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PERSPECTIVES IN RENAL MEDICINE

Progression of renal damage in human glomerulonephritides: Is there sleight of hand in winning the game?

FRANCESCO PAOLO SCHENA, LORETO GESUALDO, GIUSEPPE GRANDALIANO, and VINCENZO MONTINARO

Institute of Nephrology, University of Bari, Bari, Italy

Human glomerulonephritides (GN) remain one of the most important causes of end-stage kidney disease worldwide [1–6]. Much effort has been expended over the last twenty years trying to gain a better understanding of the mechanisms involved in the pathogenesis of this heterogeneous group of renal diseases [7].

The initial events triggering the development of the various forms of primary GN vary somewhat, as do their histological features and natural history. However, in the early phases, all GN are characterized by an inflammatory process mediated by a number of soluble factors released by activated resident cells and/or by infiltrating immune cells [7]. For this reason, hereafter we liken glomerular diseases to different card games played by the same players (resident and infiltrating cells) using the same pack of cards (the soluble mediators). If the inflammatory process persists, the renal damage will progress toward renal fibrosis at a constant rate, independently of the original events; otherwise a more or less complete recovery may be observed [8]. In short, some of the games will end at this point while others will enter a common phase and progress to renal fibrosis.

During the last decade, the explosive development of molecular biology techniques applied to routine renal biopsy has enabled identification of most of the cards and their role in the games, and an understanding of how and when they are used by the different players [9]. The aim of the present work is to describe what it is known about the players, the cards they use and their behaviors in the different games, and to give a perspective on how nephrologists can influence their game strategy.

THE PLAYERS

In all primary GN we have to consider at least three groups of players: T cells, monocytes/macrophages and resident glomerular cells (mesangial, endothelial and epithelial) [10–12]. Although all of these players are invariably involved in the various games, they may assume a different role and relevance in each. Indeed, it is the behavior of the players that makes each game different. Therefore, to understand the games we first must recognize the main features of the players and how they respond to the different cards.

T cells

T cells act as trumps in the immunological processes involved in any GN [10]. They direct the intervention of the immune system and even the activation of the resident cells, through the production and release of an array of cytokines [13]. However, different subsets of T lymphocytes may play different pathogenetic roles in glomerular diseases [14].

CD4 and CD8 are the main markers of the two major subclasses of mature T cells, helpers and suppressors, which respond differently to antigen [13]. Over the last few years it has been demonstrated that the cellular and humoral areas of the specific immune response are regulated by distinct subsets of T helper cells, termed type 1 (Th1) and type 2 (Th2) cells [14]. They both respond to antigenic stimulation with a transient burst of cytokines, that differ in the two subsets [15]. Th1 cells primarily secrete interleukin (IL)-2 and interferon (IFN)-γ, whereas Th2 cells produce IL-4, IL-5, IL-10 and IL-13 (Fig. 1) [14, 15]. Numerous factors appear to play a role in the polarization of the Th cell response toward the Th1 or Th2 phenotype, although cytokines mainly determine this differentiation [16]. Among the cytokines, IL-12 and IL-4 are the main messages directing the development of T helper cells toward the Th1 and Th2 phenotype, respectively (Fig. 1) [16]. Th1 cells are responsible for cell-mediated inflammatory reactions characterized by a marked potentiation of phagocytic cell functions, and also provide help to some B cells (Fig. 1). Th2 cells, on the other hand, promote the synthesis of antibodies and inhibit several macrophage functions (Fig. 1). Thus, Th2 cells seem, to a certain extent, to play a protective role against the damaging effects of Th1-mediated immune reactions [14].

In proliferative and progressive GN, in which a delayed hypersensitivity reaction is taking place in glomeruli, the T cells involved in the immune process are mainly of the Th1 type [17, 18]. The Th2 cell response, on the other hand, appears to play a pathogenetic role in animal models of GN associated with systemic autoimmunity (host-vs.-graft disease in mice and mercury-induced nephritis in rats) [19].

Monocytes/macrophages

Monocytes, among the cells of the immune system, are surely those whose presence has most often been described inside the glomerular tuft and the interstitial space in human GN [10, 20].

Key words: progression of renal disease, glomerulonephrides, T cells, monocytes/macrophages, glomerular cells, cytokines, growth factors.

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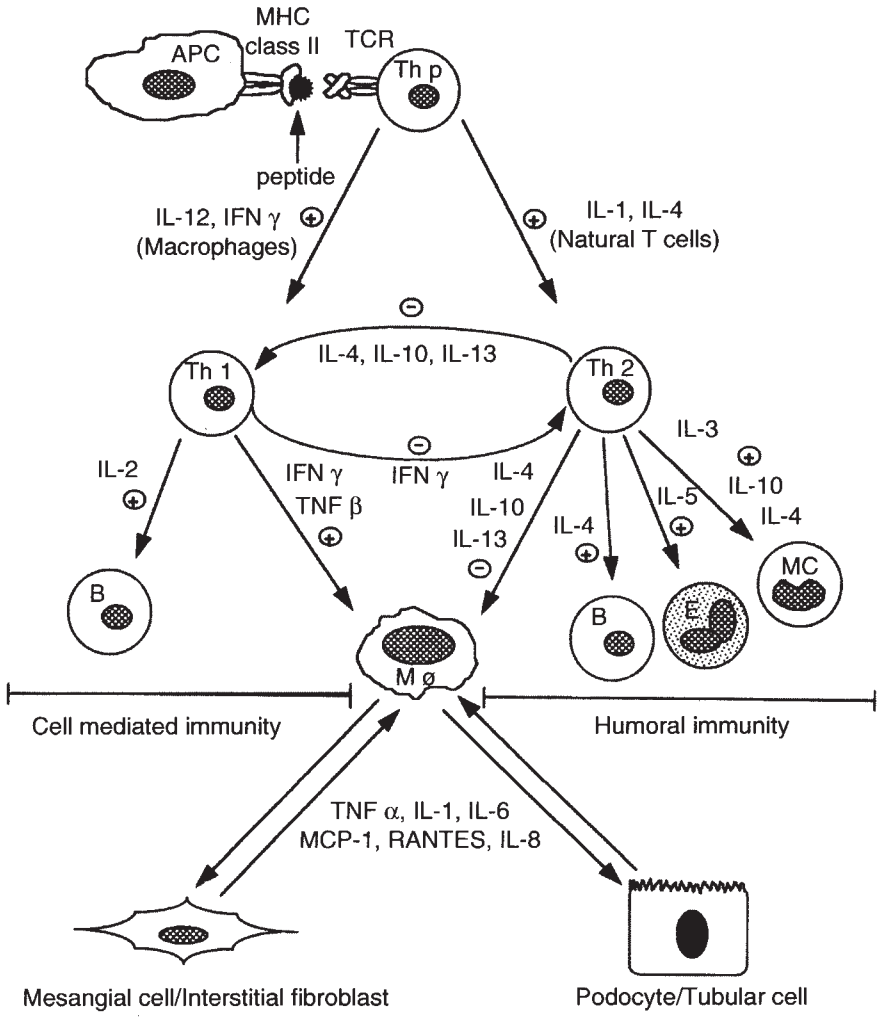

Fig. 1. Schematic representation of the regulatory interactions among T helper cell subsets (Thp, Th1, Th2), B cells (B), eosinophils (E), mast cells (MC), macrophages (Mø) and resident renal cells. Signs are: (−) suppression; (+) induction. The Th1 system modulates cell-mediated immunity and the Th2 system regulates humoral immunity. Explanation of the other observations are in the text.

They can also be considered as the real effectors of the immune system in the pathogenesis of glomerular diseases [11]. Once in the glomerulus or within the interstitium, monocytes can mediate the injury through different pathways. They can synthesize and release a variety of cytokines and growth factors that can, in turn, attract more infiltrating immune cells and activate resident cells (Fig. 1) [21]. Thus, monocytes can be described as the players with the greatest number of cards to be used. Besides the above-mentioned soluble factors, the activated monocytes/macrophages have a considerable number of weapons for directly damaging the glomerular and the interstitial structures [21]. In response to specific activating stimuli, these infiltrating cells can release free oxygen radicals and lysosomal enzymes that may endanger the other players. Although it is now clear that monocytes have an important part in the pathogenesis of GN, the mechanisms underlying their influx into the glomeruli and/or the renal interstitium are still largely unknown. However, it can be hypothesized that they move through the endothelial barrier attracted by the presence of specific chemotactic factors produced and released by resident cells or by infiltrating T cells [11].

Resident cells

In the past, resident cells were considered only as a static framework or a passive target for the action of the infiltrating cells. On the contrary, in the last decade it has been realized that mesangial, epithelial and endothelial cells are active players in GN games, participating in different ways in the pathogenesis of any glomerular disease [12]. They can proliferate in response to a variety stimuli. This is a particularly relevant trait for mesangial cells, since there is a group of GN characterized by an increased proliferation of these resident cells [22]. All may play key roles in the accumulation of extracellular matrix (ECM), since they can synthesize both the ECM components and the enzymes that catalyze ECM degradation. Moreover, they can amplify the immune-mediated inflammatory process through the synthesis and release of a variety of chemotactic factors for monocytes and T cells [23]. Finally, they can further facilitate the migration of the immune cells into the glomerular tuft, expressing a variety of adhesion molecules on their surface that favor their interaction with the infiltrating cells [12]. It is noteworthy that all these functions are strictly regulated by cytokines and growth factors

locally released by monocytes and T cells or even by the resident cells themselves. In fact, the latter can produce a relevant number of growth factors and chemokines including some of the monocyte-specific cytokines [24].

THE CARDS

All the players interact during the games using a common deck of cards that include a variety of soluble factors. These soluble mediators, which act as intercellular messengers and carry regulatory signals from cell to cell, are mainly cytokines and growth factors [25]. They can be synthesized and released to influence the functions of the cell that produced them (autocrine action) [26] or the functions of the cells in the immediate vicinity (paracrine action) [27]. Both cytokines and growth factors, by binding specific cell-surface receptors on the target cell, trigger a cascade of events that influence cellular functions [25]. Interestingly, all of these mediators can modulate their own synthesis and secretion as well as the synthesis and secretion of other cytokines and growth factors.

In our card game simile it should be assumed that: (*1*) each player can use only an assigned number of cards and present the receptor to bind a limited number of them; (*2*) each player must always react to a particular card in the same way, although different players can respond to the same card in completely different ways.

Based on their functions and origin, we can divide the cards into five groups: Th1 cytokines, Th2 cytokines, monocyte-derived cytokines, chemokines and growth factors (Table 1).

Table 1. The cards

Cytokine	Molecular wt (kD)	Sources (cells)	Targets (cells)	Effects
IL-2	15	Th1, TCD8+	T cell	-Clonal expansion
IFN-γ	20–25	Th1	Monocyte Epithelial Endothelial Mesangial	-MHC, chemokine and growth factor induction -Inhibition of Th2
IL-4	18–20	Th2	T cell, B cell Monocyte Mesenchymal Endothelial	-MHC II and antibodies induction -Proliferation -Inhibition of Th2 and monocyte
IL-10	35–40	Th2 Monocyte B cell	Th1 Monocyte B cell	-Inhibition of Th1 and monocyte -B cell activation
IL-13	10	Th2	Th1 Monocyte B cell	-Inhibition of Th1, B cell and monocyte
TNF-α	17	Monocyte	Monocyte Mesangial Endothelial	-Activation -Cytokine induction -Apoptosis
IL-1	18	Monocyte Mesangial	Monocyte Mesangial Endothelial Epithelial	-Chemotaxis -Proliferation -Chemokine and growth factor induction
IL-6	21–28	Monocyte T cell Mesangial Epithelial Endothelial	Mesangial Epithelial T cell	-Proliferation -Cytokine induction (IL-2)
MCP-1	14–21	Mesangial Epithelial Endothelial Monocyte	Monocyte	-Chemotaxis and activation
RANTES	6–8	T cell Mesangial Epithelial	T cell	-Chemotaxis and activation
IL-8	8	Mesangial Monocyte	Neutrophil	-Chemotaxis and activation
PDGF	30–34	Mesangial Endothelial Epithelial Monocyte	Mesenchymal	-Proliferation -Chemotaxis -Growth factor induction
TGF-β	25	Endothelial Mesangial Epithelial Monocyte	Endothelial Epithelial Mesangial Monocyte	-Extracellular matrix synthesis -Growth inhibition
bFGF	16–24	Endothelial Mesangial	Mesangial Endothelial	-Proliferation -Growth factor induction
EGF	6	Epithelial	Epithelial Endothelial Mesangial	-Proliferation -Growth factor induction

Th1 cytokines

Th1 cytokines are produced mainly but not exclusively by Th1 cells and present powerful pro-inflammatory effects both in the systemic circulation and within the kidney. Indeed, they activate monocyte/macrophages, T cells themselves as well as resident renal cells [15, 28]. The two most important cytokines belonging to this group are IL-2 and IFN-γ (Fig. 1).

Interleukin-2. Interleukin-2 (IL-2) is produced by Th1 and CD8+ lymphocytes, and functions as an autocrine T cell growth factor [26]. It activates T cells and stimulates their growth, giving rise to their clonal expansion [28]. Thus, IL-2 plays a key role in the delayed-type hypersensitivity response [15].

Interferon-γ. On the other hand, INF-γ primarily exerts an activating effect on monocytes and resident cells [29]. It induces MHC class I and II antigen expression, modulates tumor necrosis factor (TNF) receptors on monocytes and stimulates the synthesis and release of chemokines and growth factors by endothelial, mesangial and epithelial cells [30–32]. Moreover, IFN-γ inhibits the growth and activation of Th2 cells (Fig. 1) [28].

Th2 cytokines

Th2 cytokines have mostly inhibitory effects on monocytes/macrophages and on Th1 cells [16]. However, they can switch on humoral immunity through direct activation of B lymphocytes (Fig. 1). Moreover, they can modulate some functions of resident renal cells such as adhesion molecule expression [33].

Interleukin-4. Interleukin-4 is a glycoprotein produced by activated Th2 lymphocytes; it exerts its biological activity on resting T cells, B cells, macrophages, endothelial and mesenchymal cells [34–37]. This cytokine has been shown to increase MHC class II antigen expression and antibody production in resting B cells [38] and the proliferation of T cells directly and by increasing the production of IL-2 or the expression of IL-2 receptor [34, 39]. IL-4 increases endothelial cell adhesiveness to T cells by inducing adhesion molecules [33], while it has an anti-inflammatory effect on monocytes, inhibiting the production of IL-1β, TNFα, IL-6 and

IL-8 and reducing the expression of Fcγ receptor (Fig. 1) [37, 40, 41]. Moreover, this cytokine can stimulate the proliferation of cells of mesenchymal origin. Finally, IL-4, together with IL-10 and IL-13, inhibits the expansion of Th1 cells [15].

Interleukin-10. Interleukin-10 is produced upon immune activation by Th2 cells, monocytes/macrophages and B cells [42]. It suppresses both cytokine production and antigen specific proliferation of cultured Th1 clones. Thus, IL-10 reduces delayed-type hypersensitivity reactions and other Th1 cell-mediated responses [42]. It also inhibits the production by monocytes/macrophages of important inflammatory mediators like IL-1α, IL-6, IL-8, TNF-α. On the other hand, it exerts a stimulatory effect on B cells and mast cells [42]. Recently Del Prete et al have shown that IL-10 is produced by both Th1 and Th2 cells in the human system and that it down-regulates IFN-γ production by Th1 cells and IL-4 and IL-5 production by Th2 cells [43].

Interleukin-13. Interleukin-13 is a potent modulator of human monocyte and B cell functions. It is produced early and over prolonged periods by human Th2 cells following activation, and acts like IL-4 in suppressing the development of Th1 cells, thus favoring the generation of Th2 developmental pathways [reviewed in 44]. IL-13 inhibits the production of chemokines by human monocytes stimulated with LPS and enhances the monocyte expression of several members of the integrin superfamily and of MHC class II antigens [44].

Monocyte-derived cytokines

Activated monocytes can synthesize a variety of soluble mediators. Among them the most specific for the monocytic cell line are IL-1, IL-6 and TNF-α (Fig. 1) [11].

Tumor necrosis factor-α. This is produced mainly by monocytes/macrophages, but it can also be synthesized by lymphocytes [45]. Various different biological activities of TNF-α could be relevant in the pathogenesis of GN, including: (*1*) induction of mesangial cell contraction and proliferation; (*2*) monocyte and lymphocyte recruitment and activation directly and through the increased synthesis and release of chemokines and lipid mediators by renal resident cells; (*3*) amplification of the inflammatory response by inducing the production of prostaglandins, nitric oxide, free oxygen radicals and the expression of the MHC class I and II antigens in renal cells; (*4*) increased cell apoptosis by decreasing the mRNA levels of apoptosis-preventing gene *bcl-2* while increasing the expression of apoptosis-inducing gene *Fas* in target cells [46–48].

Interleukin-1. Interleukin-1 is a pleiotropic cytokine that is primarily released by activated monocytes/macrophages and resident cells [49–52]. Two different forms of IL-1 have been observed in humans, IL-1α and IL-1β, that present a different pattern of glycosylation and exert a variety of biological effects on different cell types [51]. Some IL-1 activities include induction of chemotaxis in polymorphonuclear cells and macrophages, proliferation of mesangial and endothelial cells and stimulation of chemokine and growth factor production by resident renal cells [53, 54]. This cytokine also induces the production of factors required for T cell activation and proliferation [55].

Interleukin-6. Interleukin-6 is produced not only by activated monocytes/macrophages, but also by T cells, endothelial and mesangial cells. It exerts its biological effects on a variety of target cells including resident glomerular and tubular cells [56]. IL-6 acts as a second signal for the production of IL-2 promoting the IL-2 dependent proliferation of T lymphocytes [57]. It induces proliferation of mesangial cells *in vitro* as well as *in vivo* [58]. IL-6 production is rapidly and transiently induced by other cytokines including IL-1 and TNF-α. Functional interaction between IFN-γ and TNF-α is involved in the regulation of IL-6 and IL-6 receptor expression in monocytes [59].

Chemokines

The chemotactic cytokines or chemokines play a pivotal role in the local dealing out of the inflammatory response, ensuring that the correct immune effector cells are recruited and activated in the right place, at the right time [60, 61]. Chemokines act as magnets for white blood cells and thus contribute to lymphocyte, granulocyte and monocyte infiltration and activation within glomeruli and interstitium. The migration of leukocytes to the interstitial tissue compartment is due to the adhesion of these cells to the endothelium and their passage through endothelial cell junctions and basement membrane. As to their action, chemokines bind the extracellular matrix components and attract the immune cells by haptotaxis (movement of target cells through a gradient of an immobilized attractant) [60]. Therefore, concentration of chemokines in the subendothelial matrix is essential for extravasation and migration of leukocytes. Among the proteins belonging to this family, attention has recently been focused on three molecules: IL-8, monocyte chemotactic peptide-1 (MCP-1) and regulated on activation normal T cells expressed and secreted factor (RANTES). These have different specificity for various immune cells: IL-8 is a powerful chemoattractant for neutrophils, while MCP-1 and RANTES act specifically on monocytes and lymphocytes, respectively [60, 62]. The production of these three chemokines is induced by cytokines, such as IFN-γ, IL-1α and β, TNF-α [23, 31, 63–65], and by other pro-inflammatory stimuli like reactive oxygen species, IgG, LDL and thrombin in renal resident cells and in infiltrating immune cells [66–71].

Growth factors

Growth factors are normally involved in development, in many physiological regenerative processes and in tissue repair after injury. Due to their specific effects, it has been suggested that some of them may be involved in the pathogenesis of renal damage in human GN [24].

Platelet-derived growth factor. Platelet-derived growth factor (PDGF) is a cationic protein, constituted by two chains (A and B) that can be combined in three different isoforms (AA, AB and BB) [72]. The PDGF receptor also consists of an α and a β subunit [72]. The former binds either A or B chains whereas the latter binds only the B chain. Initially recognized as a product of the alpha granules of platelets, PDGF is synthesized and released by circulating immune cells, activated resident glomerular cells and smooth muscle cells [reviewed in 24]. PDGF is the most potent mitogen for mesenchymal cells including mesangial cells [73]. Moreover, most of the mitogenic factors induce its production, suggesting a possible autocrine effect of PDGF in their proliferative effect [73]. Besides proliferation, PDGF can induce ECM component expression and the contraction and migration of both mesangial cells and smooth muscle cells [72]. Many stimuli induce mesangial and endothelial cell production of PDGF including basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), endothelin, thrombin and PDGF itself [73–75].

Transforming growth factor. Transforming growth factor (TGF)-β consists of three isoforms (β1, β2 and β3) and is produced by monocytes and resident cells [76]. It is secreted bound to a latency-associated peptide in an inactive form that requires activation before it can exert its biological effect. *In vitro*, the inactive form can be activated by removing the latency-associated peptide with acid treatment or proteases, but nothing is yet known about the activation mechanism *in vivo* [76]. TGF-β binds three specific cell-surface receptors (I, II and III). All of the resident cells express at least one of the receptors and respond to TGF-β. Its main effect is to stimulate ECM accumulation by increasing the synthesis and deposition of the ECM components and by reducing matrix turnover, through the inhibition of the enzymes that catalyze ECM degradation (metalloproteinases) [76, 77]. In addition, TGF-β has a potent antiproliferative action and, interestingly, an inhibitory effect on immune system activation [76]. Indeed, TGF-β knock-out mice develop a systemic autoimmune disease [78].

Basic fibroblast growth factor. Basic fibroblast growth factor (bFGF) is a peptide, belonging to the small family of heparin-binding growth factors, which exerts, both *in vitro* and *in vivo*, a mitogenic effect on a variety of cell types including mesangial, endothelial and epithelial cells, smooth muscle cells and fibroblasts [79]. It is considered one of the most potent angiogenic factors [80]. bFGF has two very peculiar features. It does not present a signal peptide bringing the protein from the Golgi system to the membrane, so that its mechanism of secretion is still largely unclear. Moreover, once bound to the receptor and internalized, it is slowly degraded to low molecular weight peptides that may persist and exert their action for up to 24 hours inside the cell [81].

Epithelial growth factor. Epithelial growth factor (EGF) acts not only on epithelial cells, but also on endothelial and mesangial cells [73]. The kidney is a significant site of EGF synthesis, as specific binding sites have been found in the glomeruli and in different segments of the tubules [82]. This peptide is a trophic factor for renal tubular cells and it plays a key role in regenerative phenomena during recovery from tubular damage [83].

THE GAMES

Minimal change disease

Minimal change disease (MCD) is the most common cause of primary nephrotic syndrome in children, accounting for 75 to 77% of all cases [84, 85], while in adults it represents less than 25% of all primary nephrotic syndromes [86]. Its pathogenesis remains unknown, although some evidence in favor of an immune pathogenetic disorder has been reported. Indeed, the association of MCD with athopy to different antigens, high serum IgE levels and an increase of Fc receptor for IgE on B cells is frequent [87]. The beneficial effects of immunosuppressors (such as corticosteroids, cyclophosphamide, etc.) or of immuno-stimulants (that is, levamisole) seem to corroborate the hypothesis that an allergen may trigger the immunological disorder responsible for MCD. An increase of glomerular capillary permeability to serum proteins, mainly to albumin, is the hallmark of this disease. No evident glomerular alterations can be seen on light microscopy, whereas foot process fusion and detachment of epithelial cells from the glomerular basement membrane (GBM) are evident on electron microscopy. The loss or the charge alteration of heparan sulphate proteoglycan costituents found, by immunohistochemistry, at the glomerular level are probably responsible for the altered glomerular capillary charge-dependent permselectivity of MCD [88–90]. On the basis of this evidence, the question "Does an abnormal immune response induce an alteration of glomerular permeability?" deserves an answer. A T cell defect, characterized by a decreased number of Th1 cells and increased number of Th2 cells, has been reported in children with MCD [91]. Moreover, in children with primary nephrotic syndrome, Stachowski et al demonstrated a shift from Th1 to Th2 subsets of CD4+ T cells, cultured with autologous monocytes, and an increased production of IL-4, IL-6 and IL-10 [92]. Furthermore, Neuhaus et al found that calcium ionophore stimulation of peripheral blood lymphocytes from children with relapsed MCD caused the release of large amounts of IL-4 [93]. Bakker et al demonstrated that infusing lymphoid cells from MCD patients in rats caused an increased glomerular permeability [94]. Thus, on the basis of these observations, it is possible that the old pathogenetic hypothesis made by Shaloub [95] that considered MCD as a T cell disorder is still applicable. The prevalence of a T cell subpopulation, during onset or relapse of the disease, may induce an increased production of specific cytokine(s), responsible for the glomerular charge-selectivity barrier alterations leading to proteinuria. Several investigators [96–98] have shown a production of a vascular permeability factor by stimulated and unstimulated peripheral blood lymphocytes from patients with MCD. Recently, Koyama et al reported that T cell hybridomas obtained from patients with MCD produce a glomerular permeability factor with properties similar to TNF-α [99]. This factor infused into rats induced massive proteinuria due to a partial fusion of glomerular epithelial cells and to the altered electron microscopic distribution of anionic charges. Moreover, Saxena and others [100, 101] found high levels of IL-1 and IL-2 in supernants of cultured activated lymphocytes from patients with MCD. Furthermore, an increased production of IL-8 has been found in peripheral blood mononuclear cells from patients with relapsing MCD [102]. Bricio et al have also demonstrated an increased production of IL-1 in cultured whole glomeruli obtained from rats with adriamycin-induced nephrotic syndrome [103]. In this case, the augmented production of IL-1 was primarily related to the glomerular macrophage infiltration and less to mesangial cell activation. Indeed, in this rat model of nephrotic syndrome, proteinuria was strictly correlated with the number of Ia-bearing infiltrating cells. Finally, indirect evidence of cytokine involvement in the pathogenesis of proteinuria in MCD is provided by development of a nephrotic syndrome in patients with various malignancies treated with recombinant IL-2 as an immunotherapy [104].

Focal segmental glomerulosclerosis

Idiopathic focal segmental glomerulosclerosis (FSGS) is another common cause of nephrotic syndrome in children and young adults [105]. Recently, it has been hypothesized that in patients with FSGS the presence of a circulating factor may alter the glomerular barrier to protein filtration [106]. This hypothesis is corroborated by the evidence that some patients with FSGS respond to therapy with plasmapheresis [107, 108] and in 20 to 40% of them FSGS recurs after transplantation [109]. Indeed, Savin et al have reported the presence of a serum factor that increases the glomerular permeability to albumin in transplanted patients with recurrent FSGS [110]. The presence of this factor

was demonstrated by incubating isolated human glomeruli with fractions of serum obtained from discarded plasma, separated by plasmapheresis, in six different patients. Although this finding may be an indirect evidence due to the technique used, the validity of the method in measuring increased glomerular permeability to albumin has previously been demonstrated in experiments where isolated murine glomeruli were incubated with protamine [111], superoxide [112], activated leukocytes [113] or Heymann antibody and complement [114]. In its active form, the 50 kDa serum factor binds to protein A and has a weak anionic charge at pH 6.0. It is assumed to be a nonimmunoglobulin protein or a fragment of an immunoglobulin and its activity is concentration dependent. This factor has a potent activity comparable to a cytokine-like molecule. Indeed, few nanograms of it cause direct injury to podocytes. It can be hypothesized that the circulating factor, present in recurrent FSGS after renal transplantation, could interact with the podocytes leading to the retraction and flattening of the foot-processes and to their detachment from the basal membrane [115]. Within several weeks after successful plasmapheresis, the podocytes revert to a normal electron microscopic morphology, and this reversible change in shape could be related to the reorganization of the podocyte cytoskeleton [108]. However, if a serum factor is responsible for the glomerular damage, why are sclerotic lesions focal and segmental instead of diffuse? A recent paper published by Fuiano et al seems to provide an answer to this question [116]. The morphological analysis of serial sections of renal biopsy specimens obtained from patients with FSGS demonstrated that the distribution of sclerotic lesions is not focal but diffuse [116]. Thus, this study supports the hypothesis that a circulating factor may damage all glomeruli although only some of them show lesions evident in a routine histological study. If the presence of this serum factor can explain the detachment of podocytes from the GBM and the glomerular sclerosis by means of a collapse phenomenon, then which factors drive this process and the development of interstitial fibrosis in FSGS? Stein-Oakley et al have recently reported an overexpression of TGF-β, PDGF and PDGF receptors both at the glomerular and at the interstitial level in human FSGS, suggesting that the combined action of both these growth factors could induce the fibrotic glomerular and interstitial histological changes observed in this GN [117].

Recent advances in the understanding of the pathogenesis of HIV-associated FSGS have shown that viral proteins, like HIV Tat, might be toxic to visceral epithelial cells or might induce the release of growth factors such as TGF-β and FGF, as observed in Tat transgenic mice and in human HIV-associated nephropathy [118–120]. Therefore, it is conceivable that other viruses, not yet identified, could play a similar pathogenetic role in those form of FSGS currently defined idiopathic.

Membranous glomerulonephritis

Membranous glomerulonephritis (MGN) is characterized by the presence of small subepithelial immune deposits in the *lamina rara externa* of the GBM that progress to large size deposits incapsulated in the GBM matrix [8]. The distruption of the GBM structure increases its permeability to albumin and other serum proteins, which then form the massive proteinuria. Approximately 25 to 50% of patients progress to end-stage kidney disease over 10 years [7]. Heymann nephritis, an experimental model induced in rats, closely mimics the morphologic changes of the GBM and the chronic course of the human disease [121, 122]. In passive Heymann nephritis, immune complexes are formed *in situ* as the result of interaction between administered antibodies and gp 330, a glycoprotein antigen present in clathrin-coated pits on the base of podocyte foot processes, and a 44 kDa receptor-associated protein [123–126]. In human membranous nephritis, different antigens have been postulated as being responsible for the immune complex formation in the blood or kidney. Recently, several reports described the association of hepatitis C virus with MGN [127]. The subepithelial immune complexes activate the complement system leading to the formation of the terminal complement complex C5b-9 that is deposited along the GBM, endocytosed by glomerular epithelial cells, and finally transported into the urinary space [128–130]. Activation of the complement cascade has also been demonstrated by the presence of C3dg in the urine [131]. Urinary excretion of C5b-9 correlates with immunologic activity of disease in both Heyman nephritis [132] and in patients with membranous nephritis [131]. In the latter, high initial urinary excretion is associated with a progressive clinical course of the disease [131].

The mechanism by which C5b-9 increases the glomerular permeability to macromolecules is poorly understood. However, *in vitro* studies have shown that C5b-9 causes the release of reactive oxygen species (ROS), IL-1β and TNF from mesangial cells [133, 134]. Histochemical studies localized ROS in podocytes within cytoplasmic membrane vesicles and along the foot process membranes in passive Heymann nephritis [135]. The authors hypothesized that C5b-9 stimulates the *de novo* synthesis of the NADPH-oxidoreductase complex by podocytes, which in turn become able to generate ROS. Lipid peroxidation in GBM, induced by ROS, could promote dimerization of type IV collagen, distortion of GBM structure, alterations in the glomerular filtration barrier and consequently proteinuria [135].

Other effects of C5b-9 on glomerular epithelial cells are probably directed toward the synthesis of cytokines and growth factors. TNF-α has been demonstrated in glomerular epithelial cells in human MGN and the integral cytokine is found in urine [136, 137]. Significantly elevated urinary levels of IL-1β are also reported in MGN in correlation with increased urinary elimination of the complement regulatory protein CD59 [138].

On the other hand, TGF-β seems to mediate ECM accumulation in experimental membranous nephropathy. Rats affected by passive Heymann nephritis show a significantly increased glomerular expression of TGF-β2 and TGF-β3, in association with development of proteinuria. The expression of these TGF-β isoforms by glomerular epithelial cells parallels the up-regulation of TGF-β receptor type I and II on the same cells. Complement depletion, by preventing C5b-9 deposition, inhibits TGF-β protein and receptor up-regulation [139].

Membranoproliferative glomerulonephritis

Membranoproliferative glomerulonephritis (MPGN) is a chronic progressive disease with a poor prognosis that occurs in older children and young adults [7]. Its pathogenesis is not completely understood. Over the past three years, several papers have reported the frequent association of this disease with hepatitis C virus infection, presence of circulating immune complexes, and often with the characteristics of cryoglobulins [140, 141]. This nephritis also occurs in a variety of diseases caused by chronic bacterial, viral, parasitic infections, autoimmune disorders or

primary or malignant hemopathies [142]. The renal manifestations are proteinuria, often in the nephrotic range, hematuria, and some degree of functional impairment and hypertension. Acute nephritic syndrome may be observed.

The light microscopic picture is characterized by mesangial cell proliferation and duplication and/or thickening of the capillary basement membrane. On the basis of ultrastructural morphology, three types have been described: type I, with subendothelial deposits; type II, with intramembranous dense deposits (dense deposit disease); and type III, a variant of type I, with subendothelial and subepithelial deposits associated with glomerular capillary basement membrane disruption. Type I, the most common form of MPGN, is probably mediated by glomerular deposition of circulating immune complexes with activation of the classic complement pathway. In type II, the occurrence of nephritic factors induces the involvement of the alternative pathway. In type III, hypocomplementemia is infrequent and the pathogenesis is poorly understood [7].

Monocytes-macrophages play an important role in this disease [20]. These cells are present in mesangium during the active injury, and move to the glomerular capillary wall attracted by chemotactic factors produced by the endothelium after immune complexes have formed *in situ* or been deposited from the circulation. During the active phase of the disease the stimulated endothelial cells produce a new basement membrane-like structure separated from the original lamina rara by the presence of deposited immune complexes and trapped inflammatory cells. This repair process completes the double contour of the glomerular capillary wall, named the "tram track." During the course of the disease either the inflammatory attacks or the repair process will predominate [7].

The chronological changes in intraglomerular CR1, CR3 and CR4 expression, as well as intraglomerular C3c deposition have been studied in serially biopsied cases of MPGN type I. Data reported by Soma et al [143] showed a marked glomerular infiltration of CR3+ and CR4+ cells (monocytes/macrophages and granulocytes) that correlated with the amount of glomerular C3c deposits. This result, and the fact that C3b is a ligand of these two adhesion molecules belonging to the $\beta 2$ integrin family, indicate that the accumulation of leukocytes in glomeruli of MPGN is partly promoted by the adhesive effect, in a $\beta 2$ integrin- and complement-dependent fashion. CR1 expression is a significant prognostic factor of MPGN type I, and the risk of renal deterioration is notably reduced in those patients with normal or decreased CR1. Some authors have also reported an interaction between intraglomerular T lymphocytes and monocytes/macrophages with continuous C3c deposition and an adverse outcome [143, 144].

IgA nephropathy

IgA nephropathy (IgAN) is a mesangioproliferative GN characterized by granular deposits of IgA and C3 in the mesangium. This disease is the most frequent form of primary GN causing end-stage renal disease among Caucasoids and Orientals. It is characterized by recurrent episodes of gross hematuria associated with upper respiratory tract infections and/or persistent microhematuria with proteinuria. IgAN is an indolent, chronic GN that may gradually progress to renal failure. The renal survival curves show 10 to 20% of the patients reaching end-stage renal disease after 10 years from the apparent clinical onset of the disease and 20 to 50% after 20 years [145].

Undoubtedly, mesangial cells play a pivotal role in IgAN, since their proliferation and activation surely is certainly a critical step in the pathogenesis of this GN [7]. It is well known that PDGF stimulates mesangial cell proliferation both *in vitro* and *in vivo* [146, 147]. Interestingly, we demonstrated an increased expression of PDGF AB within the glomeruli in this GN, while we observed an increased PDGF β receptor expression at the glomerular as well as at the interstitial level [148, 149]. The increased expression of PDGF and its receptor strictly correlated with the degree of mesangial and interstitial cell proliferation and with the extent of glomerulosclerosis and interstitial fibrosis [148, 149]. Besides PDGF, IL-6 has also been suggested to induce mesangial cell activation [56]. Indeed, several authors have demonstrated an up-regulation of IL-6 within the glomeruli, while we observed a striking up-regulation of IL-6 gene and protein expression in the tubulointerstitium [58, 150, 151]. Moreover, we reported an increased IL-6 urine excretion, strictly correlated with its renal tissue expression, suggesting the urinary presence of this cytokine as a possible marker of disease activity [151]. IL-4, a cytokine produced by infiltrating CD4+ T cells, may also be involved in turning on mesangial cells [150, 152].

The expansion of the mesangial and interstitial matrix is another histological feature of IgAN. The data obtained from *in vitro* studies would indicate TGF-β as the factor inducing these fibrotic changes [76]. Instead, studies on human disease are contradictory. In fact, Ballardie et al reported decreased TGF-β expression and concluded that reduction of this anti-mitogenic growth factor might allow the proliferation of mesangial cells [150]. In contrast, Yamamoto et al demonstrated a significant increase of all three TGF-β isoforms associated with the increase in ECM deposition [153].

The third histopathologic feature of IgAN is the presence of macrophage infiltration in the interstitium and particularly in periglomerular areas [20]. There is now increasing evidence that the mediator recruiting these cells could be MCP-1. We reported an increase in MCP-1 gene and protein expression in human IgAN, correlated with the extent of monocyte infiltration [154]. Resident cells expressing the chemokine were mainly proximal tubular cells and glomerular parietal epithelial cells. Moreover, like IL-6, MCP-1 urinary excretion also mirrored the tissue expression, suggesting that this could be used as a marker of disease activity in the follow-up for IgAN patients.

In the progressive form of this GN, tubular cells are constantly involved in progression toward fibrosis. If on the one hand they can be activated and produce pro-inflammatory cytokines, on the other their functions and trophism may also be greatly altered. EGF is the main factor regulating tubular cell growth and/or regeneration [83]. We observed a significant decrease in EGF expression in IgAN patients with moderate and severe histologic damage, mainly characterized by interstitial fibrosis and tubular atrophy, indicating this factor as a reliable marker of tubular impairment that can be easily detected in the urine [151].

Crescentic glomerulonephritis

Crescentic glomerulonephritis (CGN) is the most aggressive form of renal disease, and rapidly progresses to end-stage renal

failure. It can occur without systemic manifestations, in combination with diffuse pulmonary hemorrhage (Goodpasture's syndrome) or in the presence of diffuse necrotising vasculitis. In the first case, granular deposits of immunoglobulin and complement are found in the glomeruli. In the second, there are linear IgG deposits along the GBM and in the third, often associated with circulating antineutrophil cytoplasmic antibodies, no immune deposits can be observed within the glomerular tuft [7].

Examination of renal biopsies performed in patients with CGN showed a significant leukocyte infiltration at glomerular level. The presence of CD4+ and CD8+ T cells and monocytes/macrophages suggested the occurrence of a delayed-type hypersensitivity reaction [155]. In favor of this hypothesis, a major susceptibility to CGN has been observed in rat strains with a predominantly Th1 response to antigens [156]. A linear correlation between the number of T lymphocytes and the number of monocytes in the glomeruli indicates the importance of T cells in the recruitment of monocytes/macrophages in cell mediated immunity [157, 158]. Neale et al also demonstrated a correlation between the presence of crescents with fibrin deposits and the increased number of intraglomerular IL-2R+ mononuclear cells and decreased creatinine clearance [159]. Li et al found that T lymphocytes and macrophages in glomeruli with active crescents correlated with an increased number of intraglomerular IL-2R$^+$ mononuclear cells and decreased creatinine clearance [160].

T cells and monocytes can be therefore considered as the two main cell types involved in the pathogenesis of this GN. The migration and subsequent activation of T lymphocytes and monocytes from the blood throughout the endothelium into the glomeruli could be mediated by adhesion molecules present on the surface of lymphocytes (LFA-1) and endothelial cells (ICAM-1), and by the chemotactic action of chemokines released by activated mesangial and epithelial cells. An overexpression of MCP-1 and RANTES have been detected in glomeruli of patients with idiopathic CGN [161] and in experimental nephrotoxic nephritis [155], respectively. In addition, IL-8 expression is increased in the renal tissue of patients with GN and can be detected in their urine [162]. The relevance of the chemokines in the pathogenesis of this peculiar form of GN has been definitely established. Indeed, Wada et al demonstrated that neutralizing anti-MCP-1 and anti-IL-8 antibodies can reduce the histologic lesion and improve the clinical outcome in an experimental model of CGN [163, 164].

Once recruited and activated, both T cells and monocytes can release IL-1. This cytokine can in turn activate the resident cells and begin a positive feedback loop, recruiting and activating more infiltrating cells. IL-1 expression has been demonstrated in an experimental model of CGN [52]. Moreover, the inhibition of IL-1 by the infusion of IL-1 receptor antagonist reduces proteinuria and crescentic lesions in the anti-GBM model of CGN [165].

THE FINAL HAND IN THE GAME

Some forms of primary GN (MCD, mild IgAN) do not present a progressive feature, and for them the game could continue on the same table (the glomerulus) with the same rules and the same players forever (mild IgAN), or could eventually terminate (MCD). Other forms progress instead to a common phase played on a different table, the tubulointerstitial area, and with two new players, interstitial fibroblast and tubular cells. It is now clear that in chronic progressive GN the secondary involvement of the tubulointerstitium is a constant feature and that the degree of tubulointerstitial lesions correlates with the renal function outcome better than does the extent of glomerular damage [8]. However, the mechanisms underlying the involvement of the tubulointerstitium in progressive GN and the consequent inclusion in the game of tubular cells and interstitial fibroblasts are still largely unknown. Thus, the progression of renal damage in primary GN, although strongly influenced by the extent of glomerulosclerosis, is primarily related to the involvement of tubulointerstitial damage [8]. Several pathogenetic hypotheses have been proposed for the development of glomerulosclerosis, which is the result of chronic glomerular damage [166, 167].

In the hypothesis of progressive mesangiosclerosis, cytokines and growth factors, released by infiltrating and resident cells, play an important role. Indeed, the production of these inflammatory mediators induced by several stimuli may generate a state of chronic mesangial inflammation that, by inducing a progressive sclerosis of the mesangial area, culminates in global glomerulosclerosis. In this hypothesis, PDGF and TGF-β appear to be the key pathogenetic mediators. The intrarenal infusion of PDGF, in association with a sub-lytic dose of anti-Thy-1 serum, stimulates the proliferation of mesangial cells and the induction in their cytoplasm of α-smooth muscle actin (α-SMA), a marker of cell activation [168]. On the other hand, the *in vivo* transfection of TGF-β stimulates mesangial cells to synthesize and release extracellular matrix proteins [148]. Moreover, *in vivo* studies have demonstrated that TGF-β colocalizes with α-SMA and correlates with the glomerular deposits of type I and type III collagen [169, 170]. Accumulation of ECM components is further enhanced by the ability of TGF-β to suppress the expression of proteases and stimulate that of protease inhibitors. In some glomerular diseases, changes in the expression of metalloproteinases and their inhibitors have been found to correlate negatively or positively with matrix deposition [171, 172]. However, it should be noted that in sclerosing glomerulonephritis there are both qualitative and quantitative changes in the expression of ECM components. Moreover, an altered expression and/or affinity of some matrix receptors *in vivo* has been reported, and this could further facilitate the accumulation of collagen, laminin and fibronectin in sclerosing lesions [173].

In primary GN, the initial events that activate the local cytokine and growth factor pathways seem to be prevalently immunological (that is, circulating immune complexes deposition or their *in situ* formation and consequent complement activation). However, once the inflammatory process has been primed, other factors, such as hyperlipidemia and hypertension consequent to nephrotic proteinuria and renal damage, may contribute to the pathogenesis of the glomerulosclerosis [174]. Indeed, oxidized LDL as well as angiotensin II cause chemoattraction of monocytes/macrophages within the glomerulus, inducing MCP-1 [70, 175], while LDL can stimulate proliferation and collagen deposition inducing PDGF and TGF-β [176].

According to the second hypothesis on the development of glomerulosclerosis, suggested by Kriz, podocytes may play a crucial role [167]. Detachment of podocytes from the peripheral glomerular capillaries, induced by any kind of strain, may denude the vessel wall that, tending to attach to Bowman's capsule, causes tuft adhesion. Thus, during a mesangial injury, the glomerular peripheral capillaries, deprived of their mesangial support, may lose their centripetal attachment and extend towards the Bowman's capsule. Centrifugal expansion and ballooning of these

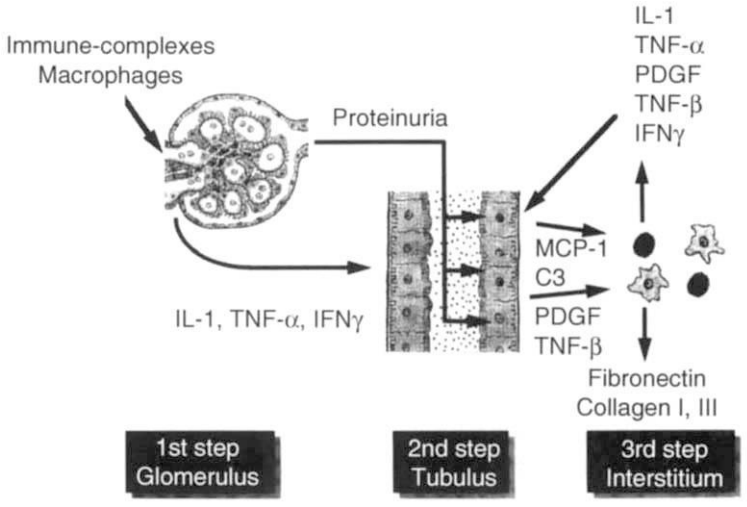

Fig. 2. The final pathway. Humoral and cellular immunity may induce glomerular damage through the release of cytokines and growth factors (1st step). Proteinuria consequent to glomerular damage [179, 180] or cytokines released by infiltrating cells and diffusing from hilar areas of glomeruli [178] may determine tubular cell activation with release of chemoattractant substances and fibrogenic factors (2nd step). Finally, the activation of interstitial fibroblasts may induce the increased production of extracellular matrix (fibronectin, collagen I, III) and fibrosis (3rd step).

capillaries will be further promoted by the loss of bandaging support of the podocytes, and eventually result in the apposition of a "naked" capillary to Bowman's capsule. In our view, while mesangial cell proliferation is not directly involved in the glomerulosclerosis process, podocyte detachment plays a pivotal role. Thus, the development of sclerosis is not a process of proliferation, but a process of collapse in which there is a disappearance of cellular elements and accumulation of hyaline material. The fate of the glomerulus with initial segmental sclerosis is characterized by the extension of the sclerotic process to the entire tuft followed by subsequent migration and colonization of cortical fibroblasts with deposition of fibrous tissue [167].

As previously reported, in primary GN that progresses together with the development of glomerulosclerosis, a second inflammatory reaction takes place within the tubulointerstitial compartment (Fig. 2) [177]. Two different models of tubulointerstitial involvement in glomerular diseases have been proposed. Lan, Nikolic-Patterson and Atkins suggested that the interstitial inflammation may originate from the hilar area of the glomeruli [178]. The cytokines produced by the mononuclear cells present within this area could diffuse and induce the up-regulation of chemokine expression in tubular cells that would in turn be responsible for the propagation of the infiltrate (Fig. 2). Other authors have recently proposed an alternative model attributing the role of messenger inducing tubular cell activation to proteinuria, consequent to the glomerular damage (Fig. 2) [179, 180]. The possibility that proteinuria may also contribute to the tubulointerstitial injury has been proposed by Rubin-Kelly and Jevnikar, who showed that massive protein reabsorption by proximal tubular cells induces lysosomal activation and, consequently, antigen presentation followed by cytokine-induced activation of T helper cells [179]. In contrast, Remuzzi et al suggested that the increased intracellular trafficking of proteins in the tubules could activate the transcription factor NF-κB and consequently increase the expression of a variety of pro-inflammatory genes, inducing the recruitment of inflammatory cells [180].

In both models proposed, the tubulointerstitial infiltrate may play a central role in the genesis of the tubular and interstitial lesions observed in chronic GN. Indeed, different studies report a strict correlation between tubular atrophy, interstitial fibrosis and the extent of interstitial infiltrate. This infiltrate is mainly constituted of a dynamic and active population of cells, predominantly monocytes and T lymphocytes, that release ROS, cytokines, growth factors and autacoids which can damage or activate the adjacent tubular and interstitial cells [181]. Recent studies suggest that infiltrating immune cells could be recruited within the interstitial areas by three powerful chemoattractants produced by activated tubular cells: MCP-1, osteopontin and RANTES [154, 182, 183]. Moreover, Savill, Mooney and Hughes hypothesized that defective apoptosis could be responsible for the accumulation of inflammatory cells in the injured kidney, protracting the inflammation [184]. A number of cytokines and growth factors could inhibit the apoptosis of the immune cells through inappropriate expression of intrinsic survival genes such as bcl-2, or failed expression of Fas receptor on their surface.

A third key feature of tubulointerstitial inflammation, besides tubular cell activation and immune cell infiltration, is the presence and activation of interstitial fibroblasts. Recently, Okada et al have suggested that interstitial fibroblasts, not generally present in the normal kidney, could derive from tubular cells through a process of transdifferentiation (Fig. 3) [185]. Upon stimulation, epithelial cells may change their phenotype, losing their tendency to form cuboidal sheets, increasing vimentin production and decreasing cytokeratin expression. Moreover, the transdifferentiated epithelial cells may express a peculiar cytoplasmic marker of fibroblast activation: the α-SMA [186]. These activated cells may proliferate and synthesize extracellular matrix components, playing a major role in the pathogenesis of interstitial fibrosis. Interstitial fibroblast activation could be mediated by a variety of soluble factors, including PDGF and TGF-β [76, 187]. The latter is known to activate fibroblasts *in vitro* and its expression is increased within the tubulointerstitial compartment in different models of progressive GN [76, 169, 170]. The former has been demonstrated *in vivo* to be effective in inducing α-SMA expression in interstitial fibroblasts and, concomitantly, interstitial fibrosis [187]. Moreover, in animal models and in human studies of GN different groups have shown a striking up-regulation of PDGF-β receptor at interstitial levels that strictly correlated with the extent of tubulointerstitial lesions [149]. Interestingly, increased expression of the glycoprotein osteopontin and SPARC at sites of

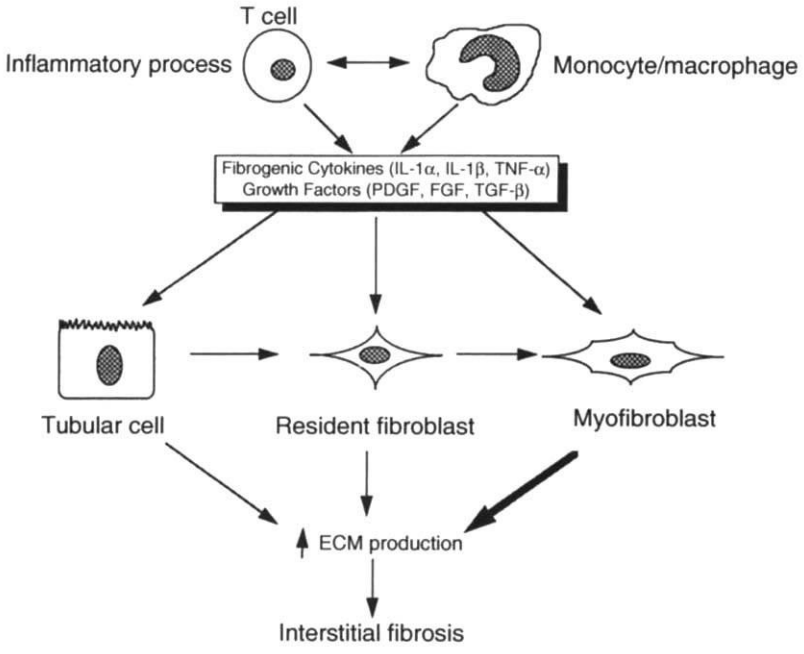

Fig. 3. The transdifferentiation hypothesis. Fibrogenic cytokines and growth factors released by infiltrating T cells and monocytes/macrophages may induce the transdifferentiation of resident cells (tubular cells and fibroblasts) into myofibroblasts leading to excessive extracellular matrix production and consequent interstitial fibrosis [185].

interstitial injury has been found in experimental animal models of nephritis and in human glomerulonephritis [188, 189]. Osteopontin is synthesized by renal tubular cells and up-regulated in tubulointerstitial injury [182] while SPARC, also termed osteonectin, modulates angiogenesis and cell proliferation, binds PDGF and collagen and promotes their deposition [185].

Therapeutic strategies

Once the players, the cards and the rules are known, there is a good chance of succeeding in influencing the course and outcome of the different games. What has been done so far, from a therapeutic point of view, has been to shoot at the players belonging to the immune system, presuming that their deaths could have a beneficial effect on the course of glomerular diseases. Instead, what needs to be done in the near future is to sit at the same table as the players and try to win the game with sleight of hand. Below are some tricks that may be used to win the game.

To stop the cascade of events featuring the inflammatory process exploding within the glomeruli or later on within the tubulointerstitial area, the pro-inflammatory signals represented by cytokines and growth factors must be blocked. The process by which these short-lived factors are produced and exert their actions is articulated in three stages: (*1*) interaction of these factors with their specific cell-surface receptors; (*2*) transmission of the external signal in the cell by the signal transduction process; (*3*) synthesis and secretion of cytokines. Therefore, development of new therapeutic strategies able to interfere with these three biological events are warranted.

Receptor blockage

A variety of natural and synthetic molecules can inhibit competitively the binding of cytokine and growth factor to their specific cell-surface receptors, inhibiting their interaction. There are already a great number of studies using this approach for the treatment of experimental models of glomerular diseases. Among the natural inhibitors of cytokine-receptor binding is the IL-1 receptor antagonist (IL-1ra) [190]. Studies on specific inhibition of IL-1 activity have been carried out in rats with progressive crescentic glomerulonephritis by administering a constant infusion of IL-1ra [190–192]. This therapy was able to reduce proteinuria and prevent crescent formation, renal impairment and renal fibrosis [165, 190]. The beneficial effects of IL-1ra were due to the marked inhibition of glomerular and interstitial ICAM-1 expression, and of the associated glomerular and interstitial leukocyte infiltration [191, 192].

Besides the natural inhibitors, there are some synthetic molecules, such as trapidil, that can interfere with the receptor binding of a specific cytokine. This anti-platelet drug has been shown *in vitro* to compete with the interaction between PDGF and its receptor and to inhibit the PDGF mitogenic effect [193]. Its administration in the anti-Thy1 experimental model significantly improved the clinical and histopathological picture of this model [194].

Administration of cytokine antagonists

Overexpression of TGF-β may cause the accumulation of extracellular matrix (ECM) in GN [153]. Administration of an antibody against TGF-β in rats with mesangioproliferative glomerulonephritis has been shown to improve the course of the disease, preventing ECM production and accumulation in the injured glomeruli [195]. However, after prolonged therapy with the antibody it is possible to observe a loss of responsiveness to TGF-β1 caused by a mutation or shedding of TGF-β receptors. For this reason, the same investigators tried to antagonize the action of TGF-β *in vivo* by administrating decorin, a natural inhibitor of this growth factor, after inducing the same experimental glomerulonephritis in rats [196]. They obtained a reduced production of extracellular matrix and attenuation of renal manifestations.

Recombinant protein therapy shows some limits, mainly represented by the repeated infusion of the highly purified protein. To overcome these limits, Isaka et al transfected *in vivo* skeletal muscle cells with decorin cDNA in rats with the same nephritis, obtaining comparable results [197]. Decorin cDNA was contained in liposomes and administered by a single intramuscular injection. Although, *in situ* transfection of DNA into skeletal muscle is a simple and safe way to deliver a protein to the systemic circulation, the long-term consequences of the constant reduction of a cytokine or growth factor could be harmful as demonstrated by the TGF-β knock-out mice, which die after birth because of an autoimmune disease [78]. Therefore, the possibility of adverse effect must be carefully evaluated for each cytokine.

Intracellular signaling blockade

The binding of a cytokine or growth factor to its receptor triggers a cascade of intracellular events causing cell activation or, in some cases, inhibition. The different receptors can elicit different intracellular pathways to transduce the signals leading to the final effect of the specific cytokine. Some of the receptors have an intrinsic enzymatic activity that, induced by the agonist binding, triggers the signaling cascade. Other receptors, instead, are associated with an enzyme whose activity can be regulated by the conformational changes of the receptor caused by the interaction with the agonist. However, the orientation of diverse intracellular proteins along finely tuned pathways requires the formation of short-lived protein complexes that relay signals throughout the cell [198]. Most of the proteins involved have enzymatic activity and can directly affect the activity of transcription factors, via protein phosphorylation or dephosphorylation, or indirectly, through second messengers like phospholipids, calcium and cyclic nucleotides. Transcription factors should in turn regulate gene expression and the consequent final cellular responses. This modular intracellular structure could represent the target of drugs able to interfere with some of the critical molecular interactions [198].

Some of these drugs are already being used in everyday clinical practice, but only recently has their intracellular action begun to be understood. Among them, ACE inhibitors have been shown to reduce proteinuria and to slow down the rate of progression of renal damage in different progressive renal diseases [199, 200]. Besides their action on blood pressure, the drugs of this class, have been shown to specifically reduce the intracellular calcium spike induced by PDGF, a key intracellular event in the mitogenic pathway of this growth factor [201].

Hydroxy methyl-glutaryl CoA (HMGCoA) reductase inhibitors have been demonstrated to reduce the progression of renal damage in a variety of experimental models of glomerulosclerosis [202, 203]. Besides their lipid-lowering effect, *in vitro* the HMG-CoA reductase inhibitors can inhibit PDGF-induced mesangial cell proliferation or LPS-stimulated NF-κB activation in mesangial cell through the inhibition of p21 ras [203, 204]. Ras is a small GTP-binding protein that plays a centrale role in the signal transduction pathways of most of the cytokines inducing cell proliferation and activation [205]. To be functionally active, however, Ras protein should be linked to specific mevalonate-derived lipids, geranyl and farnesyl pyrophosphate, whose synthesis is blocked by the HMGCoA reductase inhibition [205].

Glucocorticoids are potent inhibitors of the production of certain cytokines (IL-1, TNF-α) and chemokines, such as IL-8 and MCP-1 [206–209]. Most of these genes present in their regulatory region glucocorticoid responsive elements where the activated glucocorticoid receptor can bind, down-regulating mRNA transcription. Two other immunosuppressive drugs, cyclosporine A and FK506, instead bind to calcineurin, which is a critical factor of the T cell activation pathway regulating the T cell-specific transcription factor, NF-AT [210].

In addition to well-established drugs, there are now an increasing number of compounds targeting critical steps in the signal transduction pathways that may have therapeutical applications. Protein kinase C is a cytoplasmic enzyme that is one of the best known intracellular activators. There is an increasing body of evidence that the activation of this enzyme, and in particular of its β isoform, could be a key event in the pathogenesis of diabetic nephropathy. Indeed, a specific inhibitor of PKC-β can significantly reduce glomerular hyperfiltration and microalbuminuria, two early signs of renal involvement in diabetes [211].

Another intracellular messenger that has recently received considerable attention is cAMP. Elevated intracellular levels of this cyclic nucleotide are always associated with a resting state of the cell. Therefore, all the compounds that induce the synthesis or inhibit the degradation of cAMP, deactivating resident and infiltrating cells, could have a therapeutical relevance [212]. Prostaglandins of the E series, and in particular PGE_1, are potent activators of adenylate cyclase and thus induce an increase in cAMP intracellular concentration [212]. The use of these molecules or of their synthetic analogs has been shown to be extremely effective in different experimental models of immune-mediated progressive GN. PGE_1 infusion can reduce proteinuria and glomerular hypercellularity in nephrotoxic serum nephritis as well as in the anti-Thy1 model of mesangioproliferative GN [213, 214].

Finally, pro-inflammatory cytokines like IL-1 and TNF-α exert their action on several cell types, including mesangial cells, by inducing the synthesis and activation of phospholipase A2 (PLA_2), which generates distal effectors of the inflammatory injury [215]. The PLA_2 inhibition with synthetic molecules like lipocortin 1, quinacrine hydrochloride and RO 31-4639, therefore, could attenuate glomerular as well as tubulointerstitial inflammation [216–218].

Cytokines and gene therapy

Over the past five years remarkable progress has been made in the field of gene therapy. Not only have inherited diseases been treated with gene therapy, but also acquired disorders, such as cancer and infections, have become candidates for this kind of therapeutic approach [219, 220]. Successful gene therapy requires a delivery system by which a particular gene can be introduced into the desired cell type both efficiently and accurately. Current strategies involve the use of modified or attenuated retro- or adenoviruses or DNA-liposome mixtures as vectors for carring the genetic material into the cell [219]. The best way to achieve the local production of a specific factor is by *ex vivo* gene therapy. This approach consists of removing target cells from the body, introducing the new genetic material *in vitro*, and replacing the "modified" cells in the body, where they can produce the desired protein. Using the mesangial cell vector as a gene transfer system, Kitamura et al recently demonstrated that rat mesangial cells, transfected with TGF-β1 and infused via renal artery injection into rat kidney affected by anti-Thy-1 nephritis, expressed active

TGF-β1. This contributed to the recovery from the acute glomerular injury by reducing mitogenesis of resident cells, opposing the effect of local inflammatory cytokines and inhibiting the infiltration of macrophages that normally accumulate in anti-Thy-1 nephritis [220]. Although the effects of TGF-β1 are contradictory, this study demonstrates the feasibility of a gene transfer approach to enable the glomerular cells to produce biologically active molecules *in vivo* and to exert control over the activity of immune mediators involved in glomerular diseases.

In addition to inducing the local production of anti-inflammatory cytokines, the gene therapy approach can also enable local administration of cytokine antagonist through the local infusion of cells transfected with the specific antagonist gene. This approach has recently been applied to immunological diseases such as rheumatoid arthritis, with intra-articular infusion of synovial cells producing IL-1ra [221].

While transfecting renal resident cells with specific genes and introducing them into the kidney it is possible to induce the local production of the corresponding proteins. Expression of specific genes *in vivo* can now be blocked through the use of antisense oligonucleotides. Antisense oligonucleotides can inhibit the synthesis of a given factor by hybridizing to target sequences within the mRNA coding for that factor, destabilizing the target mRNA and consequentely decreasing its translation into protein [222]. Features that govern the identification of a target site are likely to be related to the oligonucleotide length and binding affinity and to the accessibility of the target RNA [222]. Therefore, the oligonucleotides need to be carefully selected in order to find sequences with the most potent inhibitory action and to avoid non-antisense effects. Akagi et al have used this therapeutical approach in the anti-Thy1 model in an attempt to block TGF-β expression [223]. The rats treated with TGF-β antisense oligonucleotides showed a reduced expression of this growth factor with a concomitant reduction in ECM.

Efforts to develop antisense compounds have encountered some expected problems, such as their quick degradation *in vivo* and unexpected side effects such as decreased blood clotting and increased blood pressure [224]. In addition, many synthetic oligonucleotides, because of their highly negative charge, get hung up on proteins such as growth factors and anchoring proteins (fibronectin, laminin) outside the cells, interfering with wound healing and arterial wall repair in living animals [224–226].

An emerging technology in this potential therapeutic field is the replicative antisense approach in which a gene driven by a strong viral promoter is inserted into the genome in such a way that the RNA transcribed from the inserted gene is the opposite, or "antisense," to the normal "sense" RNA. When the cell transcribes both the sense and antisense genes, the antisense RNA can interfere with the translation of the sense RNA by binding to it directly [227]. More animal studies are now warranted to test the feasibility of this therapeutic approach *in vivo*.

Administration of cytokines

Another possible strategy aimed at influencing the behavior of the players throughout the game is that of playing antagonist cards. The advent of recombinant technology has made large quantities of highly purified cytokines available for clinical trials. The progress over the last few years of therapeutic administration of cytokines alone or in combination with other drugs has yielded encouraging and exciting results in patients with cancer, inflammatory disorders (neurologic diseases, rheumatoid arthritis) and viral infections, for whom conventional treatments have failed. In these pathological conditions, the preferential activation of Th1 or Th2 response plays a key pathogenetic role and can be modulated by administering IL-4 or IFNγ, respectively [228]. Recently, IL-12 has been introduced in clinical trials as a drug to fight diseases characterized by a dominant Th2 profile [229]. In these cases, by stimulating IFNγ production IL-12 induces the differentiation of Th1 cells from uncommitted T cells, and consequently, initiates cell-mediated immunity. However, therapy with high doses of cytokines may lead to adverse clinical side effects, like those experienced with the infusion of high doses of rIL-2 that causes hypotensive reactions, development of vascular leakage syndrome induced by activation of the complement system [230], and cardiac dysfunction [231]. In animal models of GN this therapeutic approach has already been applied successfully. In an experimental passive rat model of nephrotoxic nephritis, the intravenous administration of IL-6 inhibited the effect of LPS and had a beneficial effect on glomerular injury [232].

Interestingly, it is now possible to administer cytokines orally. The heavy glycosylation and acid stability of some cytokines may serve to protect them from the low gastric pH and from complete proteolysis in intestinal environments [233]. Orally administered IFN α/β suppressed the development of collagen-induced arthritis in rats. Oral administration of IFNα may be used in the immunoprophylaxis of AIDS and chemokines as blockers of HIV-infected T cells [234]. However, this therapy may induce side effects such as leukopenia and depression of granulocyte-macrophage colony forming units in bone marrow. Other cytokines such as IL-5 and IL-6 have been administered by gavage to mice for reducing bacterial colonization of peripheral tissues following hemorrhagic shock and intestinal infections, since IL-6 is able to restore the compromised intestinal epithelial barrier [233].

Natural cytokines derived from the host species are the best choice, although questions regarding their purity must always be raised. Recombinantly-produced cytokines, which are glycosylated, may be an acceptable compromise.

Auto-vaccination

Genetic vaccination by intramuscular injection of naked DNA encoding a region of the T cell receptor has recently been used by Waisman et al in mice in experimental autoimmune encephalomyelitis [235]. They were able to obtain protection from the disease, because DNA vaccination induced a shift in the pattern of T cells cytokine production with a reduction in the Th1 cytokines, IL-2 and IFNγ, and an increased expression of IL-4 with a suppressive effect on the disease. This therapeutic strategy has the advantage of modulating the cytokine immune response from one subset to another and may help to suppress the abnormal response in disease. The consequence of the introduction of naked DNA that provides proteins must be better understood, and methods for controlling immune response need to be developed. When the naked DNA can be predictably manipulated and appropriately delivered to achieve the desired outcome of the disease, we will indeed have entered into the golden age of vaccination.

Drugs and diet

There are several other strategies that can be used or have already been used to modify the behavior of the players during the

games, even if their mechanisms of action are less clear than the ones previously described.

Some drugs may induce the prevalent production of cytokines by Th1 or Th2 cells, and in diseases with a prevalence of a one cell subset, the administration of these drugs may cause modification of this pathological balance. One of these compounds is ciprofloxacin, a quinolone carboxylic acid derivative that can modulate the immune response at two levels: the production of IL-2 by activated T cells and the production of IL-1 by activated monocytes/macrophages [236]. The possibility that ciprofloxacin may react primarily with the IL-2 producing Th1 inflammatory cells may indicate the use of this drug in diseases of prevalent function of Th2 subset.

The beneficial effects of ACE inhibitors in the progression of renal damage in chronic human glomerulonephritides have been continuously stressed in this decade, after *in vitro* studies have shown that angiotensin II stimulates PDGF and TGF-β mRNA expression in cultured mesangial cells [237] and ACE inhibitors reduce TGF-β mRNA expression [238]. Clinical trials in primary and secondary glomerulonephritides have been carried out and the results obtained showed a decrease in the progression of renal damage in patients receiving ACE inhibitors or angiotensin II receptor antagonists [239, 240]. However, in experimental models, the administration of ACE inhibitors has shown that this drug has a renoprotective effect when the treatment starts early in the course of the disease [241].

Low molecular weight heparins (that is, pentosan polysulphate) have been proposed to treat proliferative glomerulonephritides, since they have many anti-inflammatory properties [242] and an anti-proliferative effect on mesenchymal cells. Interestingly, pentosan polysulphate can block the effects of growth factors like FGF and TGF-α [243], inhibit complement activation and stop the release of elastase and other lysosomal enzymes from polymorphonuclear cells. Moreover, recently, Rix et al demonstrated that *in vitro* heparin can inhibit the ability of IFNγ to stimulate MHC class II antigens and ICAM-1 expression in endothelial cells [244].

A low protein diet has long been used as a treatment for slowing the progression of renal damage, based on the observation that a reduction in protein intake can decrease glomerular hyperfiltration. Moreover, Okuda et al recently demonstrated that the protein-restricted diet can significantly reduce TGF-β expression in rats with mesangioproliferative glomerulonephritis [245].

Plasmapheresis

The use of plasma exchange to treat renal diseases was introduced almost twenty years ago and is now gaining new interest [246]. The rationale in the use of this technique is to rapidly remove circulating factors known to induce the renal damage. Plasma exchange, therefore, has been successfully applied, in association with immunosuppresive therapy, to lowering circulating titers of autoantibodies, as in anti-GBM nephritis, or immune complexes [246, 247]. However, in some diseases characterized by elevated autoantibodies and immune complex titers, such as lupus nephritis, plasma exchange has been shown to be ineffective [248]. An interesting development of this technique has been the introduction of immunoadsorbant columns coated with protein A or the specific antigen recognized by the autoantibody, such as the α3 chain in the anti-GBM GN, to remove all of the IgG or specifically the autoantibody [249]. A new frontier for the plasma exchange has been opened in the treatment of recurrent focal glomerulosclerosis (FGS), expecially after renal transplantation, in the attempt to remove the recently isolated circulating permeability factor [110].

Conclusions

It is no surprise that the rapid evolution of biomedical research over the past few years due to advances in molecular biology techniques has allowed us to recognize the players, the cards and probably the rules regulating the different games of human GN. Moreover, we are now understanding that we could even follow the game process in the kidney by simply looking at the urinary excretion of the different soluble mediators, although this hypothesis need to be confirmed in large studies with long-term follow-up.

However, in the attempt to win the therapeutic game it is necessary to use sleight of hand to deal the cards. Over the last few years, many investigators have attempted to block cytokines and growth factors by applying various therapeutic approaches on experimental models of progressive glomerulonephritis. Encouraging preliminary results have been obtained in experimental animal models, but the transition from animals to humans requires considerable caution to win the game without organ damage or loss of life.

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Reprint requests to Prof. F.P. Schena, Institute of Nephrology, University of Bari, Polyclinic, Piazza Giulio Cesare, 11, 70124 Bari, Italy.
E-mail: nefrologia@cimedoc.uniba.it

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