin L, Christensen LL, Bloom RD, Sung RS. Kidney and pancreas transplantation in the United States, 1995-2004. Am J Transplant. 2006; 6: 1153-1169.
2. Scandling JD, Busque S, Shizuru JA, Lowsky R, Hoppe R. Chimerism, graft survival, and withdrawal of immunosuppressive drugs in HLA matched and mismatched patients after living donor kidney and hematopoietic cell transplantation. Am J Transplant. 2015; 15: 695-704.
3. Kawai T, Sachs DH, Sprangers B, Spitzer TR, Saidman SL. Long-term results in recipients of combined HLA-mismatched kidney and bone marrow transplantation without maintenance immunosuppression. Am J Transplant. 2014; 14: 1599-1611.
4. Gorer PA, Lyman S, Snell GD. Studies on the genetic and antigenic basis of tumour transplantation. Linkage between a histocompatibility gene and "fused" in mice. Proceedings of the Royal Society of London - Series B: Biological Sciences. 1948;135: 499-505.
5. Doherty PC, Zinkernagel RM. A biological role for the major histocompatibility antigens. Lancet. 1975; 1: 1406-1409.
6. Duquesnoy RJ, Marrari M. HLAMatchmaker-based definition of structural human leukocyte antigen epitopes detected by alloantibodies. Curr Opin Organ Transplant. 2009; 14: 403-409.
7. Alam SM, Travers PJ, Wung JL, Nasholds W, Redpath S. T-cell-receptor affinity and thymocyte positive selection. Nature. 1996; 381: 616-620.
8. Ashton-Rickardt PG, Bandeira A, Delaney JR, Van Kaer L, Pircher HP. Evidence for a differential avidity model of T cell selection in the thymus. Cell. 1994; 76: 651-663.
9. Germain RN, Margulies DH. The biochemistry and cell biology of antigen processing and presentation. Annu Rev Immunol. 1993; 11: 403-450.
10. Heath WR, Carbone FR. Cross-presentation, dendritic cells, tolerance and immunity. Annu Rev Immunol. 2001; 19: 47-64.
11. Andreola G, Chittenden M, Shaffer J, Cosimi AB, Kawai T. Mechanisms of donor-specific tolerance in recipients of haploidentical combined bone marrow/kidney transplantation. Am J Transplant. 2011; 11: 1236-1247.

12. Joffre O, Santolaria T, Calise D, Al Saati T, Hudrisier D. Prevention of acute and chronic allograft rejection with CD4+CD25+Foxp3+ regulatory T lymphocytes. Nat Med. 2008; 14: 88-92.

13. Benghiat FS, Graca L, Braun MY, Detienne S, Moore F. Critical influence of natural regulatory CD25+ T cells on the fate of allografts in the absence of immunosuppression. Transplantation. 2005; 79: 648-654.

14. Zheng XX, Sanchez-Fueyo A, Domenig C, Strom TB. The balance of deletion and regulation in allograft tolerance. Immunol Rev. 2003; 196: 75-84.

15. Salcido-Ochoa F, Yusof N, Hue SS, Haase D, Kee T. Are we ready for the use of foxp3(+) regulatory T cells for immunodiagnosis and immunotherapy in kidney transplantation? J Transplant. 2012; 2012: 397952.

16. Game DS, Lechler RI. Pathways of allorecognition: implications for transplantation tolerance. Transpl Immunol. 2002; 10: 101-108.

17. Herrera OB, Golshayan D, Tibbott R, Salcido Ochoa F, James MJ. A novel pathway of alloantigen presentation by dendritic cells. J Immunol. 2004; 173: 4828-4837.

18. Matzinger P, Bevan MJ. Hypothesis: why do so many lymphocytes respond to major histocompatibility antigens? Cell Immunol. 1977; 29: 1-5.

19. Sherman LA, Chattopadhyay S. The molecular basis of allorecognition. Annu Rev Immunol. 1993; 11: 385-402.

20. DAUSSET J. [Iso-leuko-antibodies]. Acta Haematol. 1958; 20: 156-166.

21. Marsh SG, Albert ED, Bodmer WF, Bontrop RE, Dupont B. Nomenclature for factors of the HLA system, 2010. Tissue Antigens. 2010; 75: 291-455.

22. Vita R, Overton JA, Greenbaum JA, Ponomarenko J, Clark JD. The immune epitope database (IEDB) 3.0. Nucleic Acids Res. 2015; 43: D405-412.

23. Robinson J, Halliwell JA, Hayhurst JD, Flicek P, Parham P. The IPD and IMGT/HLA database: allele variant databases. Nucleic Acids Res. 2015; 43: D423-431.

24. Martin M, Mann D, Carrington M. Recombination rates across the HLA complex: use of microsatellites as a rapid screen for recombinant chromosomes. Hum Mol Genet. 1995; 4: 423-428.

25. Dupont E. NIMA (non-inherited maternal antigens) versus NIPA (non-inherited paternal antigens) and tolerance of human kidney graft. Nephrol Dial Transplant. 1999; 14: 547-548.

26. Opelz G, Wujciak T, Döhler B, Scherer S, Mytilineos J. HLA compatibility and organ transplant survival. Collaborative Transplant Study. Rev Immunogenet. 1999; 1: 334-342.

27. Opelz G, Mytilineos J, Scherer S, Dunckley H, Trejaut J. Survival of DNA HLA-DR typed and matched cadaver kidney transplants. The Collaborative Transplant Study. Lancet. 1991; 338: 461-463.

28. Loupy A, Lefaucheur C, Vernerey D, Prugger C, Duong van Huyen JP. Complement-binding anti-HLA antibodies and kidney-allograft survival. N Engl J Med. 2013; 369: 1215-1226.

29. Fuggle SV, Johnson RJ, Rudge CJ, Forsythe JL. Human leukocyte antigen and the allocation of kidneys from cadaver donors in the United Kingdom. Transplantation. 2004; 77: 618-620.

30. Duquesnoy RJ, Takemoto S, de Lange P, Doxiadis II, Schreuder GM, et al. HLAMatchmaker: A Molecularly Based Algorithm For Histocompatibility Determination III Effect of matching at the HLA-A,B amino acid triplet level on kidney transplant survival. Transplantation. 2003; 75: 884-889.

31. Duquesnoy RJ, Witvliet M, Doxiadis II, de Fijter H, Claas FH. HLAMatchmaker-Based Strategy to Identify Acceptable HLA Class I Mismatches for Highly Sensitized Kidney Transplant Candidates. Transpl Int. 2004; 17: 22-30.

32. Bauer S, Groh V, Wu J, Steinle A, Phillips JH. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. Science. 1999; 285: 727-729.

33. Groh V, Steinle A, Bauer S, Spies T. Recognition of stress-induced MHC molecules by intestinal epithelial gammadelta T cells. Science. 1998; 279: 1737-1740.