Glomerular diseases and transplantation: similarities in pathogenetic mechanisms and treatment options

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Abstract

Glomerular diseases and renal transplantation have always been considered as independent fields of nephrology, due to the supposed prevalent role of antibody production and immune complex formation in glomerulonephritis versus a direct reaction of immune cell towards the grafted kidney. However, both conditions share common pathogenetical pathways, and possible new therapeutic approaches are being envisaged. Innate immunity, particularly Toll-like receptors, dendritic cells and complement pathways, B cells and antibody networks are involved in the development of glomerular damage as well as graft injury. Consequently, new treatments targeting previously not considered immune pathways, like nuclear factor-κB or the proteasome and B-cell activation with antibody production, are being tested in glomerular diseases and in transplanted kidneys.

Keywords: B cells; glomerulonephritis; innate immunity; renal transplantation; treatment

Introduction

Glomerular diseases and renal transplantation have always been considered as independent fields of nephrology. This concept has been supported by the prevalent role of antibody production and immune complex formation in glomerulonephritis (GN) versus being due to the direct reaction of immune cells towards the grafted kidney. However, recent investigations have shown that the pathogenetic mechanisms operating in both conditions share common pathways offering some new therapeutic approaches iden-
tical in both kinds of conditions. This fascinating topic was the subject of the first meeting of the recently formed ERA-EDTA Immunonephrology Working Group, which was created among experts from all over Europe to focus interest and develop investigations in the area of immune-mediated renal damage including both GN and renal transplant immune injury. Three main topics were discussed—invasive immunity, B-cell antibody networks, and new treatments—always comparing aspects in glomerular diseases and immune damage in the transplanted kidney.

The innate immunity system

The innate immunity system provides the first line of defence against invading microorganisms by means of cellular and humoral mechanisms. Recent investigations have detected a previously unsuspected role of the major actors of this ancestral system—i.e. Toll-like receptors (TLRs), dendritic cells (DCs) and complement system—in both glomerular and transplant immune damage.

Innate immunity is quick but specific; it acts through the recognition of pathogen-associated molecular patterns (PAMPs) by macrophages, DCs, leucocytes and other cells, and favours opsonization and phagocytosis [1]. Moreover, acting on antigen processing, innate immunity links to adaptive immunity, leading to maturation of DCs, favouring antigen presentation, T-cell response and finally specific adaptive immune response [2,3]. PAMPs may be recognized by serum or cell surface scavenger receptors triggering alternative complement pathway activation. Bacterial lipopolysaccharides (LPS), peptidoglycan, glycolipids, double- or single-strand RNA (virus), CpG DNA (bacteria) and also endogenous ligands produced during stress and cell damage are recognized by TLRs, whose activation, by binding to their ligands, triggers intracellular adapters—mostly myeloid differentiation factor 88 (MyD88) molecules—leading to activation of two relevant transcription factors, interferon transcription factor (IRF3) and nuclear factor-κB (NF-κB) [4]. IRF3 triggers mRNA transcription for interferon-α and interferon-γ [5]. Regulatory signals transmitted by innate immunity can be finely tuned in different conditions, resulting in opposite effects, as with DCs, favouring tolerance when in quiescent condition, versus inflammation and immune response when previously activated [6].

The clinical relationship between infection and human glomerular diseases is well known, from the recognition of the role of streptococcal infection in acute GN and the association between mucosal infection and bouts of gross haematuria in IgA nephropathy. TLR ligation by bacterial products, viruses and other ligands, induces DC maturation at the site of infection and their migration to lymph nodes, where they interact with lymphocytes and produce activation of T cells. By this mechanism, infection may exacerbate various types of GN. Alternatively, inflammation products can activate TLRs present on intrinsic renal cells, further triggering inflammation and tissue damage [7,8].

TLR7 and TLR9 are critically involved in the activation of DCs and autoreactive B cells through the recognition of endogenous DNA- or RNA-containing antigens and subsequent development of autoimmune responses against nuclear autoantigens [9]. MRL 1pr/1pr SLE-prone mice, after i.v. injection of a ligand specific for TLR3, show TLR3 staining in mesangial cells and infiltrating macrophages [10]. TLR2 agonists exacerbate accelerated nephrotic nephritis: a murine model of crescentic glomerulonephritis, induced by sheep anti-mouse antibodies, is significantly worsened by injection of a synthetic TLR2 ligand (Pam3Cys) that mimics bacterial LPS [11].

An activation of innate immunity has also been identified in IgAGN. In this disease, the mucosal IgA responses to antigens are reported to be altered resulting in failure of mucosal antigen elimination and the increase in memory cells in the bone marrow [12]. TLR ligation modulates the severity of IgAGN favouring a Th1 hyperactivation. In mice prone to IgAGN, nasal challenge with ligands for TLR9 aggravates renal injury, with increase in serum and mesangial IgA [13]. In circulating mononuclear cells of patients with IgAGN, TLR4 is upregulated particularly in phases of clinical activity with proteinuria and heavy microscopic haematuria [14]. Innate immunity is also likely to be involved in proteinuric conditions. Several TLRs are expressed on the surface of podocytes; ligation of TLR4 by its ligand LPS can induce nephrotic syndrome in animals [15], and TLR2 ligation can favour development of proteinuria in murine models of lupus nephritis [16].

Apart from a direct role of pathogens, adaptive immunity can be triggered by macromolecules released by dying cells, which can be presented to TLRs and activate DCs [17]. Kidney-resident DCs can modulate T-cell activation and proliferation by inhibiting T regulatory cells and increasing CD4+ T cells [18]. In models of crescentic GN, activation of DCs exacerbates the glomerular damage [19] which is prevented by DC inhibition [20]. DCs are detectable also in interstitial infiltrates in patients with lupus nephritis [21], interplaying with chemokines [22].

The role in renal diseases of complement enzymatic cascade, the most well-known member of the innate immune system, was identified decades ago. Complement can be activated via the classical pathway in some immune complex-mediated renal diseases, such as cryoglobulinemic nephritis and lupus nephritis. Dense deposit disease and recurrent atypical haemolytic uraemic syndrome are triggered by uncontrolled activation of the alternative pathway due to genetic downregulation of the inhibiting mechanisms, most frequently factor H. The alternative pathway may be involved in many autoimmune renal diseases and in ANCA-positive vasculitis [23,24]. In patients with IgAGN, complement activation via the lectin pathway can contribute to renal damage progression [25]. On the other hand, complement can play a role not only when activated but also when deficient. C1q deficiency can trigger the development of lupus nephritis due to the lack of some relevant function, including promotion of uptake of apoptotic cells by immature DCs to clear cellular debris [26] and inhibition of interferon-α production by DCs elicited by immune complexes or CpG DNA [27].

In summary, the current view is that innate immunity can initiate and sustain the development of some immune-mediated glomerular diseases.
In renal transplantation, attention has been mainly focused on adaptive immunity, since T cells alone are sufficient to trigger and sustain rejection. However, recent investigations have revealed a role for innate immunity as a pivotal trigger of adaptive immune response [28]. In ischaemia–reperfusion injury, damage-associated molecules, such as heat shock proteins, are generated, and they act as endogenous ligands for donor- and recipient-derived TLR4- and TLR2-bearing DCs. After this recognition, DCs mature and initiate cytokine-driven recipient adaptive alloimmune response leading to fully expressed ischaemia–reperfusion injury [29]. Complement also plays an important role in this condition. Even a short ischaemia time of 30 min can activate the classical and the lectin pathways of complement as shown by peritubular capillary and glomerular C4d and C5b-9 deposition [30].

Innate immunity significantly influences both graft rejection and graft acceptance, as demonstrated by the modulation of the suppressive activity of regulatory T cells by TLR ligation [31], which can contribute to blunting T regulator-mediated tolerance [32]. Other important mediators of innate immunity, natural killer cells, can produce graft injury acting as potent inflammatory cells, but they also regulate effector programmes of alloreactive T cells and ultimately determine whether the graft is rejected or accepted [33].

In summary, the innate immunity should be considered today as an important player in the complex machinery of alloimmune response. It can promote or inhibit adaptive immune responses, functioning as a bridge to tolerance or rejection in renal transplantation. A better knowledge of the mechanisms regulating these contrasting effects may contribute to the development of effective tolerance protocols in the next future.

**B cells and antibody network**

Circulating antibodies are strongly involved in the development of GN and renal transplantation damage. A typical model of GN caused by circulating antibodies is membranous nephropathy (MN). The disease may be secondary to infections, tumours, autoimmune diseases or exposure to drugs or toxic agents. However, the primary form is the most common. Recent studies have identified a few autoantigens in idiopathic MN. In newborns from mothers deficient in an enzyme expressed also in podocytes, neutral endopeptidase (NEP), anti-NEP circulating antibodies are associated with subepithelial immune deposits [34]. Autoantibodies directed against other podocyte enzymes, M-type phospholipase-2 receptors [35], aldose reductase and manganese superoxide dismutase [36], have been detected in adults with idiopathic MN. However, it is likely that the list of podocyte auto-antigens will be implemented in the near future. It is also possible that some exogenous antigens may participate in the development of `idiopathic’ MN. The group of Ronco hypothesized that even exogenous cationic proteins, such as bovine serum albumin, may be absorbed by the gastrointestinal mucosa, enter the circulation and be attracted by the anionic charge on the epithelial side of the glomerular basement membrane where they may be reached by antibodies (oral communication during the Immunonephrology Working Group Course 2010).

In the field of kidney transplantation, a substantial proportion of rejection episodes are mediated by circulating antibodies, which bind to renal donor antigens and trigger complement fixation and local coagulation activation leading to acute vascular rejection. In addition, complement activation recruits macrophages and neutrophils, causing additional endothelial injury and gene expression favouring vascular remodelling and leading to irreversible anatomical lesions that permanently compromise graft function [37]. The pivotal role of complement in antibody-mediated rejection is confirmed by the abundant and not evanescent deposits of C4d, a component of the classical pathway of complement, in peritubular capillaries [38,39]. Moreover, in pre-sensitized baboons, acute antibody-mediated rejection can be prevented by recombinant human C1 inhibitor [40].

There is also a growing body of evidence that B-cell production of alloantibody is an important element in the genesis of chronic rejection. Patients with preformed or de novo circulating HLA donor-specific antibodies [41] present with an increased risk of graft failure, particularly when also C4d deposits are detectable at renal graft biopsy [42]. Also, HLA class I-related chain A (MICA) can elicit antibody production, and pre-sensitization of kidney transplant recipients against MICA antigens is associated with an increased frequency of graft loss [43]. These antibodies are directed against HLA expressed on the graft endothelium and can alter/damage the cells through several mechanisms. They may elicit a complement-dependent endothelial cell injury but can also trigger transplant rejection by stimulating inflammatory and proliferative signals. There are data suggesting that the binding of antibodies to class I molecules on the surface of endothelial cells transduces signals resulting in functional changes that can favour both immunological accommodation and endothelial cell survival or rejection and endothelial cell damage, according to the concentration of the antibody and the expression of the HLA antigen [44]. On the other hand, tissue injury and remodelling caused by antibodies to donor HLA antigens can result in exposure of self-antigens that lead to post-transplant autoimmunity [45,46], and autoimmune processes may facilitate the alloimmune response to histocompatibility antigens [47]. Thus, the presence of preformed or de novo antibodies may indicate ongoing anti-graft immune aggression. However, as pointed out by Chang and Platt [48], it is also possible that antibodies favouring the resistance of the graft to immune injury (accommodating antibodies) cannot be absorbed by an allo-graft damaged by a previous immunological attack. Prospective trials evaluating the possible benefit of removing circulating antibodies will clarify whether the anti-donor antibodies we detect in the circulation are the same as those causing graft injury.

**New target for treatments**

Most of the available immunosuppressive agents used for treating primary or secondary GN have also been used in
renal transplantation and vice versa. The agents most frequently used included glucocorticoids, alkylating drugs, purine synthesis inhibitors, and calcineurin inhibitors. Of interest, a promising new trend in treating GN is to use multiple combinations of low doses of these drugs, similar to polytherapies used after transplantation. Good results have been reported, but the therapeutic index of the available treatments is still far from being satisfying. However, in both areas, several newer drugs and biological reagents are now under investigation almost at the same time.

An interesting new target for blunting pathogenetical mechanisms operating in some glomerular diseases as well as in particular settings of grafted kidney is NF-κB. It does not sound surprising, when considering that this ubiquitous transcription factor governs the expression of genes encoding for cytokines, chemokines, growth factors, adhesion molecules, and other factors involved in the immune and inflammatory response. An increased nuclear translocation of active NF-κB subunits has been detected in a number of renal diseases. In patients with IgAGN, NF-κB is activated in renal tissue and in peripheral blood mononuclear cells [49]. Also, in severe nephrotic syndromes, particularly in focal segmental glomerulosclerosis (FSGS), NF-κB is activated in peripheral lymphomononuclear cells [50], and an upregulation of NF-κB has been detected also in cultured podocytes in correlation with nephrin reduction [51]. Since NF-κB nuclear translocation is regulated by the ubiquitin–proteasome pathway, new suggestions on the possible effect of proteasome inhibitors in severe nephrotic syndrome have been recently presented (R. Coppo oral communication during the Immunonephrology Working Group Course 2010). Among proteasome inhibitors, bortezomib is the most known. It is a tripeptide which binds the catalytic site of the 26S proteasome with high affinity and specificity. The drug is approved for treating relapsing myeloma and mantle lymphoma. However, its administration may be complicated by painful peripheral neuropathy in ~30% of cases and by dose-dependent myelosuppression. Since bortezomib can deplete plasma cells [52], it may also be used in refractory lupus nephritis [53] and in antibody-mediated rejection in renal transplant recipients, where some preliminary results are highly encouraging [54].

Another interesting therapeutic target for immunemediated renal diseases is represented by suppressors of the cytokine signalling (SOCS), intracellular proteins acting as negative regulators for Janus kinase/signal transducer and activator of transcription (JAK/STAT) signalling pathways. SOCS are induced by cytokines and by TLR ligation, and regulate T-cell maturation, differentiation and function, controlling polarization of CD4+ T cells into Th1, Th2, Th17, and T regulatory cell lineages. Moreover, they promote the maturation of CD8+ T cells from naïve to ‘stem-cell memory’, central memory, effector memory states, and the activation of these lymphocytes subsets [55]. SOCS are important modulators of cell activation during renal inflammation since they may play a regulatory role in Fc receptor gamma signalling [56]. Moreover, SOCS proteins may act as negative regulators of angiotensin II signalling in renal cells [57]. In an experimental model of diabetes, the intrarenal delivery of SOCS1 and SOCS3 expressing adenovirus significantly decreased the activation of STAT1 and STAT3 and the expression of proinflammatory and profibrotic proteins, improving renal function and diabetic nephropathy kidney damage [58]. In experimental kidney transplantation, an upregulation of several protective genes including the SOCS family is detectable contemporarily with the expected activation of immune response [59]. In conclusion, suppression of the JAK/STAT pathway by increasing intracellular SOCS proteins might have therapeutic potential not only in diabetic nephropathy and other progressive renal diseases but also in renal transplantation.

Reviewing the recent contributions on new targets for therapy in both glomerular diseases and kidney transplantation injury, a relevant means is represented by the inhibitors of the mammalian target of rapamycin (mTOR). The mTOR is the downstream effector of a family of kinases, regulated by phosphatidylinositol-3 kinase (PI-3k), which in response to different stimuli, including IL-2, provides the signal for cell proliferation. Two inhibitors of mTOR, sirolimus and everolimus, are available and have been successfully used in renal transplantation. These drugs allow a reduction in calcineurin inhibitors (CNI), preventing long-term irreversible renal damage [60]. Three main schedules may be used: early elimination of CNI [61], minimization of CNI [62] and late replacement of CNI [63]. Very good graft survival, low rate of rejection and reduced renal function loss have been reported with the first two approaches. However, late replacement of CNI can only partially prevent loss of renal function, while patients with already impaired glomerular filtration rate (~40 mL/min) or proteinuria can even develop a more rapid deterioration of renal allograft function and proteinuria. The interest in mTOR inhibitors is also based on their pleiotropic effects, including limitation of viral replication, inhibition of intimal proliferation, and reduction in the incidence of neoplasia in renal transplant recipients. As far as the glomerular immunological damage is concerned, these agents have been tested in some models of GN with different effects. mTOR inhibition attenuated kidney damage progression in the anti-Thy-1.1 experimental proliferative and sclerotic GN [64] while inducing proteinuria and renal deterioration in the remnant kidney model in the rat [65]. In human GN, the efficacy of these agents remains unclear, as it has been reported that they may ameliorate [66] or induce [67] FSGS. These conflicting results may depend in part in the doses used and even more by the severity of the underlying renal lesions. Actually, mTOR inhibitor may blunt glomerular hyperfiltration and endothelial cell proliferation. These effects may be useful in an initially damaged kidney but can suppress the repair mechanisms and worsen the progression of renal disease in the presence of advanced kidney lesions.

As reviewed above, B cells contribute to the pathogenesis of idiopathic or secondary GN, and are deeply involved in antibody-mediated rejection. Therefore, there is a rationale for treatment aimed at depleting B cells in these diseases. An array of monoclonal antibodies directed against membrane proteins expressed on B cells, against B-cell-stimulating cytokines or against the late components of complement cascade is under investigation. Rituximab is a chimeric monoclonal antibody that selectively
targets CD20-positive B cells while sparing plasma cells. In patients with SLE, a number of non-controlled studies reported beneficial effects of rituximab, but a randomized controlled trial comparing the efficacy and safety of rituximab versus placebo in patients with moderately to severely active extrarenal SLE could not find any difference between the two regimens [68]. In another trial, rituximab or placebo was added to a therapy based on mycophenolate and high-dose corticosteroids in patients with proliferative lupus nephritis, but no difference was found in either efficacy or safety [69]. It is unclear whether these negative results depended on the avoidance of cyclophosphamide in the experimental arm, a too short follow-up masking any long-term benefit of rituximab, a poor depletion of B cells in tissue and/or elimination of regulatory B cells. Rituximab is presently considered as a therapeutic option in SLE; however, its effectiveness in SLE when administered without cyclophosphamide seems limited, and still unexplored risks of long-term adverse events should be considered. In refractory ANCA-associated vasculitis, rituximab was able to induce remission in a large series, and when relapses occurred, re-treatment was effective and safe, without severe adverse events. ANCA and B-cell levels did not provide guidance for re-treatment [70]. In primary GN, rituximab has been used in membranous GN with 15–20% rate of complete remission and 35–40% rate of partial remission [71]. Rituximab can induce independence from calcineurin inhibitors in patients with MN or idiopathic nephrotic syndrome needing continuous administration of these drugs [72,73]. In the published reports, rituximab was well tolerated. However, its use may be complicated by antigenicity, infusion reactions and hypogammaglobulinaemia. Rare cases of posterior leuocencephalopathy, probably caused by reactivation of J virus, have been reported.

B-cell activation is not only a new target for GN but also for graft dysfunction. There is growing evidence that chronic rejection is mediated by circulating antibodies. Cai and Terasaki [74] recommend universal testing of allograft recipients for antibodies in order to identify this risk factor and take appropriate action to minimize deterioration of transplant function. Theoretically, there are three main ways to prevent or treat the consequences of circulating antibodies: (i) removal of antibodies with plasmapheresis, immunoadsorption, and/or splenectomy; (ii) interference with mechanisms of injury by administering high-dose intravenous immunoglobulins (IVIG) or eculizumab, a monoclonal antibody directed against the C5 component of complement; and (iii) depletion of B cells and antibody-producing cells with rituximab, alemtuzumab, bortezomib or monoclonal antibodies directed against B-cell-stimulating cytokines. A reduction of preformed antibody against HLA antibodies has been reported with IVIG alone or combined with rituximab [75]. These strategies may allow a kidney transplantation to be performed in patients with a high level of anti-HLA antibodies [76,77]. Up to now, however, there is no evidence that a reduction of circulating antibodies can reduce the risk of chronic rejection. To discover how to prevent chronic rejection and improve long-term graft survival will require well-designed, long-term RCTs. Little improvement has been made in treating the cases of acute humoral rejection even though some benefits have been reported with immunoadsorption with protein A [78], plasmapheresis associated with rituximab [79] or with eculizumab [80]. Most investigators faced failure in trying to control antibody-mediated rejection; however, new hopes have been raised by the beneficial effects of proteasome inhibitors, inhibiting immunoglobulin production by plasma cells [53].

In summary, due to the complex network of immune system involvement in GN and in renal graft rejection, it is not surprising that a number of immunomodulating drugs may find a role in both conditions. However, it should be kept in mind that important differences remain between GN and renal transplant, in spite of many similarities. This may explain why the drugs that can prevent rejection, hence mostly targeting Th1 subset activation and cell–cell interaction, cannot always prevent the apparent ‘paradox’ of recurrence of GN in the same transplanted kidney. Most types of GN that recur after transplantation are likely to be related to dysregulation of synthesis of soluble factors (such as aberrant glycosylation of IgA1 in IgA nephropathy, permeability factors in FSGS, complement activating condition in haemolytic uraemic syndrome or type 2 mesangiocapillary GN, and antibody against podocyte antigens in membranous nephropathy) which may be refractory to the treatment usually adopted to avoid rejection of grafted kidneys. On the other hand, although recurrent GN is relatively frequent and may worsen the outcome of renal allograft in some patients, its impact on the fate of a renal allograft is diluted by a number of other risk factors that may have a greater impact than recurrent GN on the long-term graft survival.

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