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Proinflammatory Cytokines in Glomerulonephritis

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Summary. **The contribution of cytokines is being currently unravelled in many inflammatory situations. In particular, IL-1 and TNF have ceen shown to influence many aspects of the inflammatory response. Their participation in experimental models of glomerulonephritis (GN) has now been established. Macrophages are a major source of these cytokines, and mesangial cells have now been demonstrated to have the capacity to produce IL-1 and TNF. In experimental anti-GBM GN, augmented glomerular cytokine production is dependent of glomerular macrophage infiltration. Macrophages are also the major contributor to cytokine production in spontaneous proliferative murine models of GN. The functional contribution of these cytokines to glomerular injury in GN is as yet not fully defined.**

Tumour necrosis factor (TNF) and interleukin 1 (IL-1) are important immunomodulatory peptides with a wide spectrum of biological activities mediating a number of important immunological and inflammatory events. They act systemically and locally to initiate and amplify inflammation and share a large number of biological actions. A number of different cell types have the potential to synthesise these cytokines, however their production by inflammatory cells (principally macrophages) has been most widely studied. Despite their wide range of activities, the net effect of these cytokines is proinflammatory. Many recent studies have sought to define the involvement of these cytokines in glomerular inflammatory events in glomerulonephritis (GN). Cytokines may be produced in glomeruli by intrinsic glomerular cells or infiltrating mononuclear leukocytes and have the potential to act as mediators of injury in pathological events and in cellular repair and regeneration.

MESANGIAL CELLS AND CYTOKINES

IL-1 may be produced by endothelial cells[1] or glomerular mesangial cells.[2,3] A number of functional activities of IL-1 either on or derived from meseangial cells in culture have been studied. IL-1 stimulates mesangial PGE_2 synthesis[4] extracellular matrix synthesis[5] and neutral proteinase production.[6] Macrophage derived IL-1 stimulates mesangial cell proliferation[7] and mesangial cell production of IL-1 may potentially act as an autocoid[3] although it functions weakly in this regard. Mesangial cell derived IL-1 can also stimulate macrophages[8] and lymphocytes[9] and synergise with other growth factors including β endorphin[10] and metenkephalin[11] TNF can be synthesised by mesangial cells following LPS stimulation[12] and mesangial cells can be stimulated by exogenous TNF to produce cyclic AMP and prostaglandin.[13]

ENDOTHELIAL CELLS AND CYTOKINES

The interaction between glomerular endothelium and cytokines has not been well studied. The effect of cytokines on endothelial cells derived from other vascular structures however, has been extensively studied mainly in cell culture systems. Both TNF and IL-1 exert profound proinflammatory effects on a variety of endothelial functions.[14,15] They alter the balance of endothelial coagulation factor expression towards coagulation. The expresiion of thrombomodulin[16] and plasminogen activator (tPA)[17] is down regulated while the expression of tPA inhibitor[17] and procoagulant activity, tissue factor[18] is enhanced. The effects of these cytokines of von Willebrand factor are less clear with reports of both up[19] and down regulation.[20] Both TNF and IL-1 induce conformational and morphological changes in endothelia[21] with loss of extra cellular associated matrix proteins such as fibronectin.[22] These cytokines also enhance the endothelial capacity to facilitate leukocyte margination. The leukocyte integrin adhesion molecules, ELAM[21] and

ICAM-1[23] are both up regulated by TNF and IL-1. Amplifying this effect is the cytokine induced synthesis of colony stimulating factors by endothelia.[24,25] TNF and IL-1 stimulate endothelial release of PAF[26] and PDGF[27] and PGE_2.[28] Finally endothelial cells themselves can synthesise IL-1[1] and TNF.[29]

CYTOKINES AND GLOMERULONEPHRITIS

A number of studies suggest the involvement of cytokines in GN. The IL-1 gene is expressed in rat glomerular mesangial cells and is augmented in the cortex of rats with immune complex GN.[30] Both TNF and IL-1 gene expression is enhanced in the cortex of kidneys from mice with autoimmune GN. TNF can be measured in supernatants from isolated glomeruli and glomerular macrophages express TNF and IL-1 gene and product.[31] In rabbits and rats with anti-GBM antibody induced GN, IL-1 could be measured in supernatants of isolated glomeruli.[32,33] IL-1 bioactivity can also be detected in supernatants of glomeruli cultured from patients with crescentic GN.[34]

Mesangial cells in culture express low levels of procoagulant that can be augmented by TNF[35] and incubation of macrophages with glomerular basement membrane (GBM) and anti-GBM antibody immune complexes stimulates IL-1 and TNF production.[36] Infusion of TNF into renal arteries of normal rabbits induced glomerular neutrophil accumulation fibrin deposition and electron microscopic evidence of endothelial injury similar to that induced by LPS although proteinuria did not occur.[37] Administration of recombinant TNF to mice developing spontaneous autoimmune GN reduced injury, delayed proteinuria and prolonged survival.[38] The effect of varying doses of TNF on the outcome of GN was observed in MRL-Lpr strain mice. These studies do not necessarily address the local glomerular injurious effect of cytokines. Tomosugi et al.[40] found that pre-treatment of rabbits with anti-GBM with IL-1, TNF or LPS increased proteinuria and glomerular thrombosis. When administered alone, neither cytokine induced glomerular injury although glomerular neutrophil margination and increased glomerular thrombi was observed. The augmentation of injury induced by LPS in experimental anti-GBM GN could be prevented by the use of anti-TNF antibodies.[41] In anti-GBM GN in rats administration of anti-TNF antibody has been reported to reduce injury.[42]

CELL OF ORIGIN OF CYTOKINE PRODUCTION IN EXPERIMENTAL GN

We have examined active and passively induced models of GN in rabbits to attempt to define the cell of origin of glomerular cytokines. Accumulated evidence clearly shows the potential for glomerular intrinsic cells or infiltrating macrophage to account for augmented cytokine production.

Rabbits were injected with a nephritogenic dose of sheep anti rabbit GBM globulin (binding 73.6±10.5

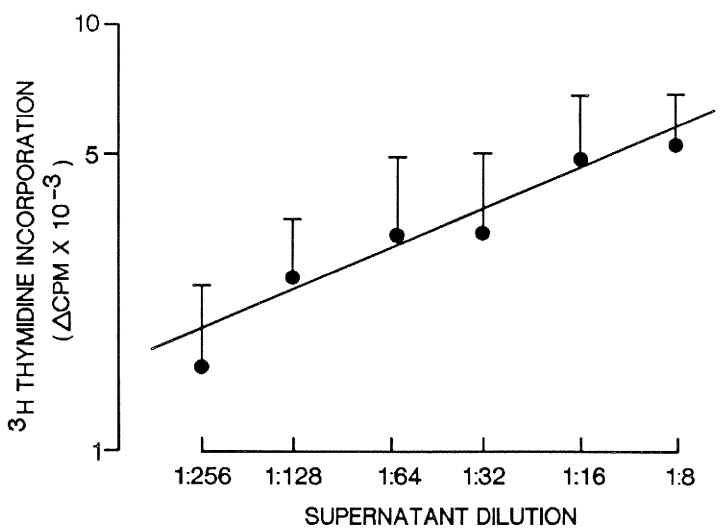

Fig. 1. The elution profile on a Sephacryl S-200 SF gel filtration column of pooled, concentrated supernatant from glomeruli of rabbits on day 8 of anti-GBM GN, showing the protein concentration (open circles) and the TNF bioactivity (closed circles). Arrows indicate the eluting positions of standards of known molecular weight.

μg/g kidney fixing antibody) that induced immediate proteinuria (heterologous phase). This phase of injury is transient and is followed by second autologous phase of injury with a severe proliferative GN and crescent formation. Glomeruli were isolated from groups of animals sacrificed during the development of injury and glomerular supernatants assessed for levels of IL-1 and TNF bioactivity. TNF bioactivity was assessed in a mouse L929 fibroblast cytotoxicity assay and levels expressed relative to a standard curve derived from the cytotoxicity of recombinant TNF. IL-1 was measured by its thymocyte co-stimulating activity. Glomerular cytokines were measureable only in glomerular supernatants from animals with autologous injury.

TNF bioactivity had biological and physico-chemical characteristics of TNF. Gel filtration on demonstrated TNF bioactivity in supernatants eluted with a peak corresponding to molecular weight of 55kd under non-denaturing conditions (Fig. 1). Chromatofocussing produced a single protein peak containing TNF bioactivity at pH 4.75 (Fig. 2). These characteristics are consistent with known properties of TNF.[43,44] Western blotting of glomerular supernatants probed with anti-TNF antibody showed a single band of 17kD identical to human recombinant

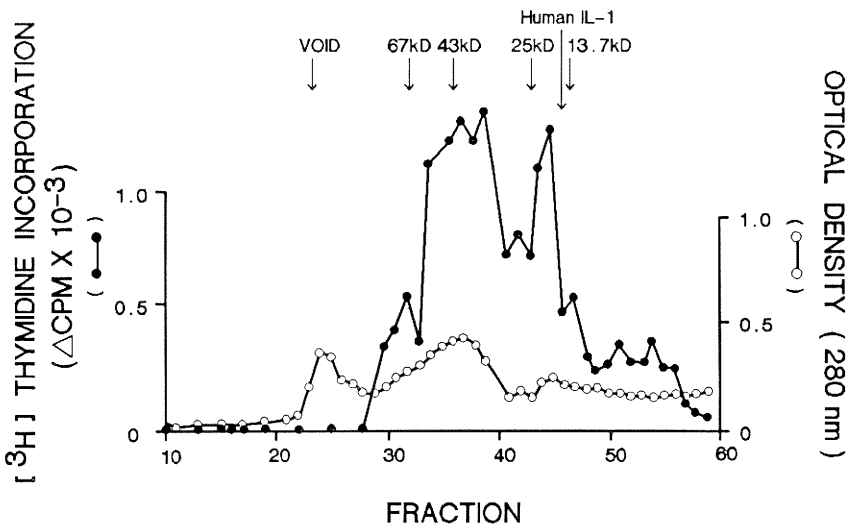

Fig. 2. The elution profile on a Polybuffer PBE 94 chromatofocusing column of the TNF bioactivity (fractions 27 to 32) from the gel filtration column, showing the protein concentration (open circles), pH gradient (closed circles) and TNF bioactivity (hatched bars).

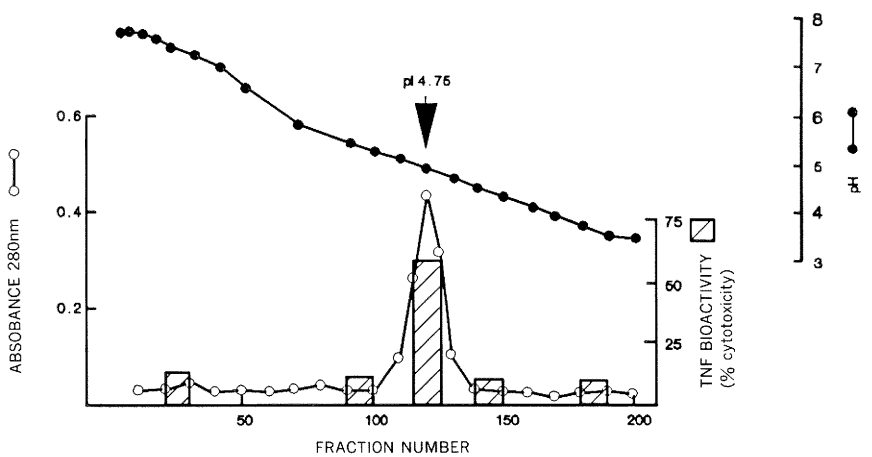

Fig. 3. Thymocyte [^{3}H] thymidine incorporation above PHA control induced by serial dilutions of glomerular supernatant from a rabbit with GN (r=0.98).

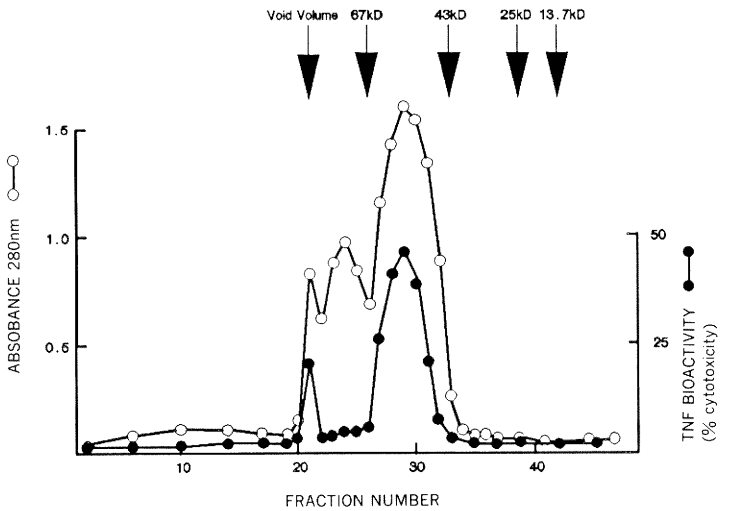

Fig. 4. The molecular weight profile of IL-1 bioactivity (thymocyte co-stimulating activity) in glomerular supernatant from rabbits with GN eluted from a Sephacryl SF-200 column.

TNF run on the same gel. To confirm the presence of TNF in glomerular supernatants, bioactivity was inhibited in a dose dependent fashion by an antibody raised against recombinant human TNF.

IL-1 bioactivity in glomerular supernatants produced titratable increases in thymocyte ^{3}H thymidine uptake (Fig. 3) in a standard bioassay shown to be insensitive to TNF, endotoxin and PDGF. This bioactivity could be substantially inhibited by a polyclonal anti-human IL-1 antibody. Gel filtration of supernatants showed bioactivity with a peak of 16.5 kD and an elution profile similar to that observed for IL-1 from macrophages[45] endothelial cells[1] and lung tissue[46] (Fig. 4).

CYTOKINE PRODUCTION BY NORMAL GLOMERULI

To assess the capacity of intrinsic cells to produce measurable cytokine activity, glomeruli from normal rabbits were incubated with endotoxin (LPS from E. coli serotype 0127138, Sigma 1 μg/ml) IL-1 and TNF production were both significantly enhanced. These studies confirmed the potential for intrinsisc glomerular cells to contribute to augmented cytokine production in GN.

TNF PRODUCTION IN AUTOLOGOUS PHASE ANTI-GBM GN (Fig. 5)

Proteinuria progressively increased (from day 6) during the course of injury in the autologous phase. Glomerular macrophages were maximal in numbers at the onset of autologous injury and then declined. Glomerular TNF production was significantly increased compared with controls on day 6, peaked on day 8 then progressively declined. Similar results were observed from IL-1 production by isolated glomeruli.

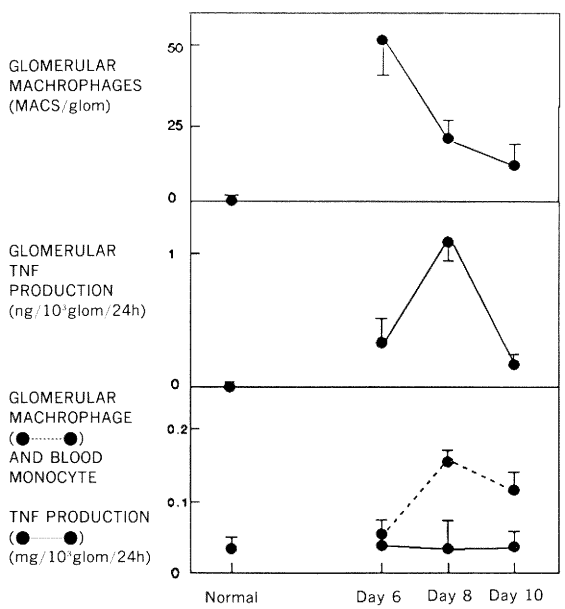

Fig. 5. Glomerular macrophage numbers, glomerular TNF production and proteinuria on days 1, 6, 8 and 10 of anti-GBM GN.

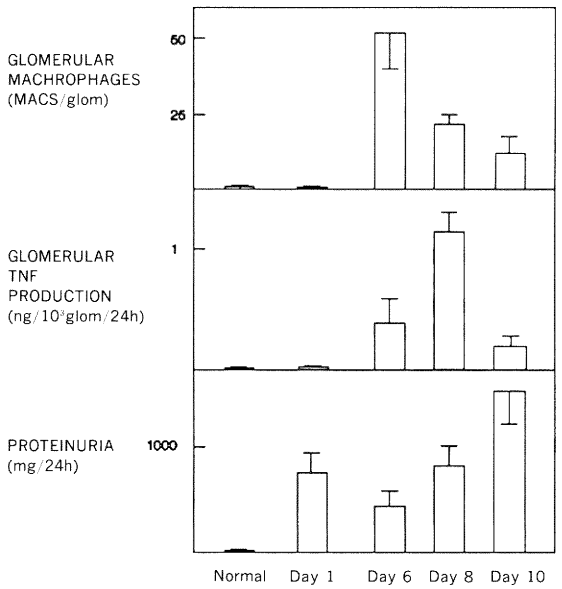

Fig. 6. Glomerular macrophage numbers, glomerular TNF production, glomerular macrophage TNF production and blood monocyte TNF production on days 6, 8 and 10 of anti-GBM GN.

TNF PRODUCTION BY GLOMERULAR MACROPHAGES (Fig. 6)

Glomerular macrophage production of TNF (as measured in supernatants) was only marginally elevated (compared with control peripheral blood monocyte) at the onset of autologous phase. However by day 8, marked augmentation of TNF production by glomerular macrophages was observed. Glomerular macrophage TNF production fell by day 10. The profile of macrophage TNF production rate was similar to glomerular TNF production. The maximal total TNF production by nephritic glomeruli could be accounted for by the glomerular macrophage TNF production.

HETEROLOGOZS INJURY AND CYTOKINE PRODUCTION

In autologous phase GN showed a correlation between glomerular macrophage ingress and total cytokine activity suggesting that macrophages may account for total glomerular cytokine production. Heterologous phase injury was studied to assess the capacity for immune reactants (initiating a similar degree of glomerular injury without an associated macrophage influx) to stimulate intrinsic glomerular cell cytokine production (Figs 3 and 5). Levels of TNF and IL-1 bioactivity were not augmented suggesting macrophages were necessary to account for cytokine production in anti-GBM GN.

To further assess the role of infiltrating macrophages a passive model of the autologous phase of anti-GBM antibody induced injury was established. The use of a passive model obviates the potential for monocyte depleting agents to affect production of disease initiating antibodies. Passive administration of homologous anti-sheep immunoglobulin antibody to rabbits injected with sheep anti-rabbit GBM globulin produces an endocapillary proliferative GN with marked monocyte influx. Glomeruli from these rabbits produced significantly elevated levels of cytokines as did macrophages isolated from nephritic glomeruli.

Bone marrow ablation with nitrogen mustard reduced circulating leukocyte counts to less than $1000/mm^3$ and prevented glomerular macrophage accumulation and cytokine production. To ensure these intrinsic glomerular cells could respond to inflammatory stimuli in diseased glomeruli another technique of macrophage depletion (bone marrow irradiation) was undertaken.

Rabbits were given bone marrow irradiation (6.5 Grays per animal) with kidney shielding. Ten days later circulating leukocyte counts were reduced to less than 1000 cells/mm^3.

When antibodies, known to initiate the passive autologous model of GN, were administered neither glomerular macrophage accumulation nor cytokine production were observed. To confirm the capacity of intrinsic cells to produce cytokines, endotoxin stimulation of isolated glomeruli resulted in augmentation of IL-1 and TNF to levels produced by LPS stimulated glomeruli from normal rabbits.

Thus studies show that in anti-GBM antibody induced GN infiltrating macrophage are required for augmented production of the proinflammatory cytokines, IL-1 and TNF. It is possible that both intrinsic cells stimulated by macrophages as well as macrophages themselves contribute to glomerular cytokine production. In other forms of GN intrinsic cell cytokine production may be increased without glomerular macrophage infiltration.

Cytokine production by inflammatory cells is much greater than that so far demonstrated for intrinsic glomerular cells.[30,31] It is not unexpected, that infiltrating macrophages account for most of the measured cytokine activity in models of GN with prominent macrophage infiltration. Protracted production of lower levels of cytokines by intrinsic cells may have biological relevance in other forms of GN where macrophages are not involved.[30]

Definitive studies of the role of proinflammatory cytokines in GN are yet to be performed. It is likely that they will augment many components of glomerular injury including permeability changes, stimulation of cell proliferation direct cytotoxicity and the shift in the balance of expression of coagulant related molecules toward fibrin deposition. In experimental autologous phase anti-GBM GN these components of injury are dependent on macrophage influx.[47,48] Macrophage activation brought about by interaction with immune complexes, immunoglobin Fc, complement components or by sensitised lymphocytes is associated with augmented production of TNF and IL-1. Some or all of these immune initiators are present in most forms of human GN and all proliferative models of experimental GN. Although cultured intrinsic cells *in vitro* can respond to similar activation stimuli and produce cytokines, these studies suggest that they may not contribute significantly to cytokine production in GN. Despite immunoglobulin and complement deposition as observed in heterologous GN or monocyte depleted autologous GN in glomerular cytokine was not increased.

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