Introduction

The involvement of Th1/Th2 cell system in the blood and macrophages leads to the production of circulating cytokines, thus inducing resident renal cells to produce other mediators. On the other hand, the deposition of circulating or in situ formed immune complexes, within the glomerular area, induces the recruitment of circulating mononuclear cells producing cytokines which act in a paracrine manner on resident cells. Structural and cellular adaptations based on molecular pathways delineate the differences between renal repair and renal destruction represented by glomerular sclerosis and tubulointerstitial fibrosis. These are two processes which exhibit common features shared by the cytokine network.

This brief overview updates the phenomenology of the activation of resident renal cells by cytokines and analyses the corresponding clinical implications in various types of human glomerulonephritides.

Th1/Th2 cell system and relationship with resident renal cells

T helper-1 and T helper-2 cell subsets depend on the cytokines they produce. Th1 cells produce interferon-γ (IFN-γ) and tumour necrosis factor-β (TNF-β), while Th2 cells produce interleukin (IL)-4, IL-5, IL-10, and IL-13. The former activate macrophages and are involved in delayed-type hypersensitivity reactions. The latter are responsible for strong antibody responses and inhibit several macrophage functions [1]. Th1-type lymphokines are involved in the genesis of organ-specific autoimmune diseases and human glomerulonephritides [2].

Cytokines generated by T helper cells activate B cells and monocytes. T-dependent B cell activation is initiated by the binding of surface Ig receptor to a specific antigen, which is then endocytosed and subsequently processed into peptides that are presented by the major histocompatibility complex (MHC) to CD4+ T cells. In addition, the T helper cell interacts with the B cell through soluble cytokines and membrane-bound ligands and adhesion molecules. Direct T–B cell interactions are required for B cells to become responsive to cytokine stimulation. The binding of CD40, expressed on the B cell, to the CD40 ligands, present on an activated T cell, plays a prominent role in such an interaction.

The wide spectrum of B cell-derived cytokines includes: (i) proinflammatory molecules (IL-1, IL-6, IL-8, TNF-α); (ii) haematopoietic growth factors [granulocyte colony-stimulating factor (G-CSF), granulocyte–macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF), IL-7]; and (iii) immunosuppressive mediators [transforming growth factor-β (TGF-β), IL-10] [3]. B cell-derived cytokines act on bystander cells, as well as the B cells themselves in an autocrine manner, and on numerous cells such as macrophages and resident renal cells in a paracrine fashion.

Monocytes can be considered the most effective cell of the immune system in the pathogenesis of glomerulonephritis (GN). They are important not only in glomerular, but also in tubular and interstitial lesions, since infiltrating and resident monocytes release proinflammatory cytokines, thus influencing the behaviour of glomerular, tubular, and interstitial cells. The initial factors responsible for the accumulation and activation of monocytes are represented by the immune complex deposition, complement activation, and lipid mediators of inflammation. They induce the generation of reactive oxygen species (ROS) which activate transcription factors such as NF-κB, its translocation to the nucleus and binding to the specific DNA sequence of monocyte chemoattractant peptide-1 (MCP-1), RANTES, and intercellular cell adhesion molecule-1 (ICAM-1) genes with enhanced transcription and generation [4].
Glomerular endothelial, epithelial, and mesangial cells, as well as renal tubular cells upon stimulation with inflammatory cytokines and ROS, are potent sources of chemokines. They recruit other monocytes from the blood into the injured area and these are then modulated by the concentration gradients of chemotactic agents into the renal tissue.

Activated monocytes release a broad spectrum of mediators of which IL-1 and TNF appear to be important in continuing the next series of reactions. These cytokines have pleiotropic activity and act both locally and distally. They cause the release of a secondary wave of cytokines which are highly chemotactic for neutrophils (IL-8) and mononuclear cells (MCP-1). The endothelium plays a critical role in communicating between the site of tissue injury and circulating leukocytes. IL-1 and TNF induce a major expression of adhesion molecules which decrease the rate of blood cell flow, initiate transendothelial passage of leukocytes and allow subsequent migration into the renal tissue [5].

In the past, resident cells were considered only a static or passive target for the action of the infiltrating cells. In the last decade, several investigators have demonstrated that mesangial, epithelial, and endothelial cells are active participants in renal inflammation, and tubular cells are active players in the later stages of this process.

Mesangioproliferative GN

The glomerular mesangium plays an important role in the evolution of immune-mediated glomerulonephritides, since mesangial cells under certain conditions produce inflammatory mediators such as IL-1, IL-6, TNF-α, platelet-derived growth factor (PDGF), IL-8, MCP-1, and complement components [6]. Mesangial cell growth, extracellular matrix, and cytokines are intricate factors of the glomerular pathology which is characteristic of several forms of GN, including IgA nephropathy (IgAN) and diabetes mellitus. In the first stage of these diseases, no infiltrating cells are present within the glomerulus and the inflammatory mediators are produced locally by mesangial cells with autocrine activity. Additionally, the recruitment of circulating cells and the involvement of other resident glomerular cells which are capable of producing cytokines stimulate mesangial cells in a paracrine manner.

IgAN is a classic mesangioproliferative GN with initial involvement of mesangial cells whose proliferation and activation is a critical step in the pathogenesis of this disease [7]. Proliferation of mesangial cells and accumulation of extracellular matrix are the first two characteristics histopathological features of IgAN. Extensive immunohistochemical studies have identified a variety of cytokines and growth factors, such as IL-4, IL-6, IL-8, PDGF, and TGF-β, that enhance or suppress mesangial cell proliferation and extracellular matrix accumulation [8].

An increased expression of IL-4 and IL-4R mRNA in mesangial cells from renal biopsies of patients with IgAN has been described recently. However, this cytokine was also detected in other resident cells such as glomerular epithelial cells, cells of Bowman’s capsule and tubular epithelial cells. Finally, an increased staining for IL-4 protein and its receptor was observed in the tubulointerstitium of patients with advanced renal damage. These data suggest the existence of an autocrine and/or paracrine pathway for IL-4 in resident glomerular and tubular cells as well as infiltrating interstitial cells in IgAN [9]. IL-6 is overexpressed in resident mesangial cells and infiltrating monocytes/macrophages in IgAN patients [10,11]. In addition, a striking upregulation of this cytokine and protein expression was found in the tubulointerstitium [12]. We reported an increased urinary IL-6 excretion, directly correlated with its renal tissue expression, in IgAN patients, and we suggested that the urinary presence of this cytokine could be a possible marker of disease activity.

PDGF stimulates mesangial cell proliferation and we demonstrated an increased expression of this growth factor within the glomeruli of patients with IgAN which was associated with an increased expression of PDGF-β receptor [13]. Additionally, PDGF-β receptor mRNA and its protein were overexpressed in the tubulointerstitium of IgAN cases with advanced renal damage; thus, the increased expression of PDGF and its receptor is correlated directly with the degree of mesangial and interstitial cell proliferation and with the extent of glomerulosclerosis and interstitial fibrosis [14].

Mesangiocapillary GN

Endothelial cells are both a target and a source of cytokines. These soluble polypeptide mediators serve to communicate with leukocytes, monocytes, and other resident cells. The spectrum of responses of endothelial cells elicited by cytokines is wide and varied, from inflammation and thrombosis to angiogenesis. The production of IL-1 and TNF facilitates thrombus formation by inducing procoagulant activity. In addition, they induce the production of prostanooids, platelet-activating factor, and nitric oxide. IL-6 production by endothelial cells is elicited by IL-1 and TNF. IL-10 is a weak stimulus for expression of chemokines. IL-4 induces urokinase-type plasminogen activator (tPA) in endothelial cells. IFN-γ induces the expression of MHC class II antigens in endothelial cells. IL-12 has a potent anti-angiogenic activity on endothelial cells. Finally, the chemokine repertoire of endothelial cells is represented by IL-8, MCP-1, and RANTES. The above mediators modulate the gene expression of adhesion molecules and other cytokines in endothelial cells [15].

Mesangiocapillary GN is a disease in which endothelial cells are the targets and active participants in renal lesions. The initial injury to the endothelium or the deposition of immune complexes generates the
local production of chemotactic factors by endothelial cells. These mediators attract monocytes and macrophages most probably accumulated in the glomerulus. These mononuclear inflammatory cells, which contribute to mesangial hypercellularity, also extend into the subendothelial space, increasing the thickness of the glomerular capillary wall. This feature gives the wall a double contoured appearance similar to a tram track.

To this figure contributes the reduplication of basement membrane which is constituted by an irregular and electron-dense deposition of material. The mesangium is also expanded in mesangiocapillary GN by the presence of electron-dense deposits, a variable increase in extracellular matrix and by an augmented mesangial cell proliferation [16].

Several stimuli can induce MCP-1 production by mesangial and endothelial cells. Hora et al. demonstrated that IgG aggregates, by Fcγ-R occupancy, induce MCP-1 mRNA expression and the release of its protein in cultured mouse mesangial cells [17]. This chemokine is also produced in vitro by endothelial cells and its production is modulated by other cytokines [18]. We also demonstrated an increased expression of MCP-1 associated with glomerular and tubulointerstitial macrophage infiltration in a secondary GN such as the cryoglobulinaemic nephritis [19].

Taking into consideration these in vitro cellular findings and immunohistochemical studies, it is possible to hypothesize that either endothelial cells or mesangial cells may be involved primarily in the initial event of mesangiocapillary GN. Also, their local production of MCP-1 and other chemoattractants enables the involvement of circulating monocyte–macrophages which accumulate in the glomerulus and migrate to the subendothelial area.

Extraglomerular crescent formation is the initial and pivotal event that occurs in crescentic GN. Several investigators found that this lesion is the expression of a local activity performed by proinflammatory cytokines, such as IL-1 and TNF-α [23,24]. They are responsible for upregulation of leukocyte adhesion molecule expression, induction of other cytokines (IL-1, IL-2, IL-6, IL-8, TNF-α, and MCP-1) and expression of the inducible form of nitric oxide synthase. These latter mediators participate in the fibrous organization of cellular crescents by the recruitment of periglomerular fibroblasts into Bowman’s space through areas of disruption of Bowman’s capsule [25].

The chronological process is characterized by the initial deposition of fibrin and the arrival of infiltrating glomerular macrophages within Bowman’s space. These humoral and cellular reactants participate in the formation of cellular crescents. In fact, the number of proliferating macrophages within Bowman’s space correlates with the total number of macrophages resident in the space and not within the glomerular tuft. T cells are prominent in active crescentic GN, and their presence suggests the participation of a delayed-type hypersensitivity mechanism in crescentic GN [2,26]. In this process, IL-1 and TNF-α promote macrophage infiltration within the glomerular tuft by upregulating ICAM-1 expression, macrophage accumulation into Bowman’s space and glomerular cell proliferation.

The disruption of Bowman’s capsule facilitates the entry of periglomerular fibroblasts, other T cells and macrophages recruited by MCP-1 and other monocyte chemoattractant mediators which are produced locally by parietal epithelial cells during crescent formation [27,28]. The proliferation of fibroblasts and deposition of large amounts of collagen and other matrix molecules such as fibronectin are driven by local production of growth factors such as acid and basic fibroblast growth factors (FGF-1 and FGF-2) [29]. In addition, TGF-β is another growth factor which plays an important role in the deposition of collagen within the fibrocellular crescents. In fact, urinary TGF-β was associated with glomerular scars in crescentic antiglomerular basement membrane GN induced in the rabbit [30]. The gradual disappearance of macrophages, T cells, and fibroblasts from the fibrocellular crescent during the process of fibrosis is mediated by apoptosis [31].

The progression of renal damage as a final pathway

Tubular epithelial cells, which derive from nephrogenic mesenchyma differentiation, participate actively in the glomerular and tubulointerstitial damage and play a pivotal role in the progression of renal damage. Two different modalities could involve the tubular cells in glomerular diseases. The cytokines produced by inflammatory cells present within the glomerulus could diffuse from the hilar area of the glomerulus into the tubulointerstitium, thus inducing the upregulation of chemokine expression in tubular cells which would in turn be responsible for the propagation of the infiltrate [32]. The second possibility is represented by the injurious effect caused by daily proteinuria to glomerular, epithelial and proximal tubular cells [33]. In this case, massive protein reabsorption of proximal tubular cells could induce lysosomal activation and, con-
sequently, antigen presentation followed by cytokine-induced activation of T helper cells [34]. Alternatively, protein reabsorption could activate the transcription factor NF-κB and the consequent overexpression of a variety of proinflammatory cytokines which induce the recruitment of other inflammatory cells [35].

An increasing number of hormonal mediators, so-called fibrogenic cytokines, are produced by tubular cells, and they play an important role in the progression of renal damage. The mediators include angiotensin II, endothelin, nitric oxide, TGF-β, PDGF, FGF, and osteopontin [36].

TGF-β1 remains the most important fibrogenic cytokine since it increases matrix synthesis, inhibits matrix degradation and is a potent chemoattractant for fibroblasts and monocytes. Interstitial cells and tubular cells appear to be the principal source of TGF-β1 production [37].

PDGF-β is thought to have a fibrogenic effect and, like TGF-β1, can transform interstitial fibroblasts into myofibroblasts, identified as interstitial cells expressing α-smooth muscle actin. In addition to the traditional interstitial fibroblasts, they have a double origin: (i) tubular cells migrate into the renal interstitium, and through a process of tubular cell transdifferentiation may be converted into fibroblasts at the site of injury [38,39]; and (ii) some fibroblasts are perivascular cells that have migrated into the interstitium [40]. IL-1 is increased in several types of human GN.

Angiotensin II is also produced by tubular cells; it stimulates TGF-β1 production in renal tubular cells [41] and upregulates TGF-β1 expression in fibroblasts [42]. Finally, endothelin-1 is another fibrogenic mediator which is expressed by proximal and distal tubular cells, in addition to a wide variety of other resident cells [43,44]. It is able to stimulate the proliferation of human renal fibroblasts [45] and to increase collagen synthesis [46].

### Summary

This review has highlighted the cytokine network which is involved in renal damage from an initial, even transient, stage to extensive glomerular and tubulointerstitial sclerosis. Studies of a variety of different proliferative glomerulonephritides have documented the prominent role of macrophages in infiltrating mesangium, subendothelial area and crescentic formation. Thus, they stimulate crescent glomerular cells to produce other cytokines and growth factors. The identification of other mediators, released by the monocytes in the interstitium, exemplifies the important role of these cells in progressive interstitial scarring through the release of fibrogenic cytokines. In addition, renal tubular cells have been found to produce a vast array of cytokines and growth factors which participate in the generation of renal interstitial scarring.

### References

6. Sato T, van Dixhoorn MG, Heemskerk E, van Es LA, Daha MR. C1q, a subunit of the first component of complement, is expressed by proximal and distal tubular cells [43,44]. It is able to stimulate the proliferation of human renal fibroblasts [45] and to increase collagen synthesis [46].