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HLA COMPLEX: BRIEF HISTORY, GENERAL CHARACTERIZATION AND IMPACT ON KIDNEY TRANSPLANTATION

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ABSTRACT

Since the beginning of renal transplantation, the incompatibility of tissues between donor and recipient is known to be one the key barriers to a successful procedure, predisposing significantly to rejection episodes. The importance of human Major Histocompatibility Complex (MHC)/Human Leukocyte Antigen (HLA) complex in kidney transplantation, is reviewed in this article. The chosen articles reveal the most important moments and achievements in this field, since the beginning of the 20th century.

This review is divided in four major topics, which englobe the main goals of this article: the history behind the discovery of the HLA; the general characterization of the HLA complex, emphasizing the relevant aspects of HLA-class I and HLA-class II structure, genetics and main functions; the impact of the HLA complex matching in kidney transplantation, including an explanation of its mechanisms and role in hyperacute rejection, cell-mediated acute rejection, antibody-mediated acute rejection and chronic allograft dysfunction; the more recent aspects on how to proceed to the immunological study of a patient before, during and after a renal transplantation, discussing the pros and cons of each step and techniques used, and the newest discoveries in the field.

The progress achieved in understanding the link between HLA complex and renal transplantation has been shown to radically improve graft survival and to reduce the incidence of rejection episodes. However, there is still a long way to improve, as the impact of the non-HLA antigens remains misunderstood and as more efficient methods to study the patients still under discussion.

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INTRODUCTION

Transplantation and immunology. Two complex and ever-expanding fields in medicine, complementing each other. The human Major Histocompatibility Complex (MHC) or Human Leukocyte Antigen (HLA) Complex impacts significantly on solid organ transplantation outcomes, particularly in kidney transplantation. Thus, this article purposes to review the importance of HLA complex in kidney transplantation, since its discovery up to today. The article will start by putting HLA in an historical perspective, revealing the most important steps and scientists linked to its discovery. Secondly, the general characteristics of the HLA will be addressed, with special interest on its molecules structure, genetics and function. This general explanation will make it possible to fully understand the impact of HLA complex in kidney transplantation, and how rejection and other postransplantation comorbidities could be prevented by understanding the immunological mechanisms of the HLA complex. Finally, the steps to study a patient's HLA specificities prior, during and after kidney transplantation will be reviewed, focusing in the techniques used in most histocompatibility laboratories.

METHODS

A systematic search was performed through the search engine Google Scholar and directly in three databases, specifically PubMed, Clinical Key and Mendeley. Recognized immunology books, such as Janeway's Immunobiology(1), Kuby Immunology(2) and Graham's The Immune System(3), were also important to elaborate this review.

Data sources

Search of these databases used the terms "HLA", "MHC", "transplantation", "kidney", "history", "rejection", "detection", "HLA antibodies" and "HLA antigens". The search strategy

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used both keywords and MeSH terms. No limits were placed on the year or language of publication. References identified from bibliographies of pertinent articles or books were also retrieved.

Selection of articles

The aforementioned database was searched and the abstracts of resulting articles were analyzed and excluded if lacking in relevancy. Then, all the selected articles were downloaded and read in full.

RESULTS

A. The History of HLA in 5 points

First steps: Discovery of the MHC in the mouse

The historical paths of Human Transplantation and Human Leukocyte Antigens (HLA*)* are closely linked. However, it all started with studies in mice (4). In 1903, **Paul Erlich** began to transplant tumours in mice. Later that year, **Carl Jensen**, a Danish biologist, recognized that the failure of tumour homografts was an immune reaction (5). Having in mind the main problem of using tumour homografts – which, from time to time, would overwhelm the recipient before they were rejected – **George Schone** started using skin grafts instead, determining, in 1912, that homografts always failed and that subsequent grafts from the same donor failed more rapidly than the first (5). Meanwhile, two geneticists studying tumour biology, **E. E. Tyzzer** and **C. C. Little**, realized, through tumour transplantations in the offspring of crosses between mice that were susceptible or resistant to an allogeneic tumour that susceptibility to the growth of allogeneic tumours was genetically determined (4). However, the nature of these genes was unknown.

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Behind all the uncertainty surrounding transplantation immunology, solid organs transplantation's potential as a last-line therapy appealed to many surgeons, leading to numerous experimental kidney transplants – the first successful ones in animal, a dog autotransplant and a dog-to-goat xenograft, by **Emerich Ulmann**, in 1902 (6); and, in humans, in 1906, by **Mathieu Jaboulay** (7), using animal donors. However, these xenografts never functioned more than a few days. **Alexis Carrel**, surgeon that dedicated almost all of his life performing transplants and winner of 1912 Nobel Prize for his pioneering work blood vessel suturing and organ transplantation, realized that, no matter how successful his autograft transplants would be, homograft ones never were. He tried to avoid this with matching of donor and recipient and recipient's irradiation – which was found to improve results, although this experiences with **James B. Murphy** were never published (8) – not never achieved great success. These misadventures from various researchers lead to disinterest in the field of transplantation in the two precedent decades.

FIGURE 1- SIR PETER MEDAWAR (1915 – 1987), ALSO KNOWN AS "FATHER OF TRANSPLANTATION". RESPONSIBLE FOR DISCOVERY OF ACQUIRED IMMUNOLOGICAL TOLERANCE

Previously to the historical works of 1960 Nobel Prize winner **Peter Medawar**'s (figure 1) in 1950's (9)(10), many other important achievements were reached in transplantation immunology, more specifically in the field of Histocompatibility.

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Peter A. Gorer (figure 2) discovered, in 1936, an antigen responsible for rejection in mice. He realized that this antigen – named by him as *antigen II* - was an important resistance factor to the growth of an allogeneic tumour in mice when present in the donor and absent in the recipient (11). Combining previous conclusions from **George D. Snell** (figure 3) findings while studying tumour resistance genes, together they realized that this antigen II was encoded by one of three alleles at the the H locus, allele that would be named *histocompatibility locus 2* or *H-2*.

FIGURE 2 - PETER GORER
(1907 – 1961)
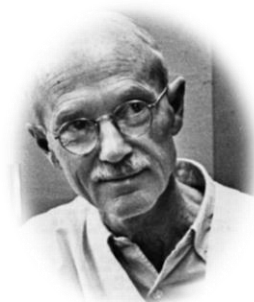
FIGURE 3 - GEORGE SNELL
(1903-1996)

The work of Gorer and Snell established that the H-2 locus encoded major histocompatibility antigens, capable of inducing quick rejection, compared with weaker or minor histocompatibility antigens, encoded by other loci. The H-2 locus therefore became the major histocompatibility locus in mice. Other H loci became minor H loci. Being found many new H-2 loci, the H-2 locus became the H-2 complex, or the *major histocompatibility complex*, *MHC*, in the mouse (4).

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Laying foundations: the 'first' HLA antigens

FIGURE 4 - JEAN DAUSSET (1916-2009)

In 1958, **Jean Dausset** (figure 4) was credited with the discovery of the first HLA antigens (12), realizing from the beginning its importance in tissue transplantation. He achieved this by using sera from patients who had received multiple blood transfusions. These sera reacted with many individuals' leucocytes, but not all, suggesting the existence of an alloantigen on human leucocytes. He named this antigen MAC, in order to honour three volunteers that helped them in his experiments. Today, we know it as HLA-A2.

Jon van Rood and **Rose Payne** followed this finding, using sera from multiparous women, and identified more alleles: respectively, 4a and 4b (nowadays known as HLA-Bw4 and -Bw6, respectively); LA-1 and LA-2 (HLA-A1 and HLA-A2, respectively).

Several other investigators also made original or first identifications of leucocyte HLA antigens in these early days of HLA. They included, among others, **Bernard Amos**, **Richard Batchelor**; **Ruggero Ceppellini**, **Paul Engelfriet, Wolfgang Mayr**, **Flemming Kissmeyer-Nielsen**, **Ray Shulman**, **Paul Terasaki, Roy Walford,** and **Erik Thorsby.**

Communication and Sharing in Science: The International Histocompatibility Workshops and HLA Nomenclature Committee

Having in mind the complexity of the relationships between different HLA antigens, and its subjacent polymorphisms and genetics, International Histocompatibility Workshops (IHWSs) were established. Their creation improved the communication of new findings and the collaboration between colleagues working in similar projects, speeding up the whole process.

The first IHWS was held in 1964, by Bernard Amos, being the major aim to compare different different techniques to detect leucocyte antigens. The results were inconsistent and only in the

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third IHWS, in 1967, lymphomicrotoxicity was recognized as the standard serological typing technique for HLA antigens. This technique was initially developed by Terasaki and **McClelland**. Later, in this review, we will address these techniques more profoundly. The second IHWS, in 1965, showed that several local specificities, defined in one laboratory, were indeed identical or almost (13). Moreover, Dausset and van Rood suggested that most of the antigens they could identify were controlled by a single chromosomal complex.

In 1968, the HLA Nomenclature Committee was created by the World Health Organization (WHO), still having the responsibility for giving official names to HLA specificities and loci. By establishing a common uniform nomenclature and, thus, avoiding various local specificities, this committee has been another crucial step to unravel the complexity of HLA genes and their products. Kissmeyer-Nielsen, F. Svejgaard, A. Hauge, M.

Research continues: HLA locus ou loci?
HLA research continued as a hot topic throughout the late 1960's and 1970's. In 1966, **Bodmer** and Payne were the first to propose two HLA loci (14). But only two years later Kissmeyer-Nielsen confirmed their existence: LA (later known as HLA-A) and 4 (later known as HLA-B) (15). In 1970, a new third locus – AJ, later know as HLA-C - was found by a Scandinavian group of researchers (16). These three loci were classified by J. Klein as Class I, more precisely, classical Class I antigens, as additional class I antigens HLA-G, -E and -F, with a more limited tissue distribution, were later found and named as non-classical Class I antigens. Class II antigens, HLA-DR,-DQ and DP, were a major topic at the sixth IHWS, in 1975, and at the seventh IHWS, in 1977, after being recognized as lymphocyte-determinants in man – contrary to Class I, which were described as serologically determinants – by **Mempel** et al.(17)

By the early 1980's, the basic knowledge about HLA was already known: the HLA chromosomal region, found to be present on the short arm of chromosome number 6, encoded

six different very polymorphic series of determinants: A, B and C that were present on most nucleated cells and DR, DQ and DP that were mainly present on antigen-presenting cells (APC), such as B cells, monocytes and dendritic cells. The term *haplotype,* for the genetic information carried by each of the two HLA chromosomal regions of an individual, had also been introduced by Cepellini, in 1967.

Nowadays, in the extended HLA complex covering a total of 7.6 Mb, as many as 252 genes have been found expressed, of which approximately 28% may have immune functions (18).

The importance of HLA in Transplantation: HLA as the MHC in human and its immuno-biological function

The first evidence that HLA antigens were strong histocompatibility antigens came from skin grafting experiments in 1960's, thanks to Dausset, van Rood and their co-workers. This was confirmed by Ceppellini et al. (19) and Amos et al (20). Both concluded that first-set skin grafts between HLA-identical siblings had a significantly longer survival time than skin grafts between siblings differing for one or two HLA haplotypes.

The first data suggesting a correlation between HLA matching and kidney allograft survival were presented by Terasaki and coworkers as early as in 1965 (21). Gathering the results from skin and kidney grafting, it could firmly be concluded that the HLA complex was the MHC in man, as it had been previously shown for H-2 in mice.

At the beginning of the 1970s, it was fully accepted that the survival of kidneys transplanted between HLA-identical siblings was superior to all other combinations. This way, HLA matching became of general use in kidney transplantations from living-related donor, as well as from cadaveric donors. However, a report from Terasaki's research group left some doubts about this last option, leaving HLA matching in cadaveric donor transplantation into a state of limbo.

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The discovery of the HLA-DR antigens changed all this matter, after studies from three independent groups, released in 1978, showing beneficial effects of matching for the HLA-DR antigens in cadaveric kidney transplantation (22–24). As for bone marrow transplantation, HLA matching is universally accepted. Further, in this review, we will discuss the current use of HLA matching in solid organs transplantation, more specifically in kidney transplantation

HLA Class I and Class II antigens' role as strong histocompatibility antigens was sure, but their immune-biological function was yet to be fully understood.

In 1963, **Benacerraf** reports that the antibody response in guinea pigs to a particular synthetic polypeptide antigen was controlled by a single gene (25). Genes like this, controlling specific immune responses, were later called *Ir* genes. For helping to discover the Ir genes, Benacerraf won the 1980 Nobel Prize, shared with Snell and Dausset.

In 1968 and 1969, **Hugh McDevitt** showed that mice's antibody response to a series of synthetic polypeptide antigens was a genetic trait that was closely linked to the H-2 complex (26,27). These contributions from McDevitt were the takeoff needed to understand MHC antigen's immune-biological function. Never the left, some doubts surrounded the dynamic between MHC and Ir genes. Later, it was shown these genes were encoded by two different loci in H-2 complex, I-A and I-E, corresponding to HLA-DQ and -DR, respectively, that is the class II antigens in mice (28).

In 1972, it was first shown that cooperation between T and B cells required MHC compatibility between the interacting cells (29). The following year, it was shown that the same was true for the interaction of macrophage-associated antigen with T cells (30). However, only in 1974, **Rolf Zinkernagel and Peter Doherty** (figure 5) introduce the term *MHC restriction* after experiments with mice infected with lymphocyte choriomeningitis virus, by showing that T-

cell recognition of antigen from the virus was restricted by the MHC antigens of the T-cell donor (31). Finally, we knew that the MHC antigens were directly involved in T-cell recognition of antigens.

To explain these results, they suggested that T-cell recognition by cytotoxic (CD8) T-cells and by helper (CD4) T-cells involved similar mechanisms, involving MHC class I and class II antigens, respectively, and that this would explain the need for histocompatibility between inter-acting immune system cells. Likewise, this could also explain how the MHC antigens are strong histocompatibility antigens and may predispose to diseases (32,33). Zinkernagel and Doherty never exactly found out the exact mechanism how the MHC antigens were involved in T-cell recognition in their experiments, but that doesn't diminish their relevance in the discovery of one of the basic understandings of today's immunology. For this, they received the Nobel Prize of Medicine in 1996.

In 1986, MHC restriction, not surprisingly, was also recognized for human T-cell-mediated immune responses, as shown by **Goulmy** et al. for cytotoxic T-cells (34) and by **Bergholtz** and Thorsby for helper T-cells (35).

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As we have referred before, HLA – human leukocyte antigen – complex is the major histocompatibility complex (MHC) in human beings and, therefore, involved in antigen processing and presentation in our species. In order to fully appreciate the impact of the HLA complex in kidney transplantation, some general aspects of this complex will be summarily reviewed. Class III HLA antigens will not be covered in this review, since their few functions don't have an obvious impact in transplantation.

Structure of HLA molecules

Class I and class II HLA molecules, are membrane-bound glycoproteins that are closely related in both structure and function. The first to describe the structure of this molecules was Bjorkman, in 1987 (36). Using X-ray crystallography, he was capable to define the structure of the HLA-A2 molecule. This achievement made it possible to explain the exact mechanism of MHC restriction (37).

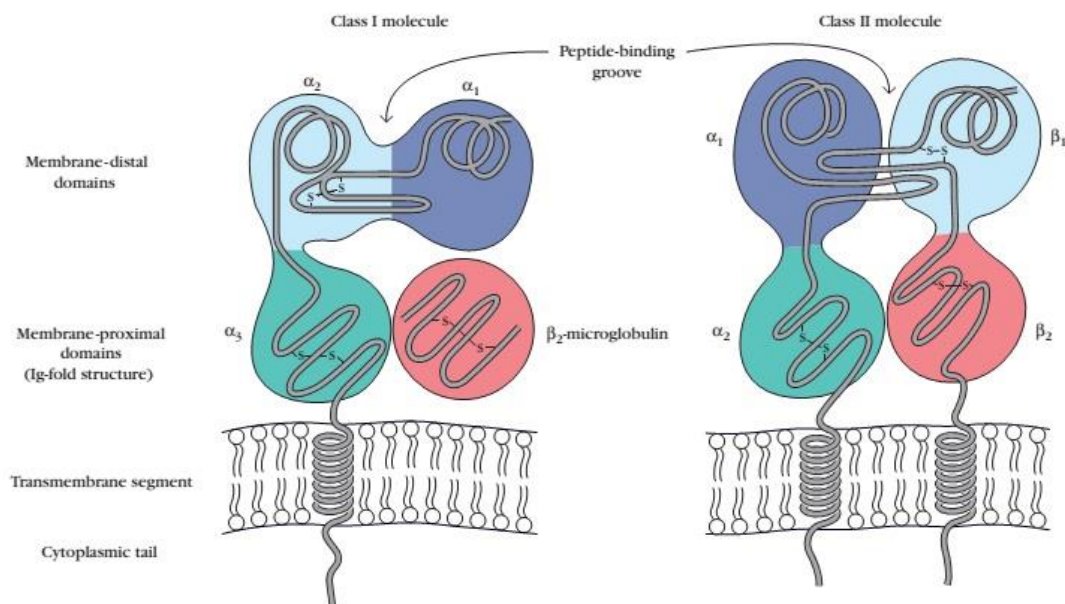FIGURE 6 - SCHEMATIC DIAGRAMS OF CLASS I AND CLASS II MHC MOLECULES
(FROM *KUBY IMMUNOLOGY SEVENTH EDITION*)

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Structure of HLA Class I

Class I MHC molecules bind peptides and present them to CD8^{+}T-cells. These peptides are often derived from endogenous intracellular proteins that are digested in the cytosol. The peptides are then transported from the cytosol into the endoplasmic reticulum (ER), where they interact with class I MHC molecules in a process known as the cytosolic or endogenous processing pathway.

They are formed by two glycoprotein chains: a heavy 45-kilodalton (kDa) α *chain* and a light 12-kDa β_2-*microglobulin* molecule. The α chain is arranged into three external domains, α1, α2, and α3, each approximately 90 amino acids long; a transmembrane domain of about 25 hydrophobic amino acids followed by a short stretch of charged, hydrophilic amino acids; and a cytoplasmic anchor segment of 30 amino acids. The α3 domain contains a sequence that interacts strongly with the CD8 cell surface molecule found on cytotoxic T-cell. As for β_2-microglobulin, it resembles α3 domain in its size and organization. It does not contain a transmembrane region and is non covalently bound to the MHC class I α chain.

An *immunoglobulin fold* can be found in class I molecules. It is formed by α3 domain and β_2-microglobulin, organized into two pleated sheets each formed by antiparallel strands of amino acids. Given the structural similarity, class I MHC molecules and β_2-microglobulin are classified as members of the immunoglobulin superfamily.

A *peptide-binding groove* is located on the top surface of the class I MHC molecule. This is grove is composed by α1 and α2domains, which form a platform of eight antiparallel strands spanned by two long-helical regions The structure forms a deep groove, or cleft, with the long helices as sides and the strands of the sheet as the bottom.

Structure of HLA Class II

Class II MHC molecules bind and present peptides to CD4^{+} T. Most of the peptides associated with class II MHC molecules are derived from self-membrane-bound proteins or foreign

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proteins internalized by phagocytosis or by receptor-mediated endocytosis and then processed through the exogenous pathway.
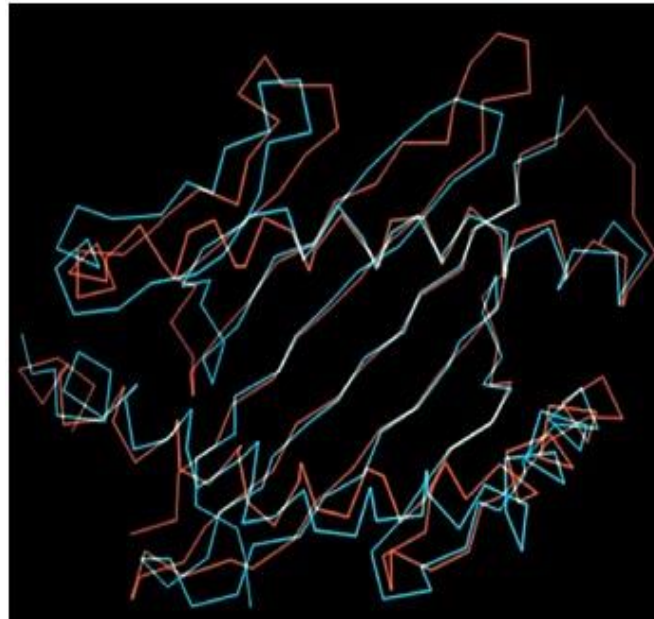
The three dimensional structure of a class II molecule (DR1) was described first by D. C. Wiley and Strominger J. L. in 1993 and it is similar to the structure of the HLA class I molecules (38). This similarity is so great that the connection regions of the molecules to peptides Class I and II may overlap.

They are formed by two different glycoprotein chains, a 33-kDa α chain and a 28-kDa β chain, which associate by noncovalent interactions. As class I chains, they also contain external domains, a transmembrane segment, and a cytoplasmic anchor segment. α chain is constituted by α1 and α2 external domains and β chain by β1 and β2 domains.

A membrane-proximal, immunoglobulin-fold structure-like, is formed by α2 and β2 domains, like the membrane-proximal α3/ β 2-microglobulin domains of class I MHC molecules. Therefore, class II MHC molecules are also classified in the immunoglobulin superfamily.

The peptide-binding groove is formed by α1 and β1 domains form, by the association of two separate chains. This is different from MHC class I, in which the peptide-binding groove is composed of two domains from the same chain (α chain). Albeit all the differences, the final quaternary structure is similar and retains the same function: of binding antigen and presenting it to T cells. Like class I molecules peptide-binding groove, class II molecules' one is composed of a floor of eight antiparallel strands and sides of antiparallel helices, presenting some differences that will be explained next.

Peptide-binding domain in Class I and Class II molecules

Unlike antibodies and T-cell receptors, peptide binding by class I and II molecules does not exhibit fine specificity. Instead, the binding between peptide and MHC molecules is regarded as being 'promiscuous', having a given MHC molecule the capacity to bind numerous different peptides, and some peptides the capacity to bind to several different MHC molecules. This promiscuity allows many different peptides to match up with the MHC binding groove and for exchange of peptides to happen on occasion, contrasting with the relatively stable, high-affinity cognate interactions of antibodies and T-cell receptors with their specific ligands.

Although both classes exhibit common peptide-binding features, there are some variances between them (figure 8). The class II molecule lacks the conserved residues in the class I molecule that bind to the terminal amino acids of short peptides. Therefore, class I presents more of a socket-like opening, whereas class II possesses an open-ended groove. As a result, class I molecules bind peptides that typically contain 8 to 10 amino acid residues, whereas the open groove of class II molecules lodges slightly longer peptides of 13 to 18 amino acids.

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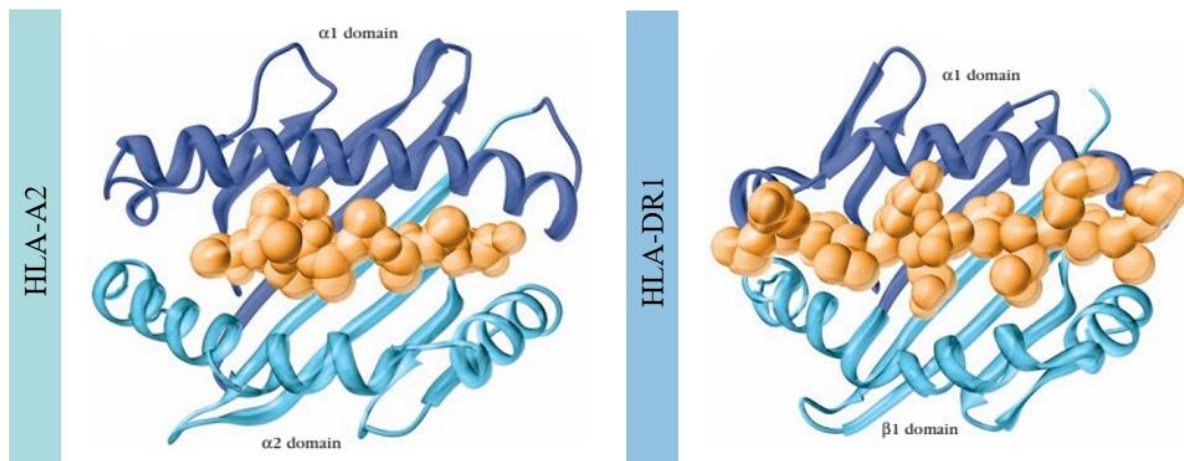
FIGURE 8 - MHC CLASS I AND CLASS II MOLECULES WITH BOUND
(FROM *KUBY IMMUNOLOGY, SEVENTH EDITION*)

Another difference is that class I binding requires that the peptide chain contain specific amino acid residues near the N and C termini, called anchor residues. They are generally hydrophobic residues (e.g., leucine, isoleucine) and they induce a conformational variance in MHC - class I -bound peptides of different length: longer peptides bulge in the middle, presumably interacting more with TCR, whereas shorter peptides lay flat in the groove. As for class II–binding peptides, they appear to lack anchor residues, then maintaining a roughly constant elevation on the floor of the binding groove.

The Genetics of the HLA Complex

The HLA complex is encoded on chromosome 6, containing more than 200 genes and extending over at least 4 million base pairs.

Class I HLA α chain molecules are encoded by the A, B, and C loci in humans, as β2-microglobulin is encoded by a gene outside the MHC, located on chromosome 15. Nonclassical class I molecules (E, G and F) that are encoded by additional genes or groups of genes within the class I region (figure 9).

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Class II HLA molecules are encoded by the DP, DQ, and DR regions, for both the α chain and the β chain. Additional nonclassical class II molecules (DM, DOA and DOB) are also encoded within this region. For each HLA class II isotype, the genes encoding the α and β chains are called A and B respectively (e.g.: HLA-DQA and HLA-DQB). When there is more than one gene, including nonfunctional genes, a number in series is added - HLA-DQA1 and HLA-DQA2, for example.

Separating the class, I and class II regions is a stretch of around 1 million base pairs called the class III region. The figure below (figure 9) shows the configuration of chromosome 6.
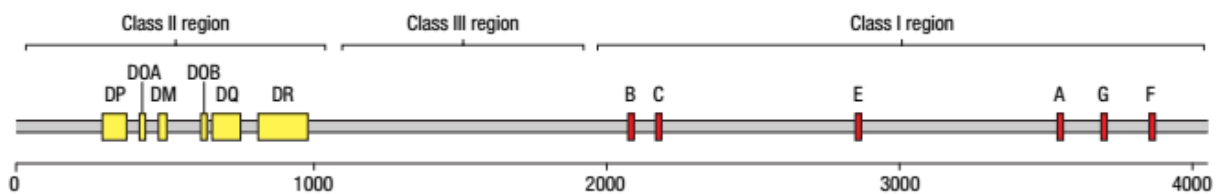
FIGURE 9- SCHEMATIC REPRESENTATION OF HLA COMPLEX, IN CHROMOSOME 6

The genes that reside within the MHC region are highly polymorphic, meaning that many alternative forms of each gene, or alleles, exist within the population. This enormous polymorphism, together with polygyny (multiple genes with the same function but with slightly different structures) results in a tremendous diversity of HLA molecules and of T-cell immunity within human populations, helping them survive epidemic disease, but also being the main immunological barrier to clinical organ transplantation. The individual genes of HLA loci lying so close together allows inheritance to be linked, meaning that most of the times individuals inherit all the alleles as a set, known as haplotype. An individual inherits one haplotype from the mother and one haplotype from the father. These genes are codominantly expressed, meaning that both haplotypes are expressed at the same time and in the same cells. This enables offspring, expressing the HLA molecules of both parental strains, to accept grafts from either parental source, each of which expresses HLA alleles viewed as "self". However, neither of the

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parents can accept a graft from its offspring because half of the HLA molecules (the ones coming from the other parent) will be viewed as "nonself," and therefore, subject to specific recognition and rejection by the immune system.

The Role of HLA molecules

The primary function of HLA molecules is to present antigens to T-cells. More specifically, Class I HLA, expressed in almost every nucleated cells, presents antigens from intracellular pathogens to cytotoxic T-cells. This is also known as the endogenous processing and presentation pathway. Class II HLA, with restricted expression in dendritic cells, macrophages and B lymphocytes (known as professional antigen-presenting cells - APCs), presents antigens from extracellular pathogens to helper T cells, in a process known as exogenous processing and presentation pathway. In some cases, exogenous antigens in certain cell types (mainly dendritic cells) can also gain access to class I presentation pathways in a process known as cross-presentation. Both of the classes will present the antigens to a T-cell receptor (TCR), that is generally CD8 for Class I and CD4 for Class II.

Besides that, HLA molecules also take part in many another responsibilities. They also work as presenters of self-class I, to demonstrate that the cell is healthy; of self-peptide in class I and II, in primary lymphoid organs to test developing T cells for autoreactivity, and in secondary lymphoid organs, to maintain tolerance to self-proteins.

Having in mind their functions and characteristics, HLA antigens display a role in many areas. They protect us from infectious diseases, by presenting the pathogens to T-cells. They play the main role in graft rejection, by distinguishing self from nonself, in a process known as allorecognition. Some of them are connected with autoimmune disorders and other diseases, such as type I diabetes, ankylosing spondylitis, celiac disease, systemic lupus erythematosus, myasthenia gravis, inclusion body myositis, Sjögren syndrome, and narcolepsy. They also play

a role in cancer, either promoting it, by mediating certain diseases (for example, gluten-sensitive enteropathy is associated with increased prevalence of enteropathy associated T-cell lymphoma) or protecting from it, by recognizing increases in antigens that are not tolerated.

Allorecognition

HLA antigens are responsible for eliciting the strongest of responses to allogeneic tissues, as the MHC in human. There are other alloantigens, known as minor histocompatibility antigens (MHA), eliciting a slower tempo, less intense immune response. MHA create potential antigenic differences between donors and recipients as they are not expressed by all members in a population. This occurs, for example, when proteins encoded on the Y chromosome (H-Y) from male grafts induce an anti-Y response in females.

Alloantigen recognition can occur via two distinct pathways (direct and indirect pathways), which depends on the source of the antigen presenting cells (donor versus recipient).

The direct pathway of allorecognition relies on the ability of recipient T cells to "directly" recognize intact non-self MHC molecules present on the surface of donor APC. It is important to know that direct recognition does not conform to the classic rules of self-MHC restriction, since antigen processing and presentation are skipped. This may be explained by MHC mimicry-structurally: an allogeneic MHC molecule linked to an allogeneic peptide is similar to a foreign peptide linked to a self-MHC molecule, allowing the same receptor of a T cell to recognize both peptide complexes, based on their common epitopes. The use of epitope-based HLA matching in transplant setting is nowadays one of the more attracting options to find acceptable mismatches for highly sensitized patients (46).

Over time, donor APC are depleted from the graft, and the response is predominated by recipient DC that migrate into the graft and continuously pick up antigens from the graft and

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present processed peptides to T cells, through the indirect pathway. This pathway is thought to be involved with chronic rejection in vascularized organs, such as the kidney (41).

Formation of HLA Alloantibodies

The impact of anti-HLA antibodies and donor specific antibodies (DSA) in kidney transplantation is undeniable, so it is important to explain how they are formed.

The antibody production starts with the interaction between B-cell receptor and antigen. The possible routes of exposure to antigens capable of forming alloantibodies are pregnancy, blood transfusion and previous transplants. The activated B cells migrate to the border of the B-cell follicle and T-cell zone of the secondary lymphoid organ to receive T-cell help. Here, B cells present the antigens, on MHC II molecules, to CD4+ T-cells. T-cells cause clonal expansion and differentiation of antigen-experienced B cells, by providing help through direct cell−cell contact and cytokine production. After T cell-dependent activation, B cells can follow two different paths. They can either migrate out the B-cell follicle and form extra follicular plasmablasts, which are responsible for early production of low-affinity antibodies; or they can undergo somatic hypermutation and class switch recombination, whereby high-affinity antibodies are selected producing memory B cells and antibody-secreting plasma cells. Not only anti-HLA class I and anti-HLA class II are formed, but also against other highly polymorphic molecules as MHC class I-related chain A (MICA) and chain B (MICB) and against non-polymorphic molecules such as angiotensin II type I receptor.

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C. Role of HLA Antigens on Kidney Transplantation

Many factors can influence kidney allograft survival. Age of the recipient and the graft, living and cadaveric donors, previous kidney disease, ethnic background, previous time spent on dialysis, ABO matching, HLA matching, anti-HLA sensitization, cold ischemia time, immunosuppression, between many others, can have an impact in graft rejection, and in the morbidity and mortality of the transplanted patients.

Since the first kidney transplants in human being that we know that graft survival in patients with 0 mismatch on HLA-DR locus is significantly better comparing with transplants incompatible for one or two DR antigens (42,43). The same has been observed for HLA-A and B matching antigens, although with less impact (43). Moreover, HLA mismatches contribute to the formation of DSA, which can lead to acute and chronic antibody-mediated rejection (AMR) and poor graft survival (44).

More recently, the importance of HLA matching was debated, given the major development on the areas of immunosuppression and patient management, and the impact of shortening cold ischemia time to a minimum. However, the importance of organ sharing based on HLA matching in kidney transplantation, according to full DR compatibility then to the best A+B matching, has been reassured (45). Some studies have shown that HLA mismatches not only affect the graft survival, but have also other adverse consequences, such as an increased risk of death with a functioning graft due to infection or cardiovascular causes, non-Hodgkin lymphoma and hip fracture (45,46). The treatment with lymphocyte-depleting antibodies probably plays a role in these adverse consequences.

Nonetheless, the impact on kidney rejection is still the most crucial consequence of HLA mismatching and allosensitization. This impact will be described next in each type of kidney rejection based on clinical, histopathological and molecular criteria.

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Hyperacute rejection

Hyperacute rejection occurs immediately following the transplantation, between minutes to hours after. This rejection is characterized by arterial and glomerular thrombi, caused by damage inflected to the endothelium, and it involves a humoral response. Preformed anti-HLA antibodies activate complement and bind to the endothelium, leading to the production von Willebrand factor, resulting in platelet aggregation, irreversible ischemia and consequent allograft rejection. The only treatment available is the removal of the graft. Since the beginning of clinical organ transplantation and routinely donor-recipient crossmatch determination (and consequent detection of DSA), this response is rarely observed in modern-day practice (47).

Accelerated acute rejection, or delayed hyperacute rejection, shares similar histopathological characteristics with hyperacute rejection, distinguish itself by happen only a few days after the transplantation. It is probably caused by delayed response to prior HLA sensitization, with a low DSA level, and it involves both cellular and humoral anamnestic responses. An aggressive therapy based on anti-lymphocyte antibodies with plasmapheresis or immunoadsorption may reverse this condition.

Acute rejection

Acute rejection typically occurs from 1 week to 3 months after transplantation, but may occur later. The incidence of acute rejection has been steadily decreasing over the last two decades. Current acute rejection rate is about 10 percent within the first year after transplantation, since the introduction of the immunosuppressive drug tacrolimus and antimetabolites such as mycophenolate mofetil. Immunosuppressive drugs act mainly on cell-mediated immune responses by inhibiting the development and proliferation of T cells, explaining why the rate of acute rejection isn't null, as an acute rejection can be either cell-mediated or antibody-mediated. The impact of B-cells and alloantibodies has been

incrementally clarified in the last 20 years, thanks to the development of methods to detect and characterize anti- HLA antibodies, DSA and, more recently, minor histocompatibility antigens antibodies. The presence of pretransplant DSA, as well as *de novo* posttransplant DSA, has been prove to be responsible not only for acute, but also chronic humoral rejection, and therefore correlated with rejection and graft outcomes (48,49).

Acute cell-mediated rejection

Approximately 90% of acute rejection episodes are predominantly cell-mediated. CD8+ cytotoxic T-cells (Tc), recognizers of HLA class I, were initially known to be the main effector of this rejection, as biopsies to rejected kidneys show its presence and the activation of perforin/granzyme degranulation pathway, the main pathway used by cytotoxic lymphocytes to eliminate virus-infected or transformed cells. However, it has been shown that the presence of Tc is not sufficient to induce a complete rejection of the organ, suggesting the need of CD+4 helper T-cells (Th), capable of recognizing of HLA Class II and of inducing a delayed hypersensitivity reaction. This reaction, mediated by various cytokines, some of them capable of increasing the expression of HLA class I (IL6, IL7, IL8, TNFα, PDGF, TGFβ) and also class II (INFγ is capable of increasing both, besides being principal macrophage-activating factor), is capable of expanding cellular infiltrate from an initial response of a small number of T-cell clones.

Histopathologically, acute cell-mediated rejection can be divided in two forms. Tubulointerstitial cell-mediated acute rejection characterized by tubulitis with interstitial edema and inflammation, mainly involving lymphocytes and monocytes. Vascular acute cell-mediated rejection usually occurs together with the tubulointerstitial form, and is characterized by lymphocytes, monocytes or both, invading the intima of endothelial cells (endothelitis). In severe cases of vascular acute cell-mediated rejection, arteritis occurs and may be associated with necrosis. In Banff 2013, acute cell-mediated rejection (category 4) is graded in IA, IB, IIA,

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IIB and III, with increased level of severity of the histological findings. Category 3 englobes histological changes 'suspicious' of cell-mediated rejection.

Acute cell-mediated rejection can be treated with immunosuppressive drugs. If it doesn't respond to corticosteroid therapy, lymphocyte-depleting antibodies or muromonab CD3 (monoclonal antibody targeted at the CD3 receptor, a membrane protein on the surface of T cells) should be used.

Acute antibody-mediated rejection

20−30% of acute rejection episodes have a humoral component, being known as acute antibody-mediated rejection (ABMR), and it can occur alone or together with cell-mediated acute rejection. Acute ABMR generally tends to occur in the first three weeks after transplant, a shorter period than acute cell-mediated rejection.

As already mentioned in this article, presensitization is a major risk factor and the immune response is mostly mediated by *de novo* DSA against HLA, endothelial cell antigens and ABO antigens.DSA are activated by the complement, resulting in endothelium injury and consequent graft damage. C4d, a split product of C4 activation, deposits on endothelial cells of peritubular capillaries in AMR, being an important finding for diagnosis of ABMR. CD4d importance has been emphasized on the last revision of Banff classification, in 2013. Still, other key pathologic characteristics can be found, such as neutrophils in peritubular capillaries and arterial fibrinoid necrosis.

In acute ABRM treatment corticosteroids may or may not be used, with one of more the following: plasma exchange, intravenous immunoglobulin, anti-CD20 (expressed on the surface of all B-cells) antibody and/or lymphocyte depleting antibody. New therapies are currently being studied, such as the use of B cell with regulatory properties and inhibitors of complement proteases (50,51).

Chronic rejection

Chronic rejection is not anymore a category in Banff classification, since 2007, but otherwise an array of progressive kidney diseases, that occur month to years after transplantation. In this new category are englobed interstitial fibrosis and tubular atrophy (IFTA), without a specific etiology. Slow cell-mediated rejection, low DSA titers or other recurrent, non-immunological, diseases are all potential causes. Later episodes of acute rejection during the first year (after three months) have also been shown to be associated with poorer long-term graft survival (52).

AMR have a major role in chronic dysfunction. Acute AMR and antibody-mediated injury, in general, have been shown to contribute majorly to chronic rejection and late graft loss (53), especially if antibodies developed early (less than one year) after transplantation (54). Early adverse events, such as acute rejection episodes, delayed graft function and no immediate function of graft, influencing long, as well as short, term kidney graft outcome, have also been shown to be influenced by anti-HLA antibodies. Transplant glomerulopathy, a major cause of chronic graft dysfunction related with endothelial cell injury but whose pathophysiological pathways are still not completely understood, may also be correlated with anti-HLA antibodies (55).

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Before a patient can have a safe renal transplanted, several aspects of the match between the donor and recipient must be evaluated, in order to reduce the chances of donor's graft rejection by the recipient.

Besides ABO blood group match, it is necessary to study the patient's HLA antigens. Before the transplant, HLA antigens have to characterize, by proceeding to an HLA typing of the recipient and the potential donor. More importantly, preformed HLA-specific antibodies in the recipient have to be identified, determining his allosensitization with a Panel Reactive Antibody (PRA) testing or screening, by using a panel of donors having as many known HLA antigens as possible. Being the donor accepted, an HLA antigen donor-recipient crossmatch should follow. By applying this test, we are practically eradicating the risk of the donor suffering an hyperacute rejection, but also reducing the number and severity of acute rejection episodes that is also translated into improved long-term graft survival. DSA, if present in high amounts, will cause immediate (hyperacute) graft loss (56) and if present in small amounts will limit the survival of an allograft (48,49,57). After the transplant, we must remain watchful and address the possible formation of *de novo* DSAs, which may also affect the graft in the short or long haul (48,57). The following scheme illustrates the study of the HLA antigens in a possible kidney transplant recipient (figure 10).
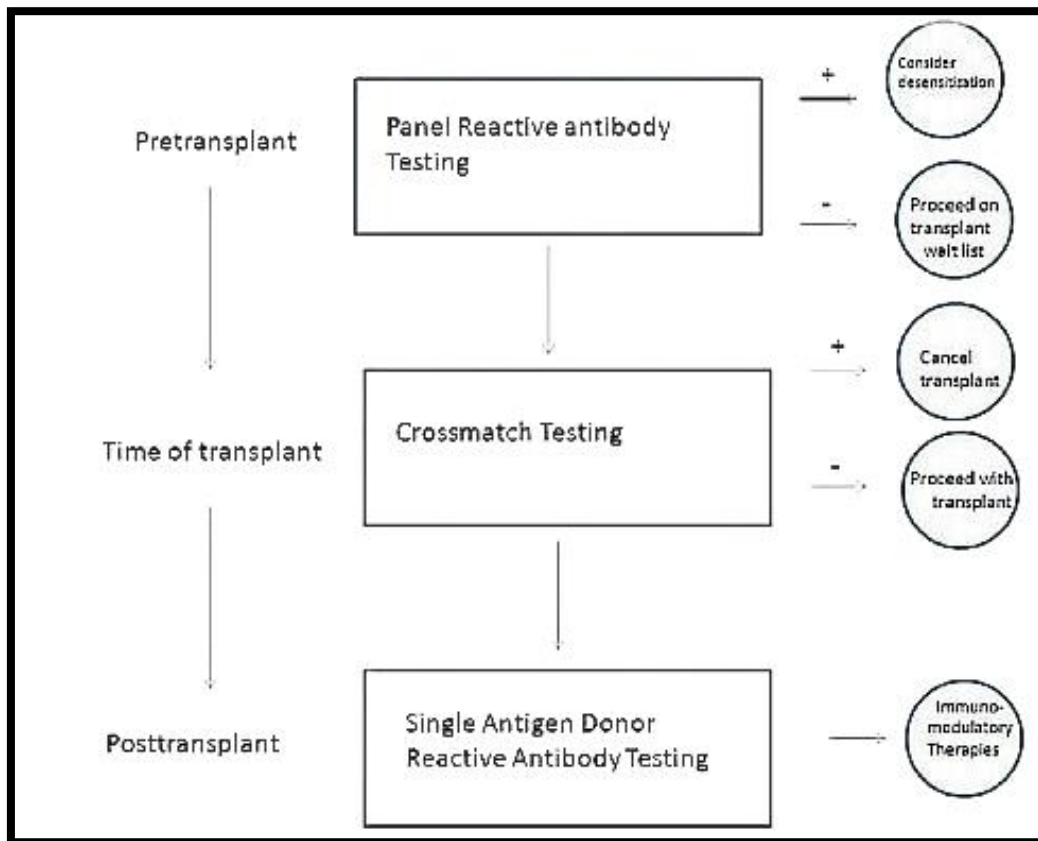
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Outcomes of kidney transplantation have steadily improved, not only because of developments immunosuppression and patient care, but also because of developments in laboratory techniques for determining the suitability of alloantigens match. Having this in mind, these techniques for the histocompatibility assessment of the kidney transplant patient will be following reviewed.

HLA Typing

There are different types and resolutions of HLA typing. Serology, based on the complement-mediated microlymphocitotoxicity (CDC) technique, is an antibody-based compatibility testing with low resolution. Molecular assays, based on DNA, are a more recent method, with a greater accuracy and with an intermediate to high resolution. The molecular typing identifies nucleotide

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polymorphisms that define the different alleles, as serology only identifies molecules that are actually expressed on the cell, by mixing the lymphocytes of the recipient with different HLA antisera.

Current DNA-based methods that are in use for HLA typing are polymerase chain reaction-sequence-specific priming (PCR-SSP)(58), polymerase chain reaction-sequence-specific oligo-hybridization (PCR-SSO), (59) and sequence-based typing (SBT) (60). The PCR-SSO and – SSP – faster than SSO and the cheapest of the three - are both are powerful methods for detecting genetic variability by identifying sequence motifs. However, to maintain the high accuracy of these methods, the number of probes and primers has to keep up with the rapidly increasing allelic diversity. The SBT is the most comprehensive method for characterizing human leukocyte antigen gene polymorphisms and it involves PCR amplification of specific coding regions of HLA genes and sequencing of the amplicons. Its disadvantages reside on needing more expensive materials and on the difficult interpretation of ambiguous allele combinations (61).

A third method, rarely used now, is the mixed lymphocyte culture (MLC). It involves culturing together lymphocytes of two individuals, where each cell population is able to recognize the 'foreign' HLA class II antigens of the other.

For solid organ transplantation, such as kidney, the typing methods should be relatively fast and only require a low - typing resolution to determine the A, B, and an intermediate-level to DRB1 loci (62).

Antibody Testing Petransplantation
The goals of the pretransplant identification of HLA-specific antibodies are to determine the breadth and strength of anti-HLA antibodies that are present, expressed by PRA, and to be

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· U   C ·

certain that there are no DSA, by performing a crossmatch – generally considered to be the most important test by the histocompatibility laboratory.

The commonly used term to describe the breadth of antibodies is PRA (panel reactive antibody) and it used to be expressed as a percent, %PRA, which was dependent on the composition of the cell panel and, therefore, was of limited value. PRA results then could not be compared between cell panels or between laboratories and did not necessarily reflect the proportion of the donor pool to which a patient is sensitized. False positive results due to the presence of autoreactive lymphocytotoxic antibodies could also give "100% PRA", despite their irrelevance to the transplant outcome. Hence sensitization nowadays is no longer defined in "%PRA", but rather in a calculated or "virtual PRA" (63). This result is obtained by comparing the unacceptable HLA specificities reported for each patient with the HLA types of blood group identical donors, and it's expressed as a percentage of HLA incompatible donors. Using this approach, a figure can be calculated objectively and as an accurate reflection of the chance of a patient receiving an HLA compatible deceased donor transplant (64).

There are various reasons to determine the PRA prior to the transplantation. Not only we know that a high level of anti-HLA antibodies has a higher incidence of rejection, but it is also important for the physician to know which patients are sensitized and, for that reason, will to have wait longer to find a compatible donor. Besides that, patients who have anti-HLA antibodies may receive additional points in the allocation scheme and are prioritized for transplantation with a deceased donor, depending on the country's kidney transplantation policies and guidelines – done in the USA (65), in Eurotransplant group (international collaborative framework formed by Austria, Belgium, Croatia, Germany, Hungary, Luxembourg, the Netherlands and Slovenia) (66) and also in Portugal (67), still being discussed in the UK (68). By determining to which specific HLA antigens a patient has antibodies, it is also possible to create a list of unacceptable within a certain population (UNet, the computer

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program operated by the UNOS, the national organ procurement and transplantation network of the USA, is one of examples) (63–65). If listed as an unacceptable antigen for a specific patient, kidneys from donors with that antigen will not be offered to that patient.

A negative crossmatch between donor and recipient is the definitive pre-transplantation test to avoid hyperacute rejection due to donor-specific HLA antibodies. The pretransplant crossmatch together with PRA can also indicate patients with an increased risk for graft loss.

Technical Aspects

We can divide the various assays for identification of HLA-specific antibodies in two major groups: cell-based assays and the more recent solid-phase immunoassays.

Cell-based assays

The cell-based assays available are complement-dependent cytotoxicity test (CDC) and flow cytometry.

Complement-dependent cytotoxicity test

Developed in 1964 by Terasaki and McClelland, the CDC is the first established method for the detection and definition of HLA-specific antibodies and it is still used for both antibody screening and crossmatching (XM) protocols, usually in addition to other, more sensitive methods.

The technique (illustrated on figure 11) is based on lymphocytes, mostly from the peripheral blood, are mostly T lymphocytes, targeted to detect only complement-fixing IgG and IgM antibodies. In the standard technique, the lymphocytes are taken from the peripheral blood, which are mostly T-cell lymphocytes, expressing only HLA class I (HLA-A, HLA-B, and HLA-C). Donor lymphocytes are collected and placed into wells. Recipient serum is then added to the wells and incubated with the lymphocytes. Rabbit complement is then added to this mixture and incubated. After the second incubation, eosin dye and formalin are added to fix the cells and evaluated under the microscope. If donor specific antibodies are present, then the combination of donor lymphocytes, recipient sera, and complement leads to a reaction in which the antigen/antibody complex recruits complement that, then, will form membrane attack complexes that destabilize the cell membrane. This allows eosin dye to enter the cell and lead to cell swelling, which is discernible under phase contrast microscopy.
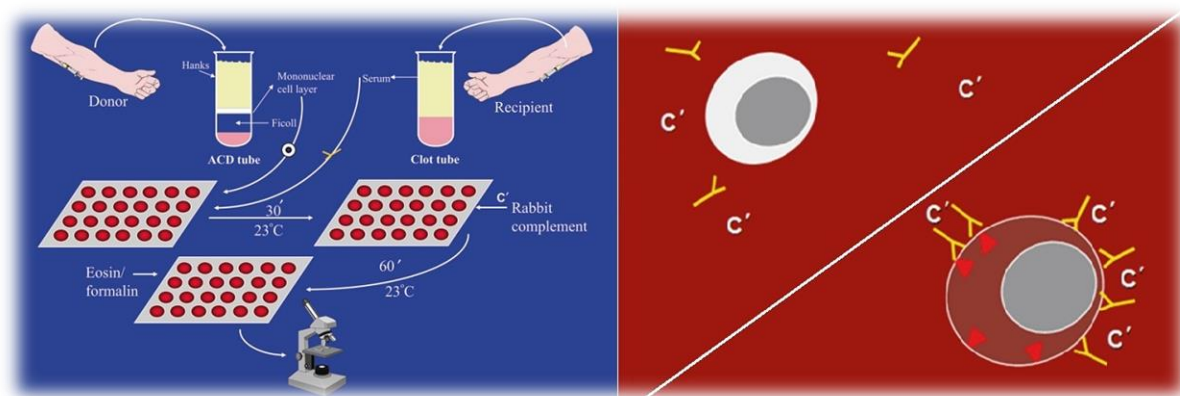
FIGURE 11 - CDC TEST
(FROM *DELOS SANTOS R, LANGEWISCH ED, NORMAN DJ. IMMUNOLOGICAL ASSESSMENT OF THE TRANSPLANT PATIENT. IN: WEIR MR, LERMA EV, EDITORS. KIDNEY TRANSPLATATION: A PRACTICAL GUIDE TO MEDICAL MANAGEMENT. NEW YORK: SPRINGER SCIENCE + BUSINESS MEDIA; 2014. P. 123–4*)

Although highly specific, as findings by Patel and Terasaki have shown (69), this test lacked adequate sensitivity, being both influenced by cell viability and the rabbit complement used, and an adequate cell panel can be difficult to obtain. In order to optimize the sensitivity of the CDC test, several washes and increasing incubation time were added to the procedures.

However, the use of anti-human globulin proved to be the most effective way to increase the sensitivity, achieved due to the cross linking and consequently enhanced complement

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activation. This technique is called *Anti-Human Globulin-Enhanced Crossmatch*. In a study that compared the standard CDC test, the standard test with the addition of antiglobulin, and the standard test with doubled incubation periods 52 of 56 sera tested (93 %) were positive when adding antiglobulin compared with 28/56 (50 %) when the incubation time was extended and 13/56 (23 %) when the standard technique was used (70).

Adding to this difficulties, in CDC test only complement-fixing antibodies are detected and these may not be HLA-specific nor related to rejection. It is generally accepted for kidney transplantation that IgG antibodies directed against donor HLA-A or -B specificities and present at the time of transplant can cause hyperacute rejection. Although fewer data are available, donor class II specific antibodies (HLA-DR, HLA-DQ, and HLA-DP), especially HLA-DR, present in the recipient may also result in rejection and are associated with worse long term outcome, (71,72), but there is still much controversy about its role and therefore the need of a B-cell CDC crossmatch (BXM) (73). Its major limitation is a rate of false positive results of up to 50%, meaning that a negative result is reassuring while a positive result may mean aborting a safe transplant (74). It is then suggested that the use of solid-phase techniques such as Luminex to investigate antibody specificity in positive B-cell crossmatches, in order to enhance the interpretation of BXM (75).

IgM autoreactive antibodies relevance to kidney transplant outcome is still uncertain (76,77). Nevertheless, it is possible to remove IgM and to avoid misleading positive results by using serum with dithiothreitol (DTT).

The use of single-antigen-expressing cell lines, in cytotoxicity and flow cytometry assays, is yet another development beyond CDC testing. Its goal is to improve the detection and characterization of alloantibodies in sensitized patients, as it only expresses a single HLA

antigen, facilitating the characterization of HLA antibody. A describe method employs the non-HLA expressing cell line K562, transfected with cDNA encoding single HLA class I alleles (78).

Flow cytometry

Flow cytometry crossmatching (FCXM) was first described in 1983 (79). The flow cytometry assay detects antibody binding to target lymphocytes through a more sensitive method, that can detect either complement and non-complement fixing antibodies, unlike CDC. Lymphocytes are mixed with recipient serum followed by the addition of a fluorochrome-tagged anti-human immunoglobulin. After washing, donor cells are run through a flow cytometer where cells are counted individually. Cells that are bound by antibodies are identified by laser activation of the fluorochrome (technique illustrated on figure 12).

The read-out may be reported simply as positive or negative or can be further quantitated. Although flow cytometry is also subject to reactions caused by non-HLA antibodies, it is appreciably more sensitive than CDC and has been proven useful in identifying patients with weak DSA who are at increased risk of AMR and graft rejection.
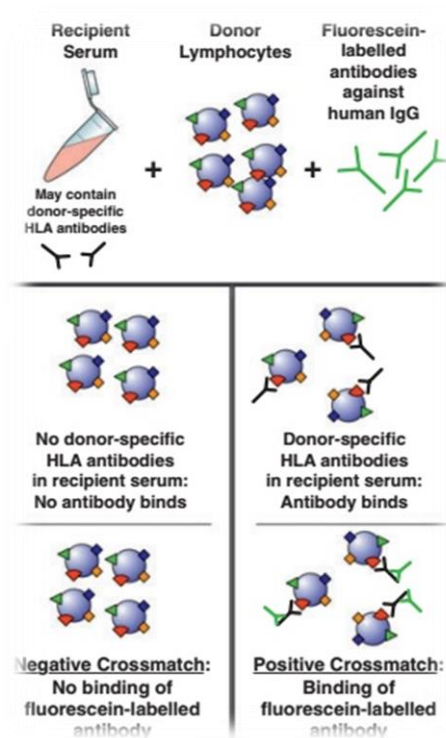
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Modifications of the flow cytometry assay include the detection of different immunoglobulin classes and subclasses, differentiation of target cells, and pronase treatment of B-lymphocytes to reduce background nonspecific reactivity antibody (80).

The relevance of distinguishing between different Ig subclasses relies on some studies that show that some IgG subclasses may have a more significant association with rejection and graft failure, but the studies are still far from being complete and relevant (81).

Solid-phase immunoassays

Solid-phase immunoassays (SPI) differ primarily on the solid matrix where the solubilized HLA molecules will bound: it can either be a microtiter plate (enzyme-linked immunosorbent assay [ELISA]) or polystyrene beads performed on a conventional flow cytometer or a small

footprint fluoroanalyzer (Luminex) (82–84). Although they were developed as qualitative assays, they provide semiquantitive results. ELISA technology is more sensitive than CDC (82), whereas Luminex bead technologies are more sensitive and specific than both CDC and flow cytometry (83).

SPI assays significantly improved the detection and characterization of alloantibodies in sensitized patients. SPIs offer a number of advantages. They are objective and with the potential to be partially automated, they do not require viable lymphocytes nor complement. They are designed to detect only HLA-specific antibodies and they also have to capability to detect non-complement fixing antibodies.

Depending of the targeted antigens, three different types of panels can be used. Pooled antigen panels have two or more different bead populations coated with either affinity-purified HLA class I or HLA class II protein molecules obtained from multiple individual cell lines and are used as a screening test for the detection of HLA antibody. Phenotype panels in which each bead population presents either HLA class I or HLA class II proteins of a cell line derived from an individual. Single-antigen beads (SAB) in which each bead population is coated with a molecule representing a single cloned allelic HLA class I or II antigen, enabling precise antibody specificity analysis. SAB arrays are the most sensitive and specific, providing the highest degree of HLA antibody resolution, and are particularly useful in the accurate identification of antibodies in highly sensitized patients.

The measure of strength in the Luminex SAB assay is MFI, which stands for mean fluorescence intensity. Increasing fluorescence intensity measured by photons emitted by specific beads bound with specific HLA molecules correlates with increasing amounts of antibody to that specific HLA antigen. However, MFI values do not necessarily reflect antibody titer, since SPI are only semiquantitative. Many studies show varying degrees of correlation between MFI, antibody level, XM results, and clinical outcomes (85) but standardized cutoff values for

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positivity are yet to be established. It has been tried to standardize MFI by conversion to molecules of equivalent soluble fluorochrome (MESF) using quantification beads known from flow cytometry (86), but this method is still variable between different centers. The need for standardization is parallel to other aspects in SPI, such as HLA source and preparation method, panel composition, and appropriate allele coverage, including DQA1, DPBA, and DPB1. This need also comprehends the cell-based assays, which needs for standardization rely on harmonization of standard operating procedures including cell isolation, cell- to-serum ratio, incubation, and wash steps (87).

Since their introduction, SPI have suffered a number of modifications in order to facilitate analysis of the results and their interpretation in the clinical setting. Heat inactivation of sera, ethylenediaminetetraacetic acid (EDTA) treatment and DTT treatment have all been used, especially on highly sensitized patients, in order to diminished the 'blocking' effect of IgM HLA-specific antibodies on the binding of IgG HLA-specific antibodies to single antigen beads. By using this modifications, we can eliminate some misleadingly low values in the assay (88).

Another modification to the bead array enables detection of only those antibodies that fix complement, by detecting the complement fragments C4d or C1q. Clinical data obtained using various modified assays correlates the presence of C4d$^+$ antibodies with lower graft survival in kidneys and with C4d deposition in the peritubular in renal grafts (89), but they do not appear to be associated with AMR in renal grafts (90). As for antibodies detected by the C1q assay ,correlations with acute rejection and long-term graft outcome have been observed is some studies (91), but others not (49). Additional studies are still required to grant both as a routine test.

Non-HLA Antigens Antibodies Assays

The interested around non- HLA antigens antibodies in kidney transplantation has grown since a report from Collaborative Transplant Study Group (92) indicating that, even if the donor was an HLA-identical sibling, a significant number of patients with pretransplantation PRA had lower graft survival. This suggests a role for immune responses to non-HLA antigens in allograft rejection.

The incidence and clinical consequence of immunization to non-HLA antigens in kidney transplant is still not fully understood, since the non-HLA antigens that have to target are still being determined. The lack of adequate diagnostic screening assays for detection of those antibodies is another difficulty that limits our knowledge.

The antigens that have been targeted, in the setting of kidney transplantation, are primarily expressed on endothelial cells and epithelial cells, generally termed anti-endothelial cell antibodies (AECA). AECA comprise both IgM and IgG isotypes and are thought to act as mediators of endothelial cell activation, apoptosis and cell injury.

AECA can be detected using an indirect immunofluorescence method on endothelial cells from human umbilical cords. This commercially available assay may permit standardization of test results among laboratories using it as a screening test to detect AECA (87). Alternatively, an endothelial FCXM technique that uses endothelial cell precursor cells found in the peripheral blood, XM-ONE, can also be used (93), with the benefit of also detecting DSA and so it can be used to test for antibodies to T lymphocytes, B lymphocytes, and endothelial cells in the same assay (94). Its capacity to bind to fully differentiated endothelial cells that line the vessels of the allograft it is still a doubt that needs to be addressed.

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Results from studies to AECA, and the differences between IgG and IgM antibodies, and different IgG antibodies subclasses, have been incoherent. A previous multicenter study showed a significant correlation between both IgM- and IgG-mediated crossmatch positivity and early acute rejection (93). Another prior study only demonstrated a correlation with IgG-positive crossmatch, especially poorly complement-fixing subclasses IgG2 and IgG4, and with early transplant dysfunction (95). More recent studies showed variable results. One found a significant relationship between a positive crossmatch and both acute rejection and transplant survival (96), but two other did not found any significant association between AECA and transplant function (97,98). At this time, it remains unclear whether to routinely test for AECA and whether their presence should alter organ allocation or therapy.

Several AECA have been identified using protein array analysis. They can be divided in polymorphic (capable of eliciting an alloresponse) or non nonpolymorphic (auto-antigens). The polymorphic major histocompatibility complex (MHC) MICA and MICB, and the nonpolymorphic AGTR1.

The MICA and MICB are surface glycoproteins with functions related to innate immunity. MICA/B are similar in structure to class I α-chains, but do not associate with β2-microglobulin or present antigen to T-cells. They are mainly found on gut epithelia and keratinocytes, as well as endothelial cells, fibroblasts, dendritic cells, and activated lymphocytes. Their expression may be upregulated in kidney epithelial and endothelial cells in times of "stress", like ischemia-reperfusion injury and acute rejection. Because they are not expressed on resting T-cells, they do not result in a positive crossmatch by CDC testing, although SPI have been developed for their detection. Enhanced MICA expression may result in kidney allograft by serving as a natural ligand for NK cell receptors known as NKG2D. That way they would mediate direct cellular cytotoxicity, possibly damaging kidney function. However, the effect of MICA/B

sensitization on transplantation outcome remains uncertain, with conflicting results both in terms of acute rejection and transplant survival. The presence of preexisting antibodies to MICA has been shown to correlate with kidney graft outcome in some reports (99–101), but not in others studies (102). The need for routine MICA (or MICB) typing at this time is unclear. In a patient with antibody-mediated rejection, involvement of MICA antibodies is a consideration when HLA antibodies cannot be implicated (103).

AGTR1 autoantibodies are agonistic antibodies directed against the second extracellular loop of this protein. They were associated before with preeclampsia, scleroderma, malignant hypertension and graft-versus-host disease. The role of AGTR1 autoantibodies in kidney transplantation was first demonstrated in 16 patients with malignant hypertension and severe vascular rejection without anti-HLA antibodies (104). Prior studies have demonstrated the association of AGTR1 antibodies with kidney transplant rejection (105,106) . The need for routine AGTR1 autoantibodies screening is still uncertain. Only in the case of an acute vascular rejection in the absence of detectable DSA or MICA antibodies, AGTR1 autoantibodies should be considered, especially if accompanied by severe hypertension(103).

Clinical interpretation

When proceeding to a patient's HLA-specific antibody screening, the laboratories are attempting to predict crossmatch outcome, by determining unacceptable antigens (UA) mismatches between recipient and donor. This is also known as virtual crossmatch, and can be used to facilitate omission of the pre-transplant laboratory crossmatch test. This omission could be important in order to diminish the impact of other factors that may affect the kidney transplantation, such as cold storage time – shown to be reduced in case of lab crossmatch omission (107).

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The SPI definitely provide the most specific and sensitive tools for the detection and characterization of HLA-specific, and therefore, allowing to determine UA not previously possible (84). However, there is still considerable debate as to the clinical significance of antibodies detected by SPI, particularly if they are not detected by cell-based assays (53,83,89). To truly understand the impact of bead assay-detected pretransplantation DSA, both short-term and longer-term outcomes need to be documented (87). Therefore, donor-specific IgG HLA antibodies detected by CDC are considered a contraindication for transplantation, whereas DSA detected by other assays represent varying degrees of risk (112).

However, this assumption that DSA may only be a risk factor and not a contraindication was also a sign of hope for highly sensitized patients. Desensitization, acceptable mismatch program, good HLA matching, between other integrative measures, are reported to result in a significant reduction in waiting time and good allograft and patient survival in this group of patients (113).

Antibody Testing Postransplantation
Following transplantation, de-novo DSA have been identified in kidney allograft recipients(114). Antibodies have been identified either by specifically crossmatching against donor cells or antibody screening assays.

The development of HLA-specific antibodies following kidney transplantation has been shown to be associated with a poorer transplant outcome (87). De novo class II DSA directed at donor DR, DQ, and, occasionally, DP antigens were the most commonly detected, with a less substantial occurrence of de novo class II DSA.

A recent study followed the evolution of *de novo* DSA and demonstrated that the mean time to appearance is 4.6 years after transplantation, with a tendency to appear sooner in case of

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nonadherence (115). The prevalence of de novo DSA at 10 years was 20% in adherent patients and 60% in nonadherent patients. Other risk factors for the development of de novo DSA, besides drug minimization and non-adherence, are HLA-DR mismatching, early cell-mediated rejection, and younger recipient ages (115). Since the production of DSA following transplantation is associated with poor outcome, there is a potential benefit to monitoring patients for production of antibodies post-transplant.

While circulating DSA can easily be detected following transplantation, the histological detection of immunoglobulin bound to the endothelium in a transplant requires a biopsy. The presence of C4d on peritubular capillaries of kidney transplant biopsies can be used as a marker of humoral rejection and therefore of posttransplant donor reactive antibody responses (116). However, the absence of detectable C4d positivity does not preclude antibody-mediated rejection, as C4d deposition in the peritubular capillaries has been showed to be only present in approximately 30% of acute rejection biopsies (117). Circulating donor reactive antibodies detected by posttransplant crossmatching and screening, are significantly associated with C4d deposition.

The inclusion of routine test for non-HLA DSA has also been considered on posttransplantation. There have been a number of reports of MICA antibodies associated with poor graft survival (101,118). However, these articles do not prove donor specificity and no correlation with a pathologic outcome was been shown. Therefore, MICA antibodies is till yet to be proven as a true cause of allograft failure.

Data suggesting the involvement of postransplantation AGTR1 autoantibodies on renal allograft failure have also been shown (105). However, it has been generally associated with patients suffering from severe hypertension, which is not enough to prove the need for postransplantation routine tests for these autoantibodies

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AECA of undefined specificity must also be considered as potential mediators of graft rejection. Recent studies have suggested that de novo AECA are associated with a risk of early graft rejection (119,120). However, these studies present the same limitations of the ones related to MICA's.

CONCLUSION

The involvement of the HLA complex in solid organ transplantation, particularly renal transplantation, is obvious from the beginning. However, as long as the knowledge about human immune system grows deeper and new detection methods are developed, perspective on how HLA complex really impact in kidney transplantation changes. Immunosuppression and patient care have taken transplantation to a high level, but everything must be taken into account, in order to eradicate graft rejection and to prevent comorbidities related to immunological responses that lead to poorer outcomes and graft survival.

Research about the impact of non-HLA antigens must continue, as well as further investigation on the complement cascade mechanisms that may be linked to rejection. Antibody-mediated rejection must be better understood. This way, histocompatibility centers could redefine and improved their strategy on how to study a patient before, during and after real transplantation. The aim is to improve drastically the efficiency of allocation programs, providing these patients a better life quality and longer graft survival.

Progress in today's transplantation techniques is not only possible, but needed, as the main goal of any physician is to give his patients the better available treatment. To quote Sir Peter Medawar, 'To deride the hope of progress is the ultimate fatuity, the last word in poverty of spirit and meanness of mind.'

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REFERENCES

1. Murphy K. Janeway's Immunobiology. Eighth. Janeway's Immunobiology. New York: Garland Science; 2012.

2. Owen J, Punt J, Stranford S. Kuby Immunology. Seventh Ed. Kuby Immunology. New York: W.H. Freeman and Company; 2012.

3. Parham P. The Immune System. 4th ed. New York: Garland Science, Taylor & Francis Group, LLC; 2014.

4. Thorsby E. A short history of HLA. Tissue Antigens [Internet]. 2009;74(2):101–16. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19523022

5. Barker CF, Markmann JF. Historical overview of transplantation. Cold Spring Harb Perspect Med [Internet]. 2013;3(4):a014977. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23545575

6. Ullmann E. Tissue and organ transplantation. Ann Surg. 1914;60:195–219.

7. Jaboulay M. Kidney grafts in the antecubital fossa by arterial and venous anastomosis

HLA Complex: Brief History, General Characterization and Impact on Kidney Transplantation
Faculdade de Medicina da Universidade de Coimbra

42

[in French]. Bull Lyon Med. 1906;107:575–7.

8.   Flexner S. Report on Cancer. Report of the Director of Laboratories to the Scientific Directors. RURG 439. Rockefeller Archives. Sleepy Hollow, NY; 1914.

9.   Billingham RE, Brent L, Medawar PB. "Actively Acquired Tolerance" of Foreign Cells. Nature [Internet]. 1953 Oct 3 [cited 2015 Nov 5];172(4379):603–6. Available from: http://dx.doi.org/10.1038/172603a0

10.   Billingham RE, Brent L, Medawar PB. Quantitative Studies on Tissue Transplantation Immunity. III. Actively Acquired Tolerance. Philos Trans R Soc B Biol Sci [Internet]. 1956 Mar 15 [cited 2016 Jan 7];239(666):357–414. Available from: http://rstb.royalsocietypublishing.org/content/239/666/357.article-info

11.   Gorer PA. The genetic and antigenic basis of tumour transplantation. J Pathol Bacteriol [Internet]. 1937 May [cited 2016 Jan 8];44(3):691–7. Available from: http://doi.wiley.com/10.1002/path.1700440313

12.   Dausset J. Iso-leuco-anticorps. Acta Haematol [Internet]. Karger Publishers; 1958 Jul 1 [cited 2016 Jan 8];20(1-4):156–66. Available from: http://www.karger.com/Article/FullText/205478

13.   Bruning JW, van Leeuwen A van RJ. Leukocyte antigens. In: Amos DB van RJ, editor. Histocompatibility Testing 1965. Copenhagen: Munksgaard; 1965. p. 275–84.

14.   Bodmer WF, Bodmer J, Adler S, Payne R BJ. Genetics of "'4'" and "'LA'" human leukocyte groups. Ann N Y Acad Sci. 1966;129:473–89.

15.   Kissmeyer-Nielsen F, Svejgaard A, Hauge M. Genetics of the Human HL-A Transplantation System. Nature [Internet]. 1968 Sep 14 [cited 2016 Jan

HLA Complex: Brief History, General Characterization and Impact on Kidney Transplantation
Faculdade de Medicina da Universidade de Coimbra

10];219(5159):1116–9. Available from: http://dx.doi.org/10.1038/2191116a0

16. Thorsby E, Sandberg L, Lindholm A, Kissmeyer-Nielsen F. The HL-A System: Evidence of a Third Sub-Locus. Scand J Haematol [Internet]. 2009 Apr 24 [cited 2016 Jan 10];7(3):195–200. Available from: http://doi.wiley.com/10.1111/j.1600-0609.1970.tb01887.x

17. Mempel, W.; Grosse-Wilde, H.; Albert, E.; Thierfelder S. Atypical MLC reactions in HL-A typed related and unrelated pairs. Transpl Proc. 1973;5:401–8.

18. Horton R, Wilming L, Rand V, Lovering RC, Bruford EA, Khodiyar VK, et al. Gene map of the extended human MHC. Nat Rev Genet [Internet]. 2004 Dec [cited 2016 Jan 10];5(12):889–99. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15573121

19. Ceppellini R, Mattiuz PL, Scudeller G, Visetti M. Experimental allotransplantation in man. I. The role of the HL-A system in different genetic combinations. Transplant Proc [Internet]. 1969 Mar [cited 2016 Jan 10];1(1):385–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/4944245

20. Amos DB, Seigler HF, Southworth JG, Ward FE. Skin graft rejection between subjects genotyped for HL-A. Transplant Proc [Internet]. 1969 Mar [cited 2016 Jan 10];1(1):342–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/4944238

21. Vredevoe, DL; Terasaki, PI; Mickey, MR; Glassock, R; Merrill, JP; Murray J. Serotyping ofhuman lymphocyte antigens. III. Long term kidney homograft survivors. In: Amos, DB; van Rood J, editor. Histocompatibility Testing 1965. Munksgaard. Copenhagen: Munksgaard; 1965. p. 25–35.

22. Persijn GG, Gabb BW, van Leeuwen A, Nagtegaal A, Hoogeboom J, van Rood JJ. Matching for HLA antigens of A, B, and DR loci in renal transplantation by

Eurotransplant. Lancet (London, England) [Internet]. 1978 Jun 17 [cited 2016 Jan 10];1(8077):1278–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/78047

23. Albrechtsen D, Flatmark A, Jervell J, Solheim B, Thorsby E. HLA-DR antigen matching in cadaver renal transplantation. Lancet (London, England) [Internet]. 1978 Apr 15 [cited 2016 Jan 10];1(8068):825. Available from: http://www.ncbi.nlm.nih.gov/pubmed/85842

24. Ting A, Morris PJ. Matching for B-cell antigens of the HLA-DR series in cadaver renal transplantation. Lancet (London, England) [Internet]. 1978 Mar 18 [cited 2016 Jan 10];1(8064):575–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/76121

25. Levine BB, Ojeda A, Benacerraf B. Studies on artificial antigens. III. The genetic control of the immune response to hapten-poly-l-lysine conjugates in guinea pigs. J Exp Med [Internet]. 1963 Dec 1 [cited 2016 Jan 11];118:953–7. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2137686&tool=pmcentrez&rendertype=abstract

26. McDevitt HO, Chinitz A. Genetic control of the antibody response: relationship between immune response and histocompatibility (H-2) type. Science [Internet]. 1969 Mar 14 [cited 2016 Jan 11];163(3872):1207–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/5765335

27. McDevitt HO, Tyan ML. Genetic control of the antibody response in inbred mice. Transfer of response by spleen cells and linkage to the major histocompatibility (H-2) locus. J Exp Med [Internet]. 1968 Jul 1 [cited 2016 Jan 11];128(1):1–11. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2138514&tool=pmcentrez&rendertype=abstract

28. McDevitt H. The discovery of linkage between the MHC and genetic control of the

immune response. Immunol Rev [Internet]. 2002 Jul [cited 2016 Jan 11];185:78–85. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12190924

29.   Kindred B, Shreffler DC. H-2 dependence of co-operation between T and B cells in vivo. J Immunol [Internet]. 1972 Nov [cited 2016 Jan 11];109(5):940–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/4538719

30.   Rosenthal AS, Shevach EM. Function of macrophages in antigen recognition by guinea pig T lymphocytes. I. Requirement for histocompatible macrophages and lymphocytes. J Exp Med [Internet]. 1973 Nov 1 [cited 2016 Jan 11];138(5):1194–212. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2139433&tool=pmcentrez&rendertype=abstract

31.   Zinkernagel RM, Doherty PC. The discovery of MHC restriction. Immunol Today [Internet]. 1997 Jan [cited 2015 Dec 3];18(1):14–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9018968

32.   Zinkernagel RM, Doherty PC. Immunological surveillance against altered self components by sensitised T lymphocytes in lymphocytic choriomeningitis. Nature [Internet]. 1974 Oct 11 [cited 2016 Jan 11];251(5475):547–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/4547543

33.   Zinkernagel RM, Doherty PC. Restriction of in vitro T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semiallogeneic system. Nature [Internet]. 1974 Apr 19 [cited 2016 Jan 11];248(5450):701–2. Available from: http://www.ncbi.nlm.nih.gov/pubmed/4133807

34.   Goulmy E, Termijtelen A, Bradley BA, van Rood JJ. Y-antigen killing by T cells of women is restricted by HLA. Nature [Internet]. 1977 Apr 7 [cited 2016 Jan 11];266(5602):544–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/300847

HLA Complex: Brief History, General Characterization and Impact on Kidney Transplantation
Faculdade de Medicina da Universidade de Coimbra

46

35. Doherty PC, Zinkernagel RM. A biological role for the major histocompatibility antigens. Lancet (London, England) [Internet]. 1975 Jun 28 [cited 2016 Jan 11];1(7922):1406–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/49564

36. Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC. Structure of the human class I histocompatibility antigen, HLA-A2. Nature [Internet]. 1987 Jan [cited 2015 Jul 3];329(6139):506–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3309677

37. Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC. The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. Nature [Internet]. 1987 Jan [cited 2016 Jan 14];329(6139):512–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2443855

38. Brown JH, Jardetzky TS, Gorga JC, Stern LJ, Urban RG, Strominger JL, et al. Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. Nature [Internet]. 1993 Jul 1 [cited 2016 Jan 16];364(6432):33–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8316295

39. Tambur AR, Claas FHJ. HLA epitopes as viewed by antibodies: What Is it all about? Am J Transplant. 2015;15(5):1148–54.

40. Duquesnoy RJ. Epitope-based human leukocyte antigen matching for transplantation. Curr Opin Organ Transplant [Internet]. 2014 Aug [cited 2016 Feb 14];19(4):418–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25010063

41. Vella JP, Spadafora-Ferreira M, Murphy B, Alexander SI, Harmon W, Carpenter CB, et al. Indirect allorecognition of major histocompatibility complex allopeptides in human renal transplant recipients with chronic graft dysfunction. Transplantation [Internet].

1997 Sep 27 [cited 2016 Feb 14];64(6):795–800. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9326400

42. Takemoto S, Terasaki PI, Cecka JM, Cho YW, Gjertson DW. Survival of nationally shared, HLA-matched kidney transplants from cadaveric donors. The UNOS Scientific Renal Transplant Registry. N Engl J Med [Internet]. 1992 Sep 17 [cited 2016 Feb 13];327(12):834–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1508242

43. Opelz G. HLA matching and cadaver kidney transplantation--status 1984. Ulster Med J [Internet]. 1985 Aug [cited 2016 Feb 13];54 Suppl:S70–5. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2447977&tool=pmcentrez&rendertype=abstract

44. Mehra NK, Siddiqui J, Baranwal A, Goswami S, Kaur G. Clinical relevance of antibody development in renal transplantation. Ann N Y Acad Sci [Internet]. 2013 Apr [cited 2016 Feb 14];1283:30–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23384346

45. Süsal C, Opelz G. Current role of human leukocyte antigen matching in kidney transplantation. Curr Opin Organ Transplant [Internet]. 2013;18(4):438–44. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23838649

46. Opelz G, Döhler B. Ceppellini Lecture 2012: Collateral damage from HLA mismatching in kidney transplantation. Tissue Antigens. 2013;82(4):235–42.

47. Nankivell BJ, Alexander SI. Rejection of the kidney allograft. N Engl J Med [Internet]. 2010 Oct 7 [cited 2016 Feb 14];363(15):1451–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20925547

48. Porter KA. The effects of antibodies on human renal allografts. Transplant Proc [Internet]. 1976 Jun [cited 2016 Jan 29];8(2):189–97. Available from:

HLA Complex: Brief History, General Characterization and Impact on Kidney Transplantation
Faculdade de Medicina da Universidade de Coimbra

48

http://www.ncbi.nlm.nih.gov/pubmed/779179

49. Otten HG, Verhaar MC, Borst HPE, Hené RJ, van Zuilen a D. Pretransplant donor-specific HLA class-I and -II antibodies are associated with an increased risk for kidney graft failure. Am J Transplant [Internet]. 2012;12(6):1618–23. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22404993

50. Haririan A. Current status of the evaluation and management of antibody-mediated rejection in kidney transplantation. Curr Opin Nephrol Hypertens [Internet]. 2015;24(6):576–81. Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=000415 52-201511000-00017

51. Shabir S, Girdlestone J, Briggs D, Kaul B, Smith H, Daga S, et al. Transitional B Lymphocytes Are Associated With Protection From Kidney Allograft Rejection: A Prospective Study. Am J Transplant [Internet]. 2015;15(5):1384–91. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25808898

52. Opelz G, Döhler B. Influence of time of rejection on long-term graft survival in renal transplantation. Transplantation [Internet]. 2008 Mar 15 [cited 2016 Feb 15];85(5):661–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18337655

53. Einecke G, Sis B, Reeve J, Mengel M, Campbell PM, Hidalgo LG, et al. Antibody-mediated microcirculation injury is the major cause of late kidney transplant failure. Am J Transplant [Internet]. 2009 Nov [cited 2016 Feb 15];9(11):2520–31. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19843030

54. Lee P-C, Zhu L, Terasaki PI, Everly MJ. HLA-specific antibodies developed in the first year posttransplant are predictive of chronic rejection and renal graft loss.

Transplantation. 2009;88(4):568–74.

55. Hanf W, Bonder CS, Coates PTH. Transplant Glomerulopathy: The Interaction of HLA Antibodies and Endothelium. J Immunol Res [Internet]. 2014;2014:549315. Available from:

http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3987972&tool=pmcentrez&rendertype=abstract

56. Kissmeyer-Nielsen F, Olsen S, Petersen VP, Fjeldborg O. Hyperacute rejection of kidney allografts, associated with pre-existing humoral antibodies against donor cells. Lancet (London, England) [Internet]. 1966 Sep 24 [cited 2016 Jan 30];2(7465):662–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/4162350

57. Huang Y, Ramon D, Luan FL, Sung R, Samaniego M. Incidences of preformed and de novo donor-specific HLA antibodies and their clinicohistological correlates in the early course of kidney transplantation. Clin Transpl [Internet]. 2012 Jan [cited 2016 Feb 15];247–56. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23721029

58. Bunce M, O'Neill CM, Barnardo MC, Krausa P, Browning MJ, Morris PJ, et al. Phototyping: comprehensive DNA typing for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5 & DQB1 by PCR with 144 primer mixes utilizing sequence-specific primers (PCR-SSP). Tissue Antigens [Internet]. 1995 Nov [cited 2016 Jan 30];46(5):355–67. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8838344

59. Ng J, Hurley CK, Baxter-Lowe LA, Chopek M, Coppo PA, Hegland J, et al. Large-scale oligonucleotide typing for HLA-DRB1/3/4 and HLA-DQB1 is highly accurate, specific, and reliable. Tissue Antigens [Internet]. 1993 Nov [cited 2016 Jan 30];42(5):473–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8146858

60. Bettinotti MP, Mitsuishi Y, Bibee K, Lau M, Terasaki PI. Comprehensive method for

HLA Complex: Brief History, General Characterization and Impact on Kidney Transplantation
Faculdade de Medicina da Universidade de Coimbra

50

the typing of HLA-A, B, and C alleles by direct sequencing of PCR products obtained from genomic DNA. J Immunother [Internet]. 1997 Nov [cited 2016 Jan 30];20(6):425–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9409447

61. Rozemuller E. Collection and analysis of SBT results data. 13th IHWS Technology Joint Report. In: Hansen J, editor. Immunobiology of Human MHC. 2006. p. 413–6.

62. Erlich HA, Opelz G, Hansen J. HLA DNA typing and transplantation. Immunity. 2001;14(4):347–56.

63. Cecka JM. Calculated PRA (CPRA): the new measure of sensitization for transplant candidates. Am J Transplant [Internet]. 2010 Jan [cited 2016 Jan 18];10(1):26–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19958328

64. Cecka JM, Kucheryavaya AY, Reinsmoen NL, Leffell MS. Calculated PRA: initial results show benefits for sensitized patients and a reduction in positive crossmatches. Am J Transpl [Internet]. 2011;11(4):719–24. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21114658\nhttp://onlinelibrary.wiley.com/store/10.1111/j.1600-6143.2010.03340.x/asset/j.1600-6143.2010.03340.x.pdf?v=1&t=hzeb15z2&s=5bd5f98cd7e526271f8ba8d1528955be587de30c

65. Report AD. 2011 SRTR & OPTN Annual Data Report. 2011;(December).

66. Claas FHJ, Doxiadis IiIN. Human leukocyte antigen antibody detection and kidney allocation within Eurotransplant. Hum Immunol [Internet]. American Society for Histocompatibility and Immunogenetics; 2009;70(8):636–9. Available from: http://dx.doi.org/10.1016/j.humimm.2009.04.010

67.   Ministry of Health Portugal. Despacho nº 10 507/2000,2.a série, nº 119. Lisbon: Diário da República; p. 8736–7.

68.   DCD Kidney Allocation Working Party. Recommendations for allocating DCD kidneys. NHSBT Kidney Advisory Group; 2014. p. 1–4.

69.   Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. N Engl J Med [Internet]. 1969 Apr 3 [cited 2015 Sep 15];280(14):735–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/4886455

70.   Johnson AH, Rossen RD, Butler WT. Detection of alloantibodies using a sensitive antiglobulin microcytotoxicity test: identification of low levels of pre-formed antibodies in accelerated allograft rejection. Tissue Antigens [Internet]. 1972 Jan [cited 2016 Jan 27];2(3):215–26. Available from: http://www.ncbi.nlm.nih.gov/pubmed/4586478

71.   Kanter Berga J, Sancho Calabuig A, Gavela Martinez E, Puig Alcaraz N, Beltran Catalan S, Avila Bernabeu A, et al. Pretransplant donor-specific HLA antibodies detected by single antigen bead flow cytometry: Risk factors and outcomes after kidney transplantation. Transplant Proc [Internet]. Elsevier Inc.; 2012;44(9):2529–31. Available from: http://dx.doi.org/10.1016/j.transproceed.2012.09.102

72.   Pollinger HS, Stegall MD, Gloor JM, Moore SB, Degoey SR, Ploeger NA, et al. Kidney transplantation in patients with antibodies against donor HLA class II. Am J Transplant [Internet]. 2007 Apr [cited 2016 Feb 2];7(4):857–63. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17295642

73.   Mahoney RJ, Taranto S, Edwards E. B-Cell crossmatching and kidney allograft outcome in 9031 United States transplant recipients. Hum Immunol [Internet]. 2002 Apr [cited 2016 Feb 2];63(4):324–35. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12039415

HLA Complex: Brief History, General Characterization and Impact on Kidney Transplantation
Faculdade de Medicina da Universidade de Coimbra

52

74. Le Bas-Bernardet S, Hourmant M, Valentin N, Paitier C, Giral-Classe M, Curry S, et al. Identification of the antibodies involved in B-cell crossmatch positivity in renal transplantation. Transplantation [Internet]. 2003 Feb 27 [cited 2016 Feb 2];75(4):477–82. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12605113

75. Eng HS, Bennett G, Tsiopelas E, Lake M, Humphreys I, Chang SH, et al. Anti-HLA donor-specific antibodies detected in positive B-cell crossmatches by Luminex® predict late graft loss. Am J Transplant. 2008;8(11):2335–42.

76. Bentall A, Cornell LD, Gloor JM, Park WD, Gandhi MJ, Winters JL, et al. Five-year outcomes in living donor kidney transplants with a positive crossmatch. Am J Transplant [Internet]. 2013 Jan [cited 2015 Dec 23];13(1):76–85. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23072543

77. Chapman JR, Taylor CJ, Ting A, Morris PJ. Immunoglobulin class and specificity of antibodies causing positive T cell crossmatches. Relationship to renal transplant outcome. Transplantation [Internet]. 1986 Dec [cited 2016 Feb 3];42(6):608–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3538548

78. Eijsink C, Zoet YM, Kardol MJ, Witvliet MD, Claas FHJ, Mulder A, et al. Identification of acceptable HLA mismatches in immunized patients using single-antigen-expressing cell lines. Tissue Antigens [Internet]. 2007 Apr [cited 2016 Jan 28];69(4):354–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17389021

79. Garovoy MR, Rheinschmidt MA, Bigos M, Perkins H, Colombe B, Feduska N, et al. Flow cytometry analysis: A high technology crossmatch technique facilitating transplantation. Trans Proc. 1983;XV:1939–44.

80. Couzi L, Araujo C, Guidicelli G, Bachelet T, Moreau K, Morel D, et al. Interpretation of

positive flow cytometric crossmatch in the era of the single-antigen bead assay. Transplantation [Internet]. 2011 Mar 15 [cited 2016 Feb 6];91(5):527–35. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21192319

81. Messina M, Ariaudo C, Pratic?? Barbato L, Beltramo S, Mazzucco G, Amoroso A, et al. Relationship among C1q-fixing de novo donor specific antibodies, C4d deposition and renal outcome in transplant glomerulopathy. Transpl Immunol [Internet]. Elsevier B.V.; 2015;33(1):7–12. Available from: http://dx.doi.org/10.1016/j.trim.2015.06.002

82. Zachary AA, Ratner LE, Graziani JA, Lucas DP, Delaney NL, Leffell MS. Characterization of HLA class I specific antibodies by ELISA using solubilized antigen targets: II. Clinical relevance. Hum Immunol [Internet]. 2001 Mar [cited 2016 Feb 6];62(3):236–46. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11250041

83. Pei R, Lee J, Chen T, Rojo S, Terasaki PI. Flow cytometric detection of HLA antibodies using a spectrum of microbeads. Hum Immunol [Internet]. 1999 Dec [cited 2016 Jan 23];60(12):1293–302. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10626745

84. Pei R, Lee J-H, Shih N-J, Chen M, Terasaki PI. Single human leukocyte antigen flow cytometry beads for accurate identification of human leukocyte antigen antibody specificities. Transplantation [Internet]. 2003 Jan 15 [cited 2015 Dec 23];75(1):43–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12544869

85. Vaidya S. Clinical importance of anti-human leukocyte antigen-specific antibody concentration in performing calculated panel reactive antibody and virtual crossmatches. Transplantation [Internet]. 2008 Apr 15 [cited 2016 Feb 7];85(7):1046–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18408587

86. Mittag A, Tárnok A. Basics of standardization and calibration in cytometry--a review. J Biophotonics [Internet]. 2009 Sep [cited 2016 Feb 7];2(8-9):470–81. Available from:

HLA Complex: Brief History, General Characterization and Impact on Kidney Transplantation
Faculdade de Medicina da Universidade de Coimbra

54

http://www.ncbi.nlm.nih.gov/pubmed/19504519

87.   Tait BD, Süsal C, Gebel HM, Nickerson PW, Zachary A a, Claas FHJ, et al. Consensus guidelines on the testing and clinical management issues associated with HLA and non-HLA antibodies in transplantation. Transplantation. 2013;95(1):19–47.

88.   Kosmoliaptsis V, Bradley JA, Peacock S, Chaudhry AN, Taylor CJ. Detection of immunoglobulin G human leukocyte antigen-specific alloantibodies in renal transplant patients using single-antigen-beads is compromised by the presence of immunoglobulin M human leukocyte antigen-specific alloantibodies. Transplantation [Internet]. 2009 Mar 27 [cited 2016 Feb 7];87(6):813–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19300182

89.   Bartel G, Wahrmann M, Exner M, Regele H, Huttary N, Schillinger M, et al. In vitro detection of C4d-fixing HLA alloantibodies: associations with capillary C4d deposition in kidney allografts. Am J Transplant [Internet]. 2008 Jan [cited 2016 Feb 7];8(1):41–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17924995

90.   Hönger G, Wahrmann M, Amico P, Hopfer H, Böhmig GA, Schaub S. C4d-fixing capability of low-level donor-specific HLA antibodies is not predictive for early antibody-mediated rejection. Transplantation [Internet]. 2010 Jun 27 [cited 2015 Dec 23];89(12):1471–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20395886

91.   Loupy A, Lefaucheur C, Vernerey D, Prugger C, Duong van Huyen J-P, Mooney N, et al. Complement-binding anti-HLA antibodies and kidney-allograft survival. N Engl J Med [Internet]. 2013 Sep 26 [cited 2016 Feb 7];369(13):1215–26. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24066742

92.   Opelz G. Non-HLA transplantation immunity revealed by lymphocytotoxic antibodies.

Lancet (London, England) [Internet]. Jan [cited 2016 Feb 8];365(9470):1570–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15866311

93. Breimer ME, Rydberg L, Jackson AM, Lucas DP, Zachary AA, Melancon JK, et al. Multicenter evaluation of a novel endothelial cell crossmatch test in kidney transplantation. Transplantation [Internet]. 2009 Feb 27 [cited 2016 Feb 7];87(4):549–56. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19307793

94. Alheim M, Johansson SM, Hauzenberger D, Grufman P, Holgersson J. A flow cytometric crossmatch test for simultaneous detection of antibodies against donor lymphocytes and endothelial precursor cells. Tissue Antigens [Internet]. 2010 Mar [cited 2016 Feb 7];75(3):269–77. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20070600

95. Jackson AM, Lucas DP, Melancon JK, Desai NM. Clinical relevance and IgG subclass determination of non-HLA antibodies identified using endothelial cell precursors isolated from donor blood. Transplantation [Internet]. 2011 Jul 15 [cited 2016 Feb 7];92(1):54–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21516064

96. Kafetzi ML, Boletis JN, Melexopoulou CA, Tsakris A, Iniotaki AG, Doxiadis IIN. Clinical evaluation of the endothelial tie-2 crossmatch in ABO compatible and ABO incompatible renal transplants. Hum Immunol [Internet]. 2013 Nov [cited 2016 Feb 7];74(11):1425–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23792052

97. Alheim M, AlMahri A, Nilsson J, Tydén G, Holgersson J. The outcome of the endothelial precursor cell crossmatch test in lymphocyte crossmatch positive and negative patients evaluated for living donor kidney transplantation. Hum Immunol [Internet]. 2013 Nov [cited 2016 Feb 8];74(11):1437–44. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23777925

HLA Complex: Brief History, General Characterization and Impact on Kidney Transplantation
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98. Zitzner JR, Shah S, Jie C, Wegner W, Tambur AR, Friedewald JJ. A prospective study evaluating the role of donor-specific anti-endothelial crossmatch (XM-ONE assay) in predicting living donor kidney transplant outcome. Hum Immunol [Internet]. 2013 Nov [cited 2016 Feb 8];74(11):1431–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23777928

99. Cox ST, Stephens HAF, Fernando R, Karasu A, Harber M, Howie AJ, et al. Major histocompatibility complex class I-related chain A allele mismatching, antibodies, and rejection in renal transplantation. Hum Immunol [Internet]. 2011 Oct [cited 2016 Feb 8];72(10):827–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21664940

100. Zou Y, Stastny P, Süsal C, Döhler B, Opelz G. Antibodies against MICA antigens and kidney-transplant rejection. N Engl J Med [Internet]. 2007 Sep 27 [cited 2015 Nov 26];357(13):1293–300. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17898098

101. Sumitran-Holgersson S, Wilczek HE, Holgersson J, Söderström K. Identification of the nonclassical HLA molecules, mica, as targets for humoral immunity associated with irreversible rejection of kidney allografts. Transplantation [Internet]. 2002 Jul 27 [cited 2016 Feb 8];74(2):268–77. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12151741

102. Lemy A, Andrien M, Wissing KM, Ryhahi K, Vandersarren A, Racapé J, et al. Major histocompatibility complex class 1 chain-related antigen a antibodies: sensitizing events and impact on renal graft outcomes. Transplantation [Internet]. 2010 Jul 27 [cited 2016 Feb 8];90(2):168–74. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20463636

103. Filippone EJ, Farber JL. Humoral Immune Response and Allograft Function in Kidney

Transplantation. Am J Kidney Dis. 2015;66(2):337–47.

104. Dragun D, Müller DN, Bräsen JH, Fritsche L, Nieminen-Kelhä M, Dechend R, et al. Angiotensin II type 1-receptor activating antibodies in renal-allograft rejection. N Engl J Med [Internet]. 2005 Feb 10 [cited 2016 Feb 8];352(6):558–69. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15703421

105. Taniguchi M, Rebellato LM, Cai J, Hopfield J, Briley KP, Haisch CE, et al. Higher risk of kidney graft failure in the presence of anti-angiotensin II type-1 receptor antibodies. Am J Transplant [Internet]. 2013 Oct [cited 2016 Feb 8];13(10):2577–89. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23941128

106. Reinsmoen NL, Lai C-H, Heidecke H, Haas M, Cao K, Ong G, et al. Anti-angiotensin type 1 receptor antibodies associated with antibody mediated rejection in donor HLA antibody negative patients. Transplantation [Internet]. 2010 Dec 27 [cited 2016 Feb 8];90(12):1473–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21030904

107. Taylor CJ, Kosmoliaptsis V, Sharples LD, Prezzi D, Morgan CH, Key T, et al. Ten-year experience of selective omission of the pretransplant crossmatch test in deceased donor kidney transplantation. Transplantation [Internet]. 2010 Jan 27 [cited 2015 Dec 23];89(2):185–93. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20098281

108. Amico P, Hönger G, Mayr M, Steiger J, Hopfer H, Schaub S. Clinical relevance of pretransplant donor-specific HLA antibodies detected by single-antigen flow-beads. Transplantation [Internet]. 2009 Jun 15 [cited 2016 Feb 8];87(11):1681–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19502960

109. Aubert V, Venetz J-P, Pantaleo G, Pascual M. Low levels of human leukocyte antigen donor-specific antibodies detected by solid phase assay before transplantation are frequently clinically irrelevant. Hum Immunol [Internet]. 2009 Aug [cited 2016 Feb

HLA Complex: Brief History, General Characterization and Impact on Kidney Transplantation
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8];70(8):580–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19375474

110. Bryan CF, McDonald SB, Luger AM, Shield CF, Winklhofer FT, Michael Borkon A, et al. Successful renal transplantation despite low levels of donor-specific HLA class I antibody without IVIg or plasmapheresis. Clin Transplant [Internet]. Jan [cited 2016 Feb 8];20(5):563–70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16968481

111. Burns JM, Cornell LD, Perry DK, Pollinger HS, Gloor JM, Kremers WK, et al. Alloantibody levels and acute humoral rejection early after positive crossmatch kidney transplantation. Am J Transplant [Internet]. 2008 Dec [cited 2016 Feb 8];8(12):2684–94. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18976305

112. Gebel HM, Bray RA, Nickerson P. Pre-Transplant Assessment of Donor-Reactive, HLA-Specific Antibodies in Renal Transplantation: Contraindication vs. Risk. Am J Transplant. 2003;3(12):1488–500.

113. Morath C, Beimler J, Opelz G, Ovens J, Scherer S, Schmidt J, et al. An integrative approach for the transplantation of high-risk sensitized patients. Transplantation [Internet]. 2010 Sep 27 [cited 2016 Feb 8];90(6):645–53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20671598

114. Worthington JE, Martin S, Al-Husseini DM, Dyer PA, Johnson RWG. Posttransplantation production of donor HLA-specific antibodies as a predictor of renal transplant outcome. Transplantation [Internet]. 2003 Apr 15 [cited 2016 Feb 8];75(7):1034–40. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12698094

115. Wiebe C, Gibson IW, Blydt-Hansen TD, Karpinski M, Ho J, Storsley LJ, et al. Evolution and clinical pathologic correlations of de novo donor-specific HLA antibody post kidney transplant. Am J Transplant [Internet]. 2012 May [cited 2016 Feb 8];12(5):1157–67.

Available from: http://www.ncbi.nlm.nih.gov/pubmed/22429309

116. Feucht HE, Schneeberger H, Hillebrand G, Burkhardt K, Weiss M, Riethmüller G, et al. Capillary deposition of C4d complement fragment and early renal graft loss. Kidney Int [Internet]. 1993 Jun [cited 2016 Feb 8];43(6):1333–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8315947

117. Nickeleit V, Zeiler M, Gudat F, Thiel G, Mihatsch MJ. Detection of the complement degradation product C4d in renal allografts: diagnostic and therapeutic implications. J Am Soc Nephrol [Internet]. 2002 Jan [cited 2016 Jan 20];13(1):242–51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11752044

118. Ozawa M, Rebellato LM, Terasaki PI, Tong A, Briley KP, Catrou P, et al. Longitudinal testing of 266 renal allograft patients for HLA and MICA antibodies: Greenville experience. Clin Transpl [Internet]. 2006 Jan [cited 2016 Feb 9];265–90. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18365384

119. Zhang X, Reed EF. Effect of antibodies on endothelium. Am J Transplant [Internet]. 2009 Nov [cited 2015 Dec 23];9(11):2459–65. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4412741&tool=pmcentrez&rendertype=abstract

120. Sun Q, Cheng Z, Cheng D, Chen J, Ji S, Wen J, et al. De novo development of circulating anti-endothelial cell antibodies rather than pre-existing antibodies is associated with post-transplant allograft rejection. Kidney Int [Internet]. 2011 Mar [cited 2016 Feb 9];79(6):655–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20980975

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