

An Insight Into the Immunologic Events and Risk Assessment in Renal Transplantation

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Abstract

Organ transplantation has always been considered to be the optimal therapeutic intervention in patients with end-stage organ failure. In the US, approximately 615,000 patients are diagnosed with end-stage renal disease and less than 30% have received a kidney transplant. One of the crucial drawbacks in successful renal transplantation is allograft rejection. Survival rates among transplant recipients have greatly improved due to better understanding of transplant biology and more effective immunosuppressive agents. Post-transplant immune monitoring and optimization of the immunosuppressive therapy using non-invasive biomarkers can effectively predict impending graft rejection and may spare the need for renal biopsy. This article provides an insight into the immunomodulations of renal transplant recipients. It depicts the immune system including several types of kidney rejection and reviews the biomarkers that may serve in near future, as surveillance tools for graft monitoring. Finally, a summary on the main immunosuppressive drugs used in kidney transplant both in the induction and maintenance phases is also covered.

Keywords: Transplant; Immunological events; Renal transplant; Transplant Rejection; Transplant biomarkers

Introduction

Renal transplant is a growing area of interest and enthusiasm in the modern era of nephrology. In the US, approximately 615,000 patients are diagnosed with end-stage renal disease (ESRD) and less than 30% have received a kidney transplant. More than 100,000 patients are on waiting list for a donor kid-

ney [1]. This article is a brief overview of the immunology that pertains to the donor recipient interaction. It also depicts the several types of kidney rejection and reviews the biomarkers that may serve in near future, as surveillance tools for graft monitoring. Finally, a summary on the main immunosuppressive drugs used in kidney transplant both in the induction and maintenance phases is reviewed.

Reviewing the Immune System

Most of the immune targets in an allograft are the polymorphic human leukocyte antigen (HLA) molecules that are cell-surface antigen-presenting proteins, encoded by chromosome 6. HLA class I molecules, mainly comprised of A, B and C antigens, are expressed by all allograft nucleated cells, such as tubular cells, and activate cytotoxic (CD8) T cells and natural killer (NK) cells. Class II HLA molecules are expressed on recipients B lymphocytes and on antigen-presenting cells (APCs). In tissues, APCs are called macrophages, in the liver, they are called Kupffer cells, in the skin, they are called Langerhans cells, in the central nervous system, they are called microglia and in the blood, they are called monocytes. Majority of class II HLAs are comprised of DP, DQ, and DR antigens. The most clinically significant antigens in kidney transplantation are A, B, and DR.

APCs process donor antigens that interact with CD4 T cells with the end result being interleukin 2 (IL-2) secretion, leading to further T-cell activation and population expansion downstream. As a result, T cells undergo maturation to effector cells and start producing cytokines such as IL-4, causing B cells to differentiate to antibody producing plasma cells. Plasma cell will then produce anti-HLA antibodies that interact with the donor HLA, leading to complement activation and triggering of an inflammatory response [2].

Talking about cytokines, these molecules are small, short-acting proteins that are produced by a wide variety of cells such as APCs, lymphocytes, and parenchymal cells. They can be divided into two types: 1) pro-inflammatory cytokines such as IL-1, IL-2, tumor necrosis factor-alpha, and interferon gamma, which are involved in cell-mediated immunity and allograft rejection; 2) anti-inflammatory cytokines such as IL-4, IL-6, and IL-10, which coordinate cellular and humoral responses and are associated with allograft protection. Due to its ability to suppress inflammation, IL-10 is thought to play a critical

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role in allograft tolerance.

Graft Rejection

There are four types of rejection described in the immunology literature.

Hyperacute rejection occurs within minutes to hours of graft reperfusion and is due to the presence of high levels of preformed anti-donor antibodies that react with donor vascular endothelium immediately after perfusion. However, in some recipients, preexisting antibodies that have waned over the years allow the graft to work for few days until the sensitized host mounts an anamnestic immune response. This is known as accelerated rejection. It is noted that these aforementioned types of rejections are very uncommon in the light of advances in antibody detection [3].

The acute rejection, also called the cellular rejection, can occur as early as 1 week after transplant and as late as months to years later. Typically, biopsy will show the presence of lymphocytic infiltrates in the interstitium and tubules (tubulointerstitial rejection). Vascular infiltration commonly occurs in the first few months after transplantation, leading to intimal thickening by inflammatory cells and transmural arterial changes. These changes can also be contributable to antibody-mediated rejection in response to donor class I or class II allo-antigens, which are particularly well expressed on the graft's endothelial cells and activation of the complement cascade [4].

Finally, the chronic allograft rejection that results from chronic graft fibrosis occurs years after transplant and can be described as long-term loss of nephron function from time-dependent immunologic and non-immunologic causes [5]. It is probably not a true rejection.

Whether an acute or a chronic rejection, there will be rising serum creatinine, progressive proteinuria, and hypertension [6] with histological findings of arteriosclerosis, glomerulosclerosis, tubular atrophy, and interstitial fibrosis [7]. Sometimes graft pain or swelling may be seen, though rare. Interestingly, a rising serum creatinine might be the only clue to graft rejection.

Due to non-specific findings of interstitial infiltration and fibrosis in both acute and chronic rejections, Banff working classification was adopted [8]. Tubulitis, arteritis, and transmural arteritis with necrosis constitute the major defining lines of Banff classification.

Immunologic Tolerance

Myeloid-derived suppressor cells (MDSCs) are a population of immune cells from the myeloid lineage with strong immunosuppressive activities by inhibiting T-cell proliferation and promoting regulatory T-cell (Treg) expansion. Treg cells (CD4⁺CD25⁺Foxp3⁺) are suppressive cells involved in tolerance. Treg cells have been shown to suppress T-cell proliferation and experimental autoimmune diseases [9].

In humans, MDSC is defined by cell surface expression of CD11 and CD33. MDSC can be further characterized into two

groups [10]: 1) granulocytic MDSC (G-MDSC) expressing CD14⁺; 2) monocytic MDSC (M-MDSC) expressing CD14⁺.

A study was conducted at Mount Sinai Medical Center to compare plasma MDSC levels in 29 renal transplant recipients with healthy volunteers (control) for 12 months. There was a significant rise in the M-MDSC levels in the renal transplant recipients. A mixed lymphocyte reaction performed on MDSC cells from transplant patients resulted in a significant inhibition in CD4 T-cell proliferation ($P = 0.003$). These findings were not seen in healthy subjects. Moreover, a positive correlation was found between the recipient MDSC and Treg *in vivo* at 12 months post-transplantation ($P = 0.03$). A limitation to the study was whether immunosuppressive agents had any influence on the levels of MDSC [11].

Chronic allograft rejection is one of the major contributors to graft loss. To overcome these events, animal research was conducted whereby graft recipients were infused with hematopoietic stem cells (HSCs) from the organ donor, showing promising results [12]. Wu et al compared plasma samples of eight kidney transplantation recipients (group 1) to four recipients with combined HSC and kidney transplantation (group 2). Though the study was limited by the sample size, none of the patients in group 2 developed immunologic rejection in the first 6 months following transplant surgery. Moreover, these patients required less immunosuppressive medications upon 29 months follow-up [13].

Biomarkers

Post-transplant immune monitoring using non-invasive biomarkers can effectively predict impending graft rejection and may spare the need for renal biopsy. Furthermore, these biomarkers can be used to monitor immunosuppressive therapy that can be regulated to avoid over- or underimmunosuppression and achieve allograft tolerance [14]. Currently, periodic monitoring of graft function by serum creatinine is used to assess graft function whereas graft biopsy is used to confirm suspected rejection.

NK cell cytotoxicity is regulated by the interaction of killer cell immunoglobulin-like receptors (KIRs) on their surface and HLA class I molecules on target cells [15].

Manna et al's study included 126 kidney transplant recipients with a 5-year follow-up, focusing on KIR genes and KIR-HLA interaction. The outcome was evaluated by serum creatinine level and glomerular filtration rate calculated using the four-variable modification of diet in renal disease (MDRD) equation. KIR2DS3, one of the 16 different KIR genes, was shown to be allograft protective ($P \leq 0.05$). Patients that expressed that specific gene had lower serum creatinine levels and a higher estimated glomerular filtration rate (eGFR) compared to KIR2DS3-negative patients. Interestingly, when KIR2DS3-positive recipients received an HLA-C1-positive kidney, the serum creatinine levels and eGFR worsened ($P = 0.0303$ and $P \leq 0.05$, respectively) after the first year post-transplant. Similar results were obtained from KIR2DL1 gene, and its association with HLA-A3 or HLA-A11 ligand or both. The presence of KIR2DL1 and HLA-A3/A11-negative kidney

reduced the risk of graft dysfunction by 33% (RR: 0.680, 95% CI: 0.465 - 0.994) [16]. This finding was in line with Kunert et al who reported that KIR2DL1-positive recipients had reduced acute rejection risk [17].

The endothelial cells are the initial site of interaction between host immune system and donor cells [18]. Immune-mediated graft vascular endothelial injury can lead to endothelial cell activation and consequently chronic rejection, organ fibrosis, and graft loss [19]. Kidney and lung endothelial cells express Endocan, a proteoglycan that has been recently studied as a potential biomarker for endothelial activity [18, 20].

A pilot study looked into plasma Endocan levels of 97 kidney transplant recipients treated for at least 3 months. Subjects with progression of chronic kidney disease (n = 40) had higher serum Endocan levels than subjects with stable graft function (n = 57) (P = 0.004). After a 3-month follow-up, subjects with an Endocan level of ≥ 643.19 pg/mL exhibited higher creatinine (P = 0.029) and lower eGFR (P = 0.006) [21]. Li et al's report described Endocan as a potential biomarker for endothelial cell injury in renal allografts that may prompt earlier changes in immunosuppressive therapies [18].

The CD30 molecule has the potential to monitor T-cell activation. It regulates immune responses between CD4 T-helper cells and CD8 cytotoxic T cells and is also responsible for the generation of memory T cells [22]. In a 6-month randomized trial of 28 pediatric renal transplant recipients, T-cell reactivity to allograft was analyzed using soluble CD30. Twenty-five percent (n = 7) of the subjects experienced biopsy-proven acute rejection (BPAR). A cutoff value of soluble CD30 concentration ≥ 40.3 U/mL on day 14 was able to predict six of seven subjects with BPAR with a sensitivity of 100% and a specificity of 76%. Fifteen subjects who were randomized to the steroid withdrawal group mounted an approximate twofold higher serum sCD30 compared to controls, suggesting the ability of steroids to limit CD30 plasma levels [23]. Thus, the measurement of soluble CD30 may be a promising early indicator for the assessment of lymphocyte activation and the risk for graft rejection [24, 25].

The kidney basement membrane is composed of collagen-IV (Col-IV) whereas the tubulointerstitial matrix is made of fibronectin (FN), laminin, and collagen type V.

Col-IV and FN are kidney-restricted self-antigens to which recipient antibodies react leading to transplant glomerulopathy (TG) [26]. Angaswamy et al retrospectively compared 26 subjects with biopsy-proven TG in kidney transplant recipients with stable control group (n = 10) that do not have any renal histopathologic abnormality. In the experimental group, post-transplantation sera of 22/26 (84%) subjects had antibodies to both Col-IV and FN (P = 0.001), and 16 out of the 26 patients (61%) developed antibodies to HLA (P = 0.026). Moreover, TG patients with antibodies to HLA were at an increased risk of developing antibodies to self-antigen. A positive association between an increase in the concentration of antibodies to self-antigen and the development of TG after kidney transplantation was found, but there were certain shortcomings in terms of the study design and its statistical power [27]. Similar results were found in the investigation of Gaston et al [28].

Matrix metalloproteinase (MMP)-2 predominantly degrades FN and laminin, whereas MMP-9 degrades collagen

types IV and V [29]. In recent years, MMPs have been studied in transplant models for acute and chronic allograft rejection [30] and their ability to activate and degrade cytokines, thus modulating immune response [31]. Natural variations in gene sequences including single nucleotide polymorphisms (SNPs) in promoters and coding regions, allow different expressions of MMPs, some of which may have an immunosuppressive nature [32].

A pilot study conducted in North India investigated the association of functional polymorphisms in MMP-2 and MMP-9. Mutant allele carriers for MMP-2 -735C>T and MMP-9 2003G>A demonstrated significantly reduced risk for allograft rejection (OR: 0.40, 95% CI: 0.18 - 0.91, P = 0.029 and OR: 0.45, 95% CI: 0.24 - 0.85, P = 0.014, respectively) [33]. A similar study involving Toll-like receptor 9 (TLR-9) investigated the relationship between TLR-9 polymorphisms and kidney allograft outcomes. Two TLR-9 gene SNPs, rs187084 and rs352140, contributed to the susceptibility for acute rejection in renal transplants (P = 0.013 and P = 0.019, respectively) [34].

Polymorphism of cytokine genes affects cytokine production and may influence the risk of acute rejection following organ transplantation [35]. Several SNPs in the genes encoding for cytokines have been described [36]. Earlier studies have shown for example that IFN- γ 874A and IL-6 G-174C variants were associated with acute rejection [37]. On the other hand, Chen et al published a study showing no statistically significant associations between SNPs in IL-2, IL-10, TGF- β 1, and IL-2RB and the occurrence of acute rejection after renal transplantation [38].

MicroRNAs are small, non-coding RNAs that can regulate the expression of a variety of genes related to B cells, IFN- γ , and TGF- β signaling, by acting on target mRNA [39, 40]. Performing microRNA profiling on peripheral blood mononuclear cells (PBMCs) of operationally tolerant renal transplant patients, miR-142-3p was the highest differentially expressed microRNA when compared with patients with stable graft function (SGF) under immunosuppression (P = 0.0098). The expression of miR-142-3p was stable over time as tested up to 13 months. Although, healthy subjects also displayed an increased expression of miR-142-3p compared with SGF patients (P = 0.0038). On further study, synthetic mimicking of Raji B-cell line leads to overexpression of miR-142-3p and up-regulation of a large number of genes [40].

Immunomodulating Drugs

Incidence of acute rejection episodes after renal transplantation is below 15% mainly due to advances in immunosuppressive therapies along with low-toxicity regimens [41].

There are two phases to immunosuppression protocol. Induction phase based on the simultaneous use of multiple drugs follows immediately after transplant and maintenance phase, characterized by a stepwise decrease in medication doses allowing partial withdrawal of dose-depending toxic immunosuppressing agents. This breakdown plan achieves immune suppression efficacy, prevents early transplant rejection and limits using these drugs to maximum toxic doses.

Induction phase agents

Lymphocytes depleting biological agents such as thymoglobulin and alemtuzumab are often used in combination in induction phase. Thymoglobulin is a polyclonal antibody preparation made in rabbits, which has antibodies against CD4, CD8, CD3 and other lymphocyte antigens. Due to the polyclonal nature of thymoglobulin, it is able to recognize a large number of immune response antigens, including cell trafficking epitopes.

Alemtuzumab is a monoclonal depleting antibody that binds to the CD52 receptor on the surface of lymphocytes. It was classically used for B-cell lymphomas; it is now used instead of thymoglobulin for induction in some transplant centers; both thymoglobulin and alemtuzumab act by complement-mediated lysis or reticuloendothelial-dependent phagocytosis of the peripheral T cells. To note, it takes several months for the peripheral T cell count to recover after the withdrawal of either of these agents [42].

One of the most commonly used monoclonal antibody basiliximab binds to the IL-2 receptor and inhibits T cells from responding to IL-2. Unlike thymoglobulin and alemtuzumab, basiliximab does not induce cytokine release nor does it deplete T cells in the peripheral circulation. Daclizumab is also an IL-2 receptor blocker on T cells. It can be used as induction immunosuppression and as an early maintenance phase immunosuppressant in patients with delayed graft function.

For sensitized recipients with preformed HLA antibodies, rituximab, a monoclonal antibody that binds the CD-20 receptor on B cells, can be initiated before transplant surgery and continued throughout the induction phase to control the antibody-mediated acute rejection.

An adjunctive therapy, eculizumab is a monoclonal antibody that binds to complement component C5 and thereby effectively inhibits the complement cascade, preventing antibody-mediated acute rejection.

Corticosteroids can be used in both the induction phase and the maintenance phase. They work mainly via two mechanisms. First, they bind to intranuclear glucocorticoid response elements (GREs), preventing cytokine expression and second they inhibit the translocation of NF- κ B, a cytokine transcription factor, to the nucleus, preventing IL-2 expression. Steroid maintenance therapy is usually limited and administered on "as needed" basis.

Maintenance phase agents

The interaction of a T-cell receptor with an APC leads to a cascade of reactions catalyzed by phosphatases. This results in the transcription of IL-2 and activation of T cells [43]. Calcineurin inhibitors (CNIs) such as cyclosporine and tacrolimus act by binding to cyclophilin and FK506 binding protein, respectively. They then prevent the phosphatase calcineurin from dephosphorylating NF-AT, preventing its translocation to the nucleus to activate IL-2 gene production.

CNIs are well known for their afferent arteriolar vasoconstricting effect, leading to reduced renal blood flow and tubular ischemia. As the side effect is dose-dependent, CNIs are usu-

ally co-administration with other maintenance drugs to limit their dosage.

Mycophenolate mofetil and myfortic (mycophenolic acid) are antimetabolites that have a cytostatic effect on lymphocytes. They act at the S phase of activated T- and B-cell cycle and interfere with *de novo* synthesis of purines, resulting in intracellular depletion of guanosine nucleotide [44]. Other common antimetabolites used include azathioprine, which is an imidazole derivative of 6-MP that prevents both *de novo* and salvage purine nucleotide synthesis.

Belatacept is an immunoglobulin that can link to the extracellular domain of CTLA-4 and blocks T-cell activation [45]. CTLA-4 is a protein receptor found on the surface of T cells. It plays a vital role in regulating T-cell stimulation. Adding belatacept to the maintenance therapy allows dose reduction of CNIs and maintenance of graft function [46].

Mammalian target of rapamycin (mTOR) is an enzyme complex that plays a critical role in regulation of T-cell cycle progression from late G1 into S phase. Sirolimus is a pro-drug that binds to mTOR inhibiting it and preventing the downstream translocation of transcription factor NF- κ B to the nucleus, thereby arresting T-cell cycle progression to S phase and IL-2 synthesis [47, 48].

Conclusion

Organ transplantation has always been considered to be the optimal therapeutic intervention in patients with end-stage organ failure. Survival rates among transplant recipients have greatly improved due to better understanding of transplant biology and more effective immunosuppressive agents. After transplant, the extents of the immune response are influenced by the amount of IL-2 being produced by the T-helper cells.

Post-transplant immune monitoring using non-invasive biomarkers can effectively predict impending graft rejection and may spare the need for renal biopsy. Several potentially useful biomarkers have been identified over the last decade, but despite the important advances achieved so far, these biomarkers lack validity and further exploring of new strategies is required.

Conflict of Interest

The authors of this manuscript have no conflicts of interest to disclose.

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