

Immunology for Renal Transplantation: A Review

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Abstract

The current success of renal transplantation (RT) is the result of advancement in the understanding of the transplant immunology from gross allograft rejection to cellular and antibody response to the current molecular level. Laboratory assay technologies have been developed to characterise patient sensitisation and to detect pre-existing donor-specific antibodies (DSA) in pre-transplant crossmatch, which has helped prevent premature transplant losses from early and late antibody-mediated rejection. After a RT, pre-existing or de novo DSA are routinely monitored for modulation of immunosuppressive regimens. Therefore, it is mandatory for the personnel involved in the management of RT recipients to have clear understanding of the components of transplantation immunology and be familiar with the modern immunological techniques used in RT.

Keywords: Allorecognition; Human leucocyte antigen; Panel reactivity; Crossmatch; Donor-specific antibody; Rejection

Introduction

Since the first successful renal transplantation (RT) performed between the identical twins in the Peter Brent Brigham Hospital, Boston on 23rd December 1954 by Murray et al., RT has become a routine for the patients with end-stage renal disease. When a kidney is transplanted from a non-identical individual, the allograft; the recipient mounts an immune response termed as an alloimmune response [1]. The alloimmune response is initiated by the T cell recognition of the alloantigen and process is commonly known as the allorecognition. Allorecognition is the first step of the complex events that leads to T lymphocyte activation, antibody production, complement activation, allograft rejection and transplant failure [2]. This review highlights the fundamental principles of transplant immunology and modern immunological assay techniques, which have important application in RT practice.

HLA Molecules

In humans, the major histocompatibility complex (MHC) genes code the strongest transplant antigens, which are called human leucocyte antigens (HLA). The HLA loci are located on the short arm of chromosome 6 [3]. The HLA haplotypes are inherited by the offspring from each parent in Mendelian co-dominant fashion. The Class I HLA are subdivided into HLA-A, -B and -C and is composed of a polymorphic heavy chain (α chain, 44 kDa) and a non-polymorphic light chain (β 2-microglobulin, 12 kDa). Class I HLA molecules are expressed on all nucleated cells in the body and are involved in presenting endogenous small antigens (typically 9 to 11 amino acids), such as viruses and self-protein fragments in the context of self MHC to CD8+ T-lymphocytes. Class II HLA molecules (HLA-DP, -DQ, and -DR) are composed of polymorphic α chain (35 kDa) and a β chain (31 kDa) and are expressed on antigen presenting cells (APC) such as dendritic cells, macrophages, B cell, endothelial cells and some epithelial cells, which allow presentation of a diverse

number of foreign peptides derived from the graft [4,5]. Proinflammatory cytokines released in response to any form of injury, upregulate the expression of HLA class II molecules on the epithelial and vascular endothelial cells, thereby enhancing allorecognition. Class II molecules present relatively larger antigens (12 to 28 amino acids) derived from extracellular proteins to CD4+ T-lymphocytes [6].

HLA Matching

The degree of HLA mismatch between the donor and recipient influences the risk of acute and chronic rejection, sensitisation and allograft survival. Traditionally, HLA-A, -B, and -DR (3 pairs and 6 antigens) are used for tissue typing and matching before RT. The sequencing of class I and II HLA loci used for histocompatibility testing include restriction fragment length polymorphism (RFLP), oligonucleotide hybridization and polymorphism-specific amplification using polymerase chain reaction and sequence-specific primers (PCR-SSP) [7-10]. Currently, the risk of rejection and sensitisation related to HLA-Cw, -DP, and -DQ mismatch is being increasingly recognised and therefore included in the tissue typing armamentarium in several transplant centres [11-13]. The major impact of HLA mismatch is seen from the match of DR antigen, and the order of importance for HLA match in RT is DR>B>A. For RT, the best long-term graft survival is seen following HLA-identical living-related RT [14,15].

Non-HLA Antigens

Despite a complete HLA match between the donor and recipient such as in HLA-identical siblings RT, acute and chronic rejection can occur, which is due to the presence of immune response to non-HLA antigens. There are reports of several non-HLA antigens and their antibodies derived from either alloimmunity or autoimmunity [16,17].

ABO blood group antigens

ABO blood group antigens play an important role in RT, which are expressed on the red cells, most epithelial and endothelial cells and

blood cells such as T cells, B cells and platelets where ABO antigens have adsorbed from plasma [18]. ABO incompatible RT leads to hyperacute rejection due to presence of preformed haemagglutinin A and/or B antibody. Akin to blood transfusion, compatibility of blood groups is essential in RT. The Rhesus factor and other red cell antigens are not relevant to organ transplants, as they are not expressed in vascular endothelium. The first attempt at ABOi RT was reported in 1955 by Chung et al., where eight of ten ABOi kidney allografts did not work successfully within the first few postoperative days [19]. In 1987, Alexandre et al. introduced an effective desensitization protocol to achieve success in ABOi living donor RT. This protocol included pretransplant repeated plasmapheresis as a strategy not only to reduce the titres of anti-A or -B antibodies, but also to decrease the antilymphocyte globulin-based induction [20]. Transplanting kidneys from A2 or A2B into blood group B or O patients were carried out in the USA in 1986 resulting in 72% 10 year graft survival of 72% for B recipients of an A2 or A2B kidneys which was equivalent to 69% graft survival to that B recipients of B kidneys [21].

Minor histocompatibility antigens

Besides the major HLA, there are small endogenous peptides, known as the minor histocompatibility antigen (MiHA), which occupy the antigen-binding sites of MHC molecules and are recognised by Cd8+ T-cells in the context of self -MHC leading to graft rejection. The H-Y MiHA is encoded by the Y chromosomes in males and can induce alloimmune response when a male organ is transplanted into a female recipient. MHC class 1 related chain A and B (MICA and MICB) are expressed in endothelial cells. Antibody-mediated rejections occurring in the absence of DSA directed against HLA on many occasions are due to antibodies directed against MICA and /or MICB leading to transplant loss [22-24].

Other antibodies such as anti-angiotensin-2 receptor, anti-glutathione S-transferase T1 and anti-endothelial antibodies are identified to be involved in causing AMR [25,26]. Anti-endothelial antibody can be detected by using donor monocyte for cross-match [27]. Some minor transplant antigens may come from mitochondrial proteins and enzymes [28]. In the future, several allo- and autoreactive antibodies are likely to be identified with the advancement of transplant immunology.

Allorecognition and T Cell Activation

In the donor kidney, particularly from a deceased donor, rapid swing in blood pressure and ischaemia leads to massive cytokine storm that activates the resident dendritic cells, vascular endothelium and tubular epithelial cells. Furthermore, ischaemia-reperfusion injury leads to cellular death and release of immunologically active molecules called damage-activates molecular patterns (DAMPs) such as heat-shock proteins, adenosine triphosphate (ATP), uric acid, ribonucleic acid (RNA), deoxyribonucleic acid (DNA), as well as proteins derived from extracellular matrix including hyaluronan fragments and heparin sulphate proteoglycans [29,30]. Epithelial, mesenchymal and endothelial cells within the donor kidney express receptors for which include toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-like receptors (NLRs), Interaction of the products of cellular injury with TLR and NLR sets of production of cytokines and chemokines, which are strong attractants for recipient innate immune cells [31-34].

Rejection of transplanted kidney involves both adaptive (T and B cells) and innate (dendritic cells, macrophages, neutrophils, mast cells and natural killer cells) immune system, The innate immune system is activated by DAMPs, cytokines and chemokines released due to ischaemia-perfusion injury and microbial products, which once activated, triggers the adaptive immune system leading to cellular rejection. On the other hand, the activation stimulus to adaptive immune system is the alloantigens [35]. Allorecognition can occur by direct or indirect pathways. In the direct pathway, recipient's T cells recognise intact allogenic HLAs expressed in the donor cells. In the indirect pathway, T cells recognise peptides derived from the donor HLAs presented by the recipient APCs. In the immediate post-transplant period, the recipient T-cells will recognise donor graft alloantigens if adequate immunosuppression is not achieved, leading to strong alloresponse and acute cellular rejection. The indirect pathway of allorecognition is predominant in the late onset of rejection, especially in chronic rejection, although this pathway may also participate in acute rejection. Therefore maintenance of immunosuppression is paramount for lifetime to prevent late acute and chronic rejection [36,37].

The activation of the immune response begins in the recipient immediately after establishment of the vascular connection between the donor and recipient. First, the recipient is exposed to a torrent of chemicals, cytokines and chemokines derived from the ischaemic damaged kidney, which attracts the innate immune cells to infiltrate the organ adding to the ischaemia-reperfusion induced injury. Simultaneously, the activated dendritic migrate out of the graft to the T-cell rich regions of the recipient lymph nodes where they encounter nave recipient T cells, which is the key initiating event of T cell activation and cellular rejection [38]. The donor dendritic cells and recipient T cells engage each other using cell surface receptors, the HLA molecules on the dendritic cell and the T cell receptor (TCR), the junction is called an "immunological synapse". The TCR is comprised of an alpha chain and a beta chains and several associated molecules called the CD3 chains [39,40].

Included in the synapse are the juxtaposed T cell (TCR/CD3 complex) and the dendritic cell (HLA/peptide complex), which initiates the antigen-specific intracellular signal (signal 1). Simultaneously, additional molecules coalesce in the synapse to generate second signal called co-stimulation signal (signal 2), which is essential for complete T cell activation. Lack of signal 2 leads to either anergy or apoptosis [36,39]. The receptor-ligand interaction between T-cells and the antigen presenting cells (APCs) which are involved in generation of co-stimulatory signals are CD28-B7 and CD154-CD40. CD28 and CD154 are expressed on T cells and their ligands B7 and CD40 are expressed on APCs. CD28 consists of two ligands, B7-1 (CD80) and B7-2 (CD86). T cells also express cytotoxic T-lymphocyte associated antigen-4 (CTLA-4), which is homologous to CD28, but has a higher affinity than CD28 to bind B7. Binding of CTLA-4 to B7 (both CD80 CD86) leads to generation of inhibitory signal to terminate T cell response [41,42].

Within seconds of assembly of immunological synapses, the combination of signal 1 and 2 activates three downstream signal transduction pathways within the cytoplasm of the T cell: the calcium-calineurin pathway, the RAS-mitogen activated protein kinase (MAPK) pathway, and the IKK-nuclear factor κB (NF- κB) pathway [43-45]. The signals generated from through these three pathways reach the nucleus and activates gene transcription factors including the nuclear factor of activated T cells (NFAT), activated protein-1, and

NF- κ B, respectively. As a result of gene transcription, several new molecules and cytokines including CD25, CD154, interleukin-2 (IL-2) and interferon- γ (IFN- γ) are secreted and their receptors expressed [46]. IL-2 binds to its own receptor on the surface of T cell in autocrine fashion (signal 3), which, activates the mammalian target of rapamycin (mTOR) pathway, phosphoinositide-3-kinase (PI3K) pathway and Janus kinase/signal transducers and activators of transcription protein pathway (JAK/STAT), which allow the activated T cells to progress through the cell division cycle and clonal expansion of donor HLA/peptide-specific effector (CD8+ cytotoxic T cells) T cells [47,48]. These cells produce CD8+ T-cell mediated cytotoxicity, help macrophage-induced delayed type hypersensitivity response (CD4+Th1) and help B cells for antibody production (CD4+Th2) [49,50]. A subset of activated T cells (CD4 or CD8) survive in an inactive state for a long period, called the memory T cells, but will quickly expand and mount an aggressive immune when re-exposed to the same foreign protein [51].

Tacrolimus and cyclosporine inhibit calcineurin leading to blockade of IL-2 gene transcription and development of cytotoxic T lymphocytes [52]. Anti-IL-2 receptor blockade with monoclonal antibodies such as basiliximab and daclizumab and mTOR inhibitor such as sirolimus and everolimus have been successful in inhibiting T cell activation and reduce the incidence of acute rejection [53,54].

T Cell-Mediated Cytotoxicity

Physiological cytotoxicity is mediated by CD8+ T cells because they bind to the class I MHC of all nucleated cells. Killing occurs either by a calcium-dependent secretory mechanism or a calcium-independent mechanism that requires direct contact [55]. The influx of calcium during the activation causes exocytosis of cytolytic granules containing a lytic protein called perforin and serine proteases granzymes. Perforin forms a defect in the target cell membrane allowing granzyme activity to lyse the cell. In the absence of calcium, cytotoxic T cells upregulate fas ligand upon activation, which can then bind to fas on the target cells, leading to apoptosis, which is a programmed cell death that involves fragmentation of nuclear contents [56,57].

Role of B Lymphocytes

B cells recognise antigens in its native form without the requirement of processing and presentation on MHC molecules [58]. Cross-linking of antigen to the antigen-specific surface antibodies stimulates B cell activation, proliferation, and differentiation in to a plasma cell. Some activated B cells become memory B cells. B cells also internalise antigens, process them and present to the T cells through the indirect pathway of allorecognition. They receive signals from T cells via CD40 by binding to the T cell CD40L, which upregulate expression of B7 molecules on B cells and facilitate antigen presentation and T cell costimulation [59]. The anti-HLA antibodies produced by plasma cells bind alloantigen and cause graft injury either by activating the complement cascade known as complement-dependent cytotoxicity (CDC) or via Fc receptor in the natural killer (NK) cells, neutrophils and antibody-dependent cellular cytotoxicity [60].

Donor-specific Antibodies

Plasma cells can produce antibodies against both HLA and non-HLA antigens. Non-HLA antibodies directed against donor endothelial antigens such as MHC class I polypeptide-related sequence

A or B (MICA and MICB), smooth muscle antigen (vimentin), collagen-V and cell surface receptor such a type I angiotensin II receptor are reliably detected by currently available techniques [61]. Donor-specific antibodies (DSA) are measures routinely post-transplantation to detect the reactivity of the recipient B cells against the donor antigens. If the titre of the specific DSA rises suggesting inadequate immunosuppression, several therapeutic options, including plasmapheresis, thymoglobulin, intravenous immunoglobulin (IVIG), and anti-CD20 antibody (rituximab) can be attempted [62-64]. Emerging therapies include proteasome inhibitors such as Bortezomib [65]. Several studies have suggested that DSA to HLA antigens and endothelial antigens may be a driver not only of acute antibody-mediate rejection (AMR) but also chronic rejection [66].

Sensitisation and Panel Reactivity

Sensitisation is defined by the presence of antibodies in the recipient blood against a panel of selected HLA antigens representing donor population, which is reported as percentage panel reactivity (PRA). PRA estimates the likelihood of positive crossmatch to potential donors. The higher is the PRA level; the lower becomes the chance of receiving compatible kidney and longer the waiting time on the waitlist [67]. Sensitisation is caused by exposure to HLA antigens through failed previous transplants, pregnancy and blood transfusion [68,69]. The percent PRA in an individual may vary from time to time due either to a change in the antibody titre, or a change in the usage of HLA antigens in the assay. The technology of PRA assay has advanced significantly from the initial CDC assay to solid phase based enzyme-linked immunosorbent assay (ELISA), to a current multiplexed particle-based flow cytometry (Luminex). Single antigen beads are increasingly used to characterise the preformed HLA antibodies before RT and to detect development DSA after RT [70].

Crossmatch

When a potential donor is identified, a crossmatch with fresh serum from recipient and lymphocytes from donor has to be performed to exclude preformed DSA, which can lead hyperacute AMR. The final crossmatch must be negative to proceed with RT. CDC crossmatch and flow cytometry (FCXM) are two commonly used methods, although the choice is dependent on individual centre experience and their availability [71,72].

T cells express class I antigens only, while B cells express both HLA class I and class II antigens. Furthermore, there is significantly increased expression of HLA class I antigens in B cells compared to T cells. A positive T cell crossmatch is considered as true and significant sensitisation with DSA against HLA class I antigens. A negative T cell crossmatch and a positive B cell crossmatch may represents presence of either HLA class II antibodies or low titre of HLA class I antibodies. A positive T cell crossmatch associated with a negative B cell crossmatch is likely to be due to the presence of non-HLA antibodies [73-75].

The antigens corresponding to preformed HLA antibodies are considered unacceptable for that patient. So a patient will not be offered a kidney from a deceased donor, who expresses an unacceptable HLA antigen (positive virtual crossmatch). Only those patients whose HLA antibodies are not donor directed will appear on the matching run (negative virtual crossmatch). Virtual crossmatch is increasing used in clinical practice as this has improved the efficiency

of organ allocation by decreasing the risk of positive crossmatch and cold ischaemia time [76,77].

Complement-dependent Cytotoxicity Crossmatch

The donor T and B lymphocytes are isolated from lymph nodes and blood, and placed in wells, where the recipient serum and rabbit complement are added. The cytotoxicity is determined by counting the lysis of the lymphocytes compared to the control. Antihuman globulin is added to increase the sensitivity (AHG-CDC), as antihuman globulin can induce cross-linking of antibodies and increases the visual cytotoxicity [78]. If the CDC crossmatch becomes positive, this is repeated with addition of dithiothreitol (DTT), which reduces the disulphide bonds of the immunoglobulin (Ig)M if it present and makes the crossmatch negative in the presence of IgM antibodies [79]. A positive CDC crossmatch before and after treatment with DTT indicate the presence of DSA of IgG nature. IgM antibodies are generally not considered to cause real sensitisation. In the presence of a positive CDC cross match secondary to a cytotoxic IgG anti-HLA antibody, RT should not proceed, unless a desensitisation protocol is adopted to remove the preformed DSA and a negative crossmatch is achieved [80].

Flow Cytometric Crossmatch

In RT, Flow Cytometric Cross Match (FCXM) represents the most sensitive method of demonstrating the binding HLA antibodies and has revolutionised the HLA antibody assessment and facilitated improved allograft survival among highly sensitised recipients. The FCXM is performed by incubating donor lymphocytes with recipient serum after addition of fluorochrome-labeled antihuman IgG. The amount of measured fluorescence, that is the channel shift in the fluorescence intensity, is proportional to the amount of antibody bound to the target cell [81,82]. In order to help differentiate the class of antibody that may be binding to the lymphocytes, labelled monoclonal antibodies are used to identify T-cells (CD3) or B-cells (CD19). FCXM is much more sensitive than CDC or AHG-CDC in detecting low level antibodies. Non-cytotoxic antibodies can also be detected with FCXM since it is not dependent on complement activation of the antibody. Single antigen bead (Luminex) can be used to further to determine if the DSA is responsible for the channel shift in the FCXM [83,84].

The CDC and FCXM differ in their degree of sensitivity. FCXM significantly reduces the incidence of post-RT AMR, but it may also be too sensitive to detect clinically irrelevant antibodies there by denying opportunity to some potential transplants [85]. Crossmatch tests can also be performed with the recipient's previous sera. A negative crossmatch for current sera and positive for historical sera suggests previous antibodies which may have waned in titre [86]. The specific memory B cells could rapidly expand and produce antibodies when re-exposed to specific alloantigen. There is an increased risk of AMR, but this situation is not a contra-indication for a RT. Close monitoring of DSA and immunosuppression is essential [87,88].

Tolerance

In order to meet the growing need of organs for transplantation, induction of tolerance to an organ allograft is under investigation. Tolerance is characterised by the specific lack of unrestrained adverse immune reactivity, the maintenance of which does not require chronic immunosuppression. Transplantation of donor bone marrow to

condition recipients leads to a state of mixed chimerism, where the recipient and donor haemopoietic cells co-exist. This induces tolerance allowing successful withdrawal of chronic immunosuppression in both animal and human models post-transplantation [89,90]. Blockade of CD28 using CTLA4Ig has been successful in inducing tolerance of human pancreatic cells in diabetic mice [91]. Several types of T cells with immunosuppressive properties have been identified, but FOXP3⁺ regulatory T (TREG) cells have emerged as a dominant cell type; they are critically involved in the induction and maintenance of immune tolerance. Manipulation of this cell type for the induction of transplant tolerance including renal transplant tolerance has attracted considerable attention [92]. Belatacept, a recombinant fusion protein composed of the Fc fragment of a human IgG1 immunoglobulin linked to the extracellular domain of CTLA-4, which is a molecule crucial for T-cell costimulation, is effective in induction of tolerance through co-stimulation blockade and inhibition of generation of allogenic-specific TREG cells in human [93,94].

Xenotransplantation

Transplanting organs from animal sources, xenotransplantation (XT), remains in the experimental stage. Hyperacute rejection due to the presence of galactose- α 1,3-galactose (Gal) antibodies in man against the Gal- α 1,3-Gal antigen present in the pig organs [95,96], is the major barrier to XT. This is being overcome by breeding transgenic pigs that express human decay accelerating factor on their vascular endothelium [97]. Alternatively the production of cloned α -1,3-Gal knockout pigs, through elimination of the gene that encodes for the α 1,3-galactosyl transferase enzyme necessary for the generation of α -Gal epitope, may prevent complement activation and hyperacute rejection [98]. Xenozoonoses derived from the transfer of porcine endogenous retrovirus is the major risk of xenotransplantation [99]. Other strategies used are the establishment of xenogenic tolerance through mixed chimerism [100] and thymic transplantation [101].

Conclusion

Understanding of the immunology related to RT, advances in the techniques of detection and characterisation of antibodies before and after RT and the crossmatch techniques have significantly improved the outcomes of RT over last two decades. A late allograft loss from chronic antibody-mediated rejection still remains a major problem, which needs further research to advance our understandings of the immunological process involved that would help to reduce the transplant losses.

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