Differential expression of macrophage inflammatory protein-2 and monocyte chemoattractant protein-1 in experimental glomerulonephritis

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Differential expression of macrophage inflammatory protein-2 and monocyte chemoattractant protein-1 in experimental glomerulonephritis. We examined the relation between glomerular expression of chemokines from α-subfamily (macrophage inflammatory protein-2, MIP-2) and β-subfamily (monocyte chemoattractant protein-1, MCP-1) and infiltration of neutrophils and monocytes in antibody mediated glomerulonephritis in rats. In the accelerated model of nephrotoxic nephritis (NTN), glomerular expression of MIP-2 and MCP-1 genes correlated with the sequential migration of neutrophil and monocyte influx, respectively. These relationships were investigated further in the heterologous phase of NTN by applying various treatments known to modulate the severity of injury. Pretreatment with bacterial lipopolysaccharide resulted in greater injury, MIP-2 expression increased 25- to 50-fold, and the glomerular neutrophil count increased two- to fourfold. Both MIP-2 mRNA levels and neutrophil infiltration were reduced by additional pretreatment with IL-6, IL-1 receptor antagonist, soluble IL-1 receptor or soluble TNF receptor (Spearman correlation coefficient $r = 0.897$, $P < 0.005$). In the heterologous phase of NTN, different pre-treatments only resulted in trivial changes in MCP-1 expression and monocyte infiltration. In conclusion, glomerular MIP-2 gene expression correlates with neutrophil infiltration both temporally during the evolution of nephritis, and when glomerular injury is modified by treatment. Glomerular MCP-1 gene expression correlates with monocyte influx. The data show chemokines of α- and β-subfamilies co-operative to cause selective and sequential migration of different leukocyte subsets during development of antibody mediated glomerulonephritis.

Acute glomerulonephritis is characterized by a series of inflammatory events that can result in severe tissue injury. Glomeruli are infiltrated by leukocytes, initially with neutrophils and later by monocytes, both of which can cause injury by releasing proteases and reactive oxygen or nitrogen species [reviewed in 1, 2]. The processes responsible for localization are increasingly well understood at the molecular level, principally from *in vitro* studies of binding of leukocytes to human umbilical vein endothelium. Leukocytes first adhere lightly to activated endothelium, largely through interactions with selectins and their counter-receptors

[3]. Next, leukocytes are activated at the endothelial cell surface by a variety of substances, including complement breakdown products, platelet activating factor and chemotactic cytokines (chemokines). They then adhere more firmly to the endothelium before migrating into the interstitial space; these last two processes involve binding of integrins to counter-receptors of the immunoglobulin superfamily [4].

Studies of a variety of experimental models of inflammation, including glomerulonephritis, in rodents have confirmed the relevance of these processes *in vivo*. For example, neutrophil migration can be prevented by passive immunization against ICAM-1 in the heterologous phase of nephrotoxic nephritis in rats [5]. However, none of these studies explain what controls the kinetics of leukocyte influx during the evolution of an inflammatory response, and specifically why neutrophils are prominent in the infiltrate during the early stages, whereas monocytes are found later. *In vitro* evidence suggests that chemokines are prime candidates for regulating such selectivity. Chemokines are potent chemoattractants *in vitro*, and *in vivo* when injected into local tissues [6, 7]. They have been categorised into α- and β-subfamilies by the arrangement of cysteine residues in the primary structure. The α-chemokines are potent chemoattractants for neutrophils, and include interleukin 8 (IL-8) and macrophage inflammatory protein-2 (MIP-2) [8, 9]. Beta-chemokines, such as monocyte chemoattractant protein-1 (MCP-1), have no activity on neutrophils but are potent monocyte chemoattractants [7].

Chemokines from both subfamilies are synthesized in inflamed tissues *in vivo* [10]. Interleukin 8 can be detected in the glomeruli of rabbits with acute immune complex induced glomerulonephritis, and passive immunization against IL-8 in this model decreased neutrophil infiltration and attenuated glomerular injury [11]. Interleukin 8 has not been detected in mice or rats, and MIP-2 appears to be its functional homologue [9]. In rats, MIP-2 mRNA can be detected in rejecting cardiac allografts [12], and in pulmonary inflammation induced either by LPS or mineral dust [13, 14]. In each of these settings, MIP-2 expression is associated with neutrophil infiltration. MCP-1 is expressed in the glomeruli of rats with anti-Thy 1.1 antibodies induced glomerulonephritis [15], nephrotoxic nephritis [16], in isolated rat kidneys [17] perfused *in vitro* with LPS and in the glomeruli of patients with proliferative

glomerulonephritis [16]. It has also been detected in immune complex-induced lung injury in rats [18], and in the joints of patients with rheumatoid arthritis [19].

The relation between the kinetics of chemokine expression to leukocyte influx into glomeruli has rarely been studied *in vivo*, and there are no reports of experiments designed to examine the differential expression of α- and β-chemokines during the evolution of acute glomerulonephritis. Nephrotoxic nephritis (NTN) in rats induced by injection of heterologous antibodies to the GBM is ideal for such studies. The intensity of infiltration can be quantified accurately, and the respective roles of neutrophils and monocytes in injury have been characterized [20, 21]. Two models of NTN are used in our study. In the heterologous phase is transient and results directly from binding of injected antibodies to GBM; neutrophil is the major type of leukocytes infiltrating the glomeruli. Small numbers of monocytes infiltrate glomeruli but do not appear to be required for injury [20]. The accelerated NTN can be induced if rats are pre-immunized against the foreign IgG, and in this case the early neutrophil influx merges into a more sustained monocyte response [21].

The present experiments were designed to examine the role of α- and β-chemokines in neutrophil and monocyte recruitment in NTN using MIP-2 and MCP-1 as candidate molecules. The results show that MIP-2 and MCP-1 are differentially expressed in nephritic glomeruli with kinetics that correlate closely with infiltration of neutrophils and monocytes respectively. The degree of chemokine expression is reflected in the intensity of leukocyte infiltration. These data support the hypothesis that chemokines are powerful determinants of the nature of a leukocyte infiltrate during pathogenesis of glomerulonephritis.

Methods

Glomerulonephritis

Accelerated NTN was induced in male Sprague-Dawley rats (weight 190 to 250 g) which had been immunized by subcutaneous injection of 1 mg normal rabbit IgG (Sigma, St. Louis, MO, USA) in Freund's complete adjuvant (Sigma) seven days previously. Each rat was injected with 1.5 ml of NTS intravenously. Groups of five rats were killed 4, 6, 24, 48 and 96 hours after injection of NTS. Additional groups of five normal rats, and five rats injected with normal rabbit IgG in Freund's complete adjuvant only were used as controls.

The relation between chemokine expression and glomerular leukocyte infiltration in the heterologous phase of NTN was analyzed using biopsies from previous experiments, which were designed to determine whether treatments that inhibit TNF and IL-1 reduce injury. The effects of these treatments on injury has already been reported [22, 23] and here we report the effect on chemokine expression. Heterologous phase of NTN was induced by a single intravenous injection of 10 mg of NTAb in male Sprague Dawley rats (weight 180 to 200 g). From previous experiments this dose was known to cause minimal albuminuria in normal rats and severe nephritis in rats pretreated with an intraperitoneal injection of 0.25 μg of LPS, small doses of recombinant IL-1β or TNF-α one hour previously [24]. Rats were killed four hours after injection of NTAb for quantitation of glomerular neutrophil and monocyte infiltration and extraction of glomerular RNA. Studies were also performed on groups of rats in which the effects of LPS were attenuated by treatments with

IL-6, IL-1ra, sIL-R or sTNFR. In each case, these were administered intravenously 30 minutes before injection of LPS and caused a 79 to 98% reduction in glomerular injury (assessed by proportion of glomeruli affecting by capillary thrombosis).

Reagents

Rabbit antiserum to rat GBM, nephrotoxic serum (NTS), was prepared as described previously. Immunoglobulin G (IgG) was purified from NTS using a Staphylococcus protein-A column, with special care to avoid contamination with endotoxin [25]. The resulting IgG (anti-GBM antibody, nephrotoxic antibody, NTAb) contained less than 50 pg/ml of LPS.

A stock solution of 2.5 mg/ml of LPS (Salmonella typhimurium; Sigma) was prepared and stored at 4°C until used. Human recombinant IL-6 was prepared at the Glaxo Institute of Molecular Biology (Geneva, Switzerland). It was active down to a concentration of 0.1 pM, as determined by the hybridoma growth activity of 7TD1 cells, and had a specific biological activity of 2.8 $\times 10^{10}$ units/mg [26]. Recombinant soluble murine IL-1 receptor (sIL-1R) was produced from BHK-21 cells transfected with PCDM8 vector with the extracellular region of the murine IL-1 receptor type I (muIL-1RtI) [27] and subsequently purified on an immunoaffinity column consisting of anti-murine IL-1 receptor monoclonal antibody M1 (Immunex) coupled to CNBr-activated sepharose, previous studies had shown that doses of 1.25 to 4 mg/kg of sIL-1R were effective in reducing the severity of experimental arthritis in rats and mice [28], and so a dose of 2.5 mg/kg was used in our experiments. Recombinant human IL-1 receptor antagonist (IL-1ra) was synthesized as reported previously [29]. Twenty-five-fold molar excess of IL-1ra was effective in half maximal inhibition of IL-1β-induced PGE2 production by human dermal fibroblast. Recombinant human IL-1ra has been shown to be effective in suppression glomerular injury in the heterologous phase of NTN and accelerated NTN in rat [30, 31]. Soluble TNF receptor (sTNFR) was prepared at Centocore Inc. (PA, USA) by fusion of the extracellular domain of human P55 TNF receptor to the J sequence and constant domains of human IgG1, which has a high affinity and neutralizing capacity towards human TNFα and -β, and murine TNF [32]. The amount of sTNFR used in this study was shown to be effective in neutralizing TNF in systemic circulation completely as measured by WEHI 164 clone 13/2F2 bioassay [23]. The effectiveness of these recombinant IL-1 and TNF inhibitors to inhibit rat IL-1 and rat TNF *in vivo* was further supported by the finding that they are as effective as polyclonal antiserum to rat IL-1β and antiserum that cross react with rat TNF in reducing glomerular injury in the heterologous phase of NTN [33]. IL-6, sIL-1R, IL-1ra and sTNFR are all free from endotoxin.

The cDNA probe for rat MCP-1 [34] and MIP-2 [35] were 665 bp and 1021 bp insert subcloned into pBLUEScript plasmid, respectively. The cDNA probe for human tubulin kα1 [36], which was used as a control, was a 1.6 kb insert subcloned into pSP64 plasmid.

Quantitation of glomerular neutrophil and monocyte infiltration

Rats were anaesthetized with ether, and the kidneys were perfused *in situ* with 50 ml of phosphate buffered saline (PBS) at room temperature, followed immediately by 10 ml of ice cold PBS. Samples of renal tissue were snap frozen in liquid nitrogen for immunohistochemistry, or were fixed in formalin for light
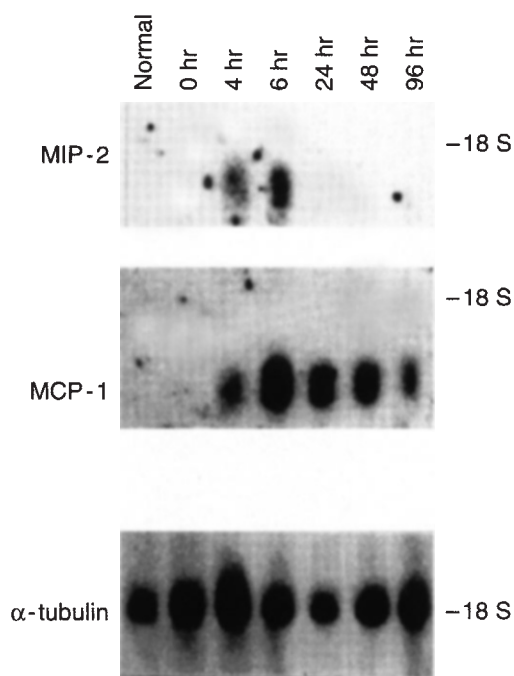
Fig. 1. *Northern blot analysis of glomerular RNA during induction of accelerated nephrotoxic nephritis.* The same blot was sequentially hybridized for MIP-2, MCP-1 and tubulin mRNA. Position of the ribosomal band 18S was shown as size markers. Tubulin mRNA was used as control for any differences of loading or transfer of RNA. In each lane, 26 μg of total RNA extracted from glomeruli pooled from 3 rats were used. Densitometric analysis of this Northern blot is shown as Blot A in Table 1. Another Northern blot of RNA from another 2 rats at the same time points showed similar results.

Table 1. Densitometric analysis of glomerular chemokine mRNA levels in accelerated nephrotoxic nephritis

| mRNA | Northern blot | N | Normal | Hours after induction | | | | |
				0	4	6	24	48	96
MIP-2	A	3	0	0	51%	100%	0	0	0
	B	2	0	0	100%	66%	0	0	0
MCP-1	A	3	0	0	11%	100%	50%	30%	15%
	B	2	0	0	59%	100%	21%	10%	bg

Results were from two sets of Northern blots. Blot A is the Northern blot shown in Figure 1, each glomerular RNA sample was extracted from glomeruli pooled from 3 rats. For Blot B, RNA were pooled from 2 rats at each time point. Densitometric values of MIP-2 and MCP-1 mRNA levels were normalized to tubulin mRNA level. The highest value was assigned as 100%. Abbreviations are: *N*, number of animals at each time point that the RNA derived from; bg, densitometry was not possible at 96 hr sample for MCP-1 mRNA in Blot B, because of a patch of non-specific background radioactivity.

$$\frac{\text{MCP-1 or MIP-2 mRNA}}{\text{tubulin mRNA}}.$$

These results were then expressed as a percentage of the highest value.

Statistical analysis

The data are presented as means together with standard errors (SE). The probabilities that differences between groups were significant were calculated using Wilcoxon rank sum test. Semiquantitive analysis of MIP-2 mRNA and neutrophil infiltration was assessed by Spearman correlation.

Results

Accelerated nephrotoxic nephritis

In the first experiment, groups of three rats were killed at each time point for measurement of steady-state mRNA levels for MIP-2 and MCP-1. Neither chemokine mRNA could be detected by Northern analysis of glomerular RNA from normal or pre-immunized rats, but both were detected in the glomerular RNA from nephritic rats (Fig. 1). However, MIP-2 mRNA was found only at four and six hours after induction of nephritis, whereas MCP-1 mRNA could be detected at all time points between 4 and 96 hours. The highest MCP-1 mRNA level was at six hours (Table 1). These results were confirmed in a second experiment from Northern blots of glomerular RNA from another two rats at each time point (Table 1).

The kinetics of glomerular neutrophil and monocyte infiltration of at each time point is shown in Figure 2 and are consistent with previous reports [21, 39]. There was a large but transient increase in glomerular neutrophil count which rose to maximal values between four and six hours, and had almost completely resolved 24 hours after induction of nephritis. By contrast, although the monocyte numbers had increased by four hours, maximal values were found at 24 hours and were sustained for the rest of the experiment. Thus, the time course of MIP-2 expression coincided exactly with the glomerular neutrophil infiltration whereas MCP-1 expression coincided with that of monocytes.
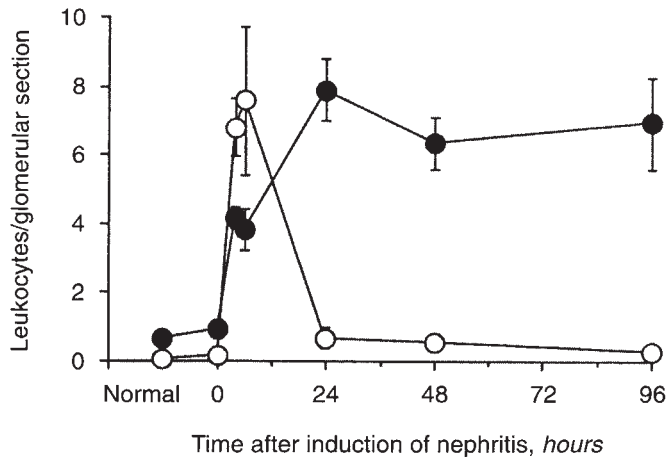
microscopy as previously described [24]. Neutrophil infiltration was assessed in chloroacetate esterase stained sections by counting the number of neutrophils in 50 consecutive glomeruli [24]. Monocytes were identified in frozen sections by indirect immunoperoxidase staining with the ED1 monoclonal antibody (Serotec, Oxford, UK) [37] and the degree of infiltration was again assessed by counting 50 glomeruli.

Glomerular mRNA analysis

The remaining kidney tissue was used to purify glomeruli by differential sieving [38]. RNA was extracted from the glomeruli using 4 M guanidine thiocyanate solution and purified by the caesium chloride gradient ultracentrifugation method [38]. Aliquots of glomerular RNA were analyzed by Northern blotting as described previously [38]. The filters were sequentially hybridized with 32P-CTP cDNA probes for MIP-2, MCP-1 and tubulin. Multiple exposure times were used to ensure optimal conditions for densitometry. Filters were stripped of radioactivity by washing in boiling 0.01% SDS, 0.01 × SSC (1 × SSC = 15 mM trisodium citrate, 150 mM sodium chloride) solution before repeat hybridization with another cDNA probe. Degree of hybridization was assessed by scanning optimally exposed autoradiographs with a Chromoscan 3 densitometer (Joye Loebl, England). Differences in RNA loading were corrected by reference to hybridization of the tubulin probe using the formula:

Fig. 2. *Glomerular infiltration by neutrophils (○) and monocytes (●) during induction of accelerated nephrotoxic nephritis in rats. Results were expressed as mean ± SEM for 4 rats at each time point.*

Fig. 3. *Northern blot analysis of MIP-2 and MCP-1 mRNA during induction of the heterologous phase of nephrotoxic nephritis. Each sample of glomerular RNA (25 μg) was extracted from glomeruli pooled from 4 rats. Samples included glomerular RNA from rats that received injections of NTAb, LPS+NTAb and IL-6+LPS+NTAb, which were killed four hours following induction of nephritis. RNA from LPS-stimulated bone marrow-derived macrophages were used as positive control for hybridization. Densitometric analysis of NTAb, LPS+NTAb and IL-6+LPS+NTAb groups were shown in experiment A of Table 2. This Northern blot is representative of two Northern blots.*

Heterologous phase of nephrotoxic nephritis

Next we performed experiments to ascertain whether the degree of chemokine expression correlated with the intensity of leukocyte infiltration. This was done in the heterologous phase of NTN because in this model, the intensity of leukocyte infiltration and the severity of injury are increased by pretreatment with small doses of LPS [24], an effect which is attenuated by treatments that inhibit IL-1β or TNF [33]. Four hours after induction of nephritis, MIP-2 gene expression was 25- to 50-fold higher in nephritic rats pretreated with LPS compared to those given NTAb alone. This change was associated with a two- to fourfold increase in glomerular neutrophil count (Fig. 3 and Table 2), and with significantly more glomerular injury [22, 23]. Inhibition of IL-1 with 500 μg IL-1ra or 500 μg sIL-1R, and inhibition of TNF with 400 μg sTNFR reduced the degree of glomerular neutrophil infiltration. Pretreatment with IL-6 also reduced glomerular IL-1β and TNFα gene expression and circulating TNFα concentration; the effects of these treatments on glomerular injury have been described in detail elsewhere [22, 23]. All the treatments also reduced MIP-2 gene expression substantially, and when results from all heterologous phase experiments were considered together, there was a strong correlation between MIP-2 expression and glomerular neutrophil numbers (Fig. 4) (Spearman correlation coefficient r = 0.897, P < 0.005). However, there was a small but definite increase in the glomerular neutrophil count when MIP-2 mRNA was barely detectable. This clearly suggest factors other than MIP-2 contribute to neutrophil recruitment. The change in MIP-2 expression following LPS pretreatment (25- to 50-fold higher in LPS pretreated group) contrasts with relatively little change in MCP-1 mRNA level (1.4- to 3.6-fold higher in LPS pretreated group). Furthermore, LPS pretreatment had no effect on the glomerular monocyte counts (Fig. 3 and Table 2). Inhibitors of IL-1 and TNF only caused small changes (28 to 72% reduction) of MCP-1 gene expression and again had no effect on glomerular monocyte numbers. These results again show that glomerular expression of MIP-2 and MCP-1 genes is dissociated; this is particularly interesting because heterologous phase injury is neutrophil rather than monocyte dependent.
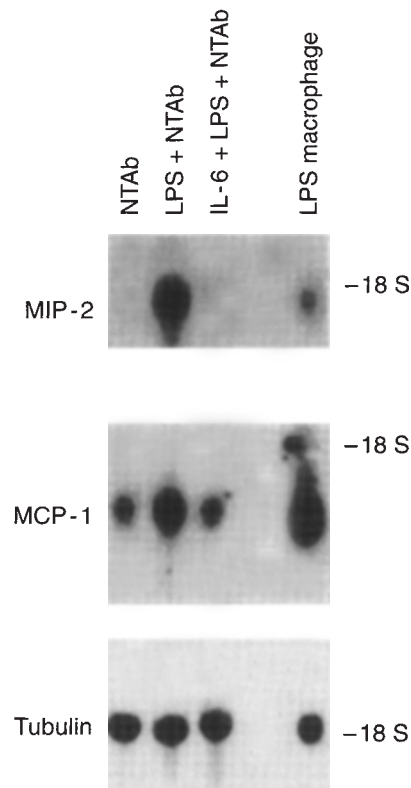
Discussion

A large number of different inflammatory mediators are potent chemoattractants for leukocytes. Most are equally active on neutrophils and monocytes, and are unlikely to be responsible for the difference in kinetics of their recruitment to an inflamed site. The chemokines are the obvious exception to this rule and so the experiments reported here were designed to analyze the potential role of MIP-2 (α-chemokine) and MCP-1 (β-chemokine) in selective recruitment of neutrophils and monocytes into inflamed glomeruli. It would have been ideal to use immunohistology and specific immunoassays to assess cytokine expression, but unfortunately suitable reagents are not available. We therefore chose Northern blot analysis of glomerular mRNA as a surrogate measure, first because it provides a robust assay not subject to the uncertainties of a reverse transcription-polymerase chain reaction based approach. Secondly, it avoids the potential *in vitro* artifacts inherent in cell culture methods that are used to assess glomerular cytokine synthesis.

The kinetics of glomerular MIP-2 and MCP-1 gene expression in the accelerated model of NTN correlated with the individual time courses of glomerular neutrophil and monocyte infiltration respectively. Thus, the data are consistent with the idea that

Table 2. Glomerular chemokine mRNA levels and leukocyte infiltration when the severity of injury was modulated in the heterologous phase of nephrotoxic nephritis

Treatment	Time hours	Number of rats	Neutrophils/glomerular section	Monocytes/glomerular section	MIP-2 mRNA	MCP-1 mRNA
Experiment A						
NTAb	4	4	1.74 ± 0.12[a]	1.06 ± 0.02	2%	29%
LPS + NTAb	4	4	3.54 ± 0.46	1.10 ± 0.06	100%	100%
IL-6 + LPS + NTAb	4	4	1.44 ± 0.40[a]	1.28 ± 0.14	4%	33%
Experiment B						
NTAb	4	4	1.12 ± 0.22[a]	1.20 ± 0.1	2%	28%
LPS + NTAb	4	4	4.40 ± 0.28	1.28 ± 0.18	100%	100%
IL-1ra + LPS + NTAb	4	4	2.42 ± 0.60[a]	1.18 ± 0.04	27%	72%
sIL-1R + LPS + NTAb	4	4	2.72 ± 0.32[a]	1.16 ± 0.08	45%	42%
Experiment C						
NTAb	4	4	1.52 ± 0.16[a]	ND	4%	74%
LPS + NTAb	4	4	4.30 ± 0.38	ND	100%	100%
sTNFR + LPS + NTAb	4	4	2.32 ± 0.22[a]	ND	3%	28%

Twenty-five μg of total RNA from glomeruli pooled from 4 rats of each treatment group were analyzed by Northern blots. Densitometric analysis of MIP-2 and MCP-1 mRNA were normalized to tubulin level. The highest value was assigned as 100%. Results for experiment A were from densitometric analysis of the Northern blot shown in figure 3. Results for experiments B and C were from analysis of another two Northern blots. Neutrophils and monocytes per glomerular section was calculated from the mean of 50 consecutive glomerular sections from each rat. Results are expressed as mean ± standard error for each group of rats. Abbreviations are: IL-6, interleukin-6; IL-1ra, IL-1 receptor antagonist; sIL-1R, soluble murine IL-1 receptor type I; sTNFR, soluble TNF receptor; LPS, bacterial lipopolysaccharide; NTAb, nephrotoxic antibody (anti-GBM antibody); ND, not done.
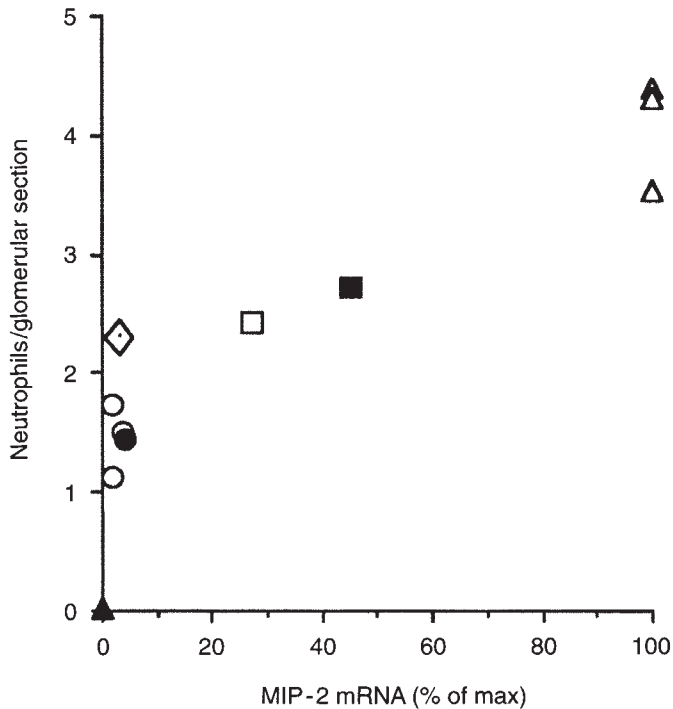[a] $P = 0.05$ Wilcoxin rank sum test (two tailed) of comparison with LPS + NTAb group

Fig. 4. *Glomerular MIP-2 mRNA levels and neutrophil infiltration in the heterologous phase of nephrotoxic nephritis in rats.* These data represent results from 3 experiments shown in Table 2. Symbols are: (▲) normal; (○) NTAb; (△) LPS/NTAb; (●) IL-6/LPS/NTAb; (□) IL-1ra/LPS/NTAb; (■) sIL-1R/LPS/NTAb; (◇) sTNFR/LPS/NTAb. Each point represents mean value for 4 rats in each treatment group (Spearman correlation coefficient $r = 0.897, P < 0.005$).

increased MIP-2 expression contributed substantially to the neutrophil influx, and that generation of MCP-1 was important for monocyte recruitment. An alternative explanation is that the leukocytes themselves were the source of their respective chemokines since they are known to synthesize them [7, 40]. However, this is unlikely because mesangial cells synthesize MIP-2 [40] and MCP-1 [41–43] when stimulated *in vitro* with IL-1 or with TNF-α, interferon-γ or IgG aggregates.

Glomerular MIP-2 expression also correlated with the intensity of neutrophil infiltration and the severity of injury when the heterologous phase of NTN was modified by different treatments. Thus, pretreatment of nephritic rats with LPS caused a 25- to 50-fold increase in MIP-2 expression and a two- to fourfold increase in glomerular neutrophils, whereas there was only 1.4- to 3.6-fold change in MCP-1 expression and no difference in monocyte counts. Monocytes is the minor component of the leukocyte infiltrates in this model. We have previously also examined the effect of pretreatment with IL-6 and LPS on glomerular monocyte number at later time point in the heterologous phase of NTN, but there was no difference between the groups [22]. Overall, there was a strong correlation between MIP-2 expression, neutrophil counts and injury. This is consistent with the idea that MIP-2 expression is an important determinant of heterologous phase injury, in which the severity of glomerular injury directly correlates with glomerular neutrophil infiltration and can be abrogated by depletion of neutrophils [20]. Feng and colleagues have recently confirmed that this by passive immunization against MIP-2 and results in reduction of neutrophil influx by 40% and reduction of fibrin deposit by 54% in this model [35].

These data suggest that chemokines are important in the recruitment of leukocytes *in vivo*, but do not exclude a contribution of other chemoattractants. In fact, there is recent evidence that chemokines synergize with other chemoattractants, for example, MCP-1 synergizes with platelet activating factor to stimulate monocytes [44]. In this way, when different chemoattractants synergize in the recruitment of leukocytes, chemokines may determine the subsets of leukocytes being recruited.

Our data also support the role of the cytokine network in the

regulation of chemokines expression *in vivo*. LPS pretreatment in the heterologous phase of NTN up-regulated glomerular expression of IL-1β and TNFα mRNA, and also TNFα concentration in serum [22]. Pretreatment with inhibitors of IL-1 and TNF inhibit the effect of LPS on the glomerular expression of chemokine genes in this study. At least three factors could be involved in down-regulation of glomerular chemokine mRNA by IL-6: first, it could result from reduced circulating TNFα concentrations [22]; second, it could be caused by reduction in glomerular IL-1β and TNFα; and third, it might be a direct effect of IL-6 on chemokine synthesis. It should be noted that none of the pretreatment completely abrogated expression of MIP-2 mRNA.

Our study was design to compare kinetics of expression of candidate members of α- and β-subfamily chemokines during the evolution of acute glomerulonephritis. It does not catalogue the range of chemokines expressed and does not exclude participation of other chemokines in the pathogenesis of antibody mediated glomerulonephritis. Other chemokines have now been shown to involved in the pathogenesis of NTN. For example, cytokine-induced neutrophil chemoattractant (CINC or KC), which was originally cloned from a rat glomerular epithelial cell line, has recently been shown to contribute to neutrophil infiltration and proteinuria in NTN in rats [45]. Nevertheless, the close temporal relationship between glomerular expression of MIP-2 mRNA, glomerular neutrophil infiltration and injury in NTN supports the notion of a pathogenesis role for MIP-2. Proof of this comes from recent experiments by Feng and colleagues, in which passive immunization against MIP-2 reduced injury in the heterologous phase of NTN [35].

In summary, we have demonstrated that the differential glomerular expression of MIP-2 and MCP-1 mRNA correlates with the selective migration of neutrophils and monocytes in two models of antibody mediated glomerulonephritis.

Acknowledgments

Appendix

Abbreviations used in this paper are: CINC, cytokine-induced neutrophil chemoattractant; GBM, glomerular basement membrane; IL-1ra, IL-1 receptor antagonist; MCP-1, monocyte chemoattractant protein-1; MIP-2, macrophage inflammatory protein-2; muIL-1RtI, murine IL-1 receptor type I; NTAb, nephrotoxic antibody; NTN, nephrotoxic nephritis; NTS, nephrotoxic serum; PBS, phosphate buffered saline; sIL-1R, soluble IL-1 receptor; sTNFR, soluble TNF receptor.

References

1. WILSON CB: The renal response to immunologic injury, in *The Kidney*, edited by BRENNER BM, RECTOR FC, Philadelphia, W.B. Saunders, 1991, p 1062
2. CATTELL V: Macrophages in acute glomerular inflammation. *Kidney Int* 45:945–952, 1994

3. BEVILACQUA MP: Endothelial-leukocyte adhesion molecules. *Annu Rev Immunol* 11:767–804, 1993
4. BRADY HR: Leukocyte adhesion molecules: potential targets for therapeutic intervention in kidney diseases. *Curr Opinion Nephrol Hypertens* 2:171–182, 1993
5. MULLIGAN MS, JOHNSON KJ, TODD RF III, ISSEKUTZ TB, MIYASAKA M, TAMATANI T, SMITH CW, ANDERSON DC, WARD PA: Requirements for leukocyte adhesion molecules in nephrotoxic nephritis. *J Clin Invest* 91:577–587, 1993
6. BAGGIOLINI M, DEWALD B, MOSER B: Interleukin-8 and related chemotactic cytokines-CXC and CC chemokines. *Adv Immunol* 55: 97–179, 1994
7. YOSHIMURA T, LEONARD EJ: Human monocyte chemoattractant protein-1: structure and function, in *Cytokines (vol 4): Interleukin 8 (NAP-1) and Related Chemotactic Cytokines*, edited by BAGGIOLINI M, SORG C, Basel, Karger, 1992, p 131
8. SHERRY B, HORII Y, MANOGUE KR, WIDMER U, CERAMI A: Macrophage inflammatory proteins 1 and 2: An overview, in *Cytokines (vol 4: Interleukin 8 (NAP-1) and Related Chemotactic Cytokines*, edited by BAGGIOLINI M, SORG C, Basel, Karger, 1992, p 117
9. YOSHIMURA T, JOHNSON DG: cDNA cloning and expression of guinea pig neutrophil attractant protein-1 (NAP-1). *J Immunol* 151:6225–6236, 1993
10. OPPENHEIM JJ, ZACHARIAE COC, MUKAIDA N, MATSUSHIMA K: Properties of the novel proinflammatory supergene "intercrine" cytokine family. *Annu Rev Immunol* 9:617–648, 1991
11. WADA T, TOMOSUGI N, NAITO T, YOKOYAMA H, KOBAYASHI K, HARADA A, MUKAIDA N, MATSUSHIMA K: Prevention of proteinuria by the administration of anti-interleukin 8 antibody in experimental acute immune complex-induced glomerulonephritis. *J Exp Med* 180: 1135–1140, 1994
12. WIEDER KJ, HANCOCK WW, SCHMIDBAUER G, CORPIER CL, WIEDER I, KOBZIK L, STROM TB, KUPIEC-WEGLINSKI JW: Rapamycin treatment depresses intragraft expression of KC/MIP-2, granzyme B, and INF-γ in rat recipients of cardiac allografts. *J Immunol* 151:1158–1166, 1993
13. HUANG S, PAULAUSKIS JD, GODLESKI JJ, KOBZIK L: Expression of macrophage inflammatory protein-2 and KC mRNA in pulmonary inflammation. *Am J Pathol* 141:981–988, 1992
14. DRISCOLL KE, HASSENBEIN DG, CARTER J, POYNTER J, ASQUITH TN, GRANT RA, WHITTEN J, PURDON MP, TAKIGIKU R: Macrophage inflammatory protein 1 and 2: Expression by rat alveolar macrophages, fibroblasts, and epithelial cells and in rat lung after mineral dust exposure. *Am J Respir Cell Mol Biol* 8:311–318, 1993
15. STAHL RAK, THAISS F, DISSER M, HELMCHEN U, HORA K, SCHLONDORFF D: Increased expression of monocyte chemottractant protein-1 in anti-thymocyte antibody-induced glomerulonephritis. *Kidney Int* 44:1036–1047, 1993
16. ROVIN BD, RUMANCIK M, TAN L, DICKERSON J: Glomerular expression of monocyte chemoattractant protein-1 in experimental and human glomerulonephritis. *Lab Invest* 71:536–542, 1994
17. XIA Y, FENG L, YOSHIMURA T, WILSON CB: LPS-induced MCP-1, IL-1β, and TNF-α mRNA expression in isolated erythrocyte-perfused rat kidney. *Am J Physiol* 264:F774–F780, 1993
18. JONES ML, MULLIGAN MS, FLORY CM, WARD PA, WARREN JS: Potential role of monocyte chemoattractant protein 1/JE in monoycte/macrophage-dependent IgA immune complex alveolitis in the rat. *J Immunol* 149:2147–2154, 1992
19. AKAHOSHI T, WADA C, ENDO H, HIROTA K, HOSAKA S, TAKAGISHI K, KONDO H, KASHIWAZAKI S, MATSUSHIMA K: Expression of monocyte chemotactic and activating factor in rhematoid arthritis. *Arthritis Rheum* 36:762–771, 1993
20. COCHRANE CG, UNANUE ER, DIXON FJ: A role of polymorphonuclear leukocytes and complement in nephrotoxic nephritis. *J Exp Med* 122:99–116, 1965
21. SCHREINER GF, CONTRAN RS, PARDO V, UNANUE ER: A mononuclear cell component in experimental immunological glomerulonephritis. *J Exp Med* 147:369–383, 1978
22. KARKAR AM, TAM FWK, PROUDFOOT A, MEAGER A, REES AJ: Modulation of antibody-mediated glomerular injury in vivo by interleukin-6. *Kidney Int* 44:967–973, 1993
23. KARKAR AM, TAM FWK, STEINKASSERER A, KURRLE R, LANGNER K, SCALLON BJ, MEAGER A, REES AJ: Modulation of antibody-mediated

glomerular injury in vivo by IL-1ra, soluble IL-1 receptor and soluble TNF receptor. *Kidney Int* 48:1738–1746, 1995

24. TOMOSUGI NI, CASHMAN SJ, HAY H, PUSEY CD, EVANS DJ, SHAW A, REES AJ: Modulation of antibody-mediated glomerular injury in vivo by bacterial lipopolysaccharide, tumor necrosis factor, and IL-1. *J Immunol* 142:3083–3090, 1989

25. EL NAHAS AM, LECHLER R, ZOOB SN, REES AJ: Progression to renal failure after nephrotoxic nephritis in rats studied by renal transplantation. *Clin Sci* 68:15–21, 1985

26. PROUDFOOT AE, BROWN SC, BERNARD AR, BONNEFOY JY, KAWASHIMA EH: Recombinant human IL-6 expressed in E. coli undergoes selective N-terminal degradation: Evidence that the protein consists of a stable core and a nonessential flexible N-terminal. *J Protein Chem* 12:489–497, 1993

27. DOWER SK, WIGNALL JM, SCHOOLEY K, MCMAHAN CJ, JACKSON JL, PRICKETT KS, LUPTON S, COSMAN D, SIMS JE: Retention of ligand binding activity by the extracellular domain of the IL-1 receptor. *J Immunol* 142:4314–4320, 1989

28. SCHORLEMMER HU, KANZY EJ, LANGNER KD, KURRLE R: Immunomodulatory activity of recombinant IL-1 receptor (IL-1-R) on models of experimental rheumatoid arthritis. *Agents Actions* 39:C113–C116, 1993

29. STEINKASSERER A, SOLARI R, MOTT HR, APLIN RT, ROBINSON CC, WILLIS AC, SIM RB: Human interleukin-1 receptor antagonist: High yield expression in E. coli and examination of cyteine residues *FEBS J* 310:63–65, 1992

30. TANG WW, FENG L, VANNICE JL, WILSON CB: Interleukin-1 receptor antagonist ameliorates experimental anti-glomerular basement membrane antibody-associated glomerulonephritis. *J Clin Invest* 93:273–279, 1994

31. LAN HY, NIKOLIC-PATERSON DJ, ZARAMA M, VANNICE JL, ATKINS RC: Suppression of experimental crescentic glomerulonephritis by interleukin-1 receptor antagonist. *Kidney Int* 43:479–485, 1993

32. BUTLER DM, SCALLON B, MEAGER A, KISSONERGHIS M, CORCORAN A, CHERNAJOVSKY Y, FELDMANN M, GHRAYEB J, BRENNAN FM: TNF receptor fusion proteins are effective inhibitors of TNF-mediated cytotoxicity on human KYM-1D4 rhabdomyosarcoma cells. *Cytokine* 6:616–623, 1994

33. KARKAR AM, KOSHINO Y, CASHMAN SJ, DASH AC, BONNEFOY J, MEAGER A, REES AJ: Passive immunization against tumour necrosis factor-alpha (TNF-α) and IL-1 β protects from LPS enhancing glomerular injury in nephrotoxic nephritis in rats. *Clin Exp Immunol* 90:312–318, 1992

34. YOSHIMURA T, TAKEYA M, TAKAHASHI K: Molecular cloning of rat monocyte chemoattractant protein-1 (MCP-1) and its expression in rat spleen cells and tumor cell lines. *Biochem Biophys Res Commun* 174:504–509, 1991

35. FENG L, XIA Y, YOSHIMURA T, WILSON CB: Modulation of neutrophil influx in glomerulonephritis in the rat with anti-macrophage inflammatory protein-2 (MIP-2) antibody. *J Clin Invest* 95:1009–1017, 1995

36. COWAN NJ, DOBNER PR, FUCHS EV, CLEVELAND DW: Expression of human alpha tubulin genes: Interspecies conservation of 3' untranslated regions. *Mol Cel Biol* 3:1738–1745, 1983

37. DIJKSTRA CD, DOPP EA, JOLING P, KRAAL G: The heterogeneity of mononuclear phagocytes in lymphoid organs. Distinct macrophage subpopulation in the rat recognised by mononuclear antibody ED1, ED2, ED3. *Immunology* 54:589–599, 1985

38. TAM FWK, SMITH J, CASHMAN SJ, WANG Y, THOMPSON EM, REES AJ: Glomerular expression of interleukin-1 receptor antagonist and interleukin-1β genes in antibody-mediated glomerulonephritis. *Am J Pathol* 145:126–136, 1994

39. CATTELL V, SMITH J, COOK HT: Prostaglandin E1 suppresses macrophage infiltration and ameliorates injury in an experimental model of macrophage-dependent glomerulonephritis. *Clin Exp Immunol* 79:260–265, 1990

40. TAM FWK, KARKAR AM, LARGEN PJ, YOSHIMURA T, CATTELL V, REES AJ: Glomerular expression of rat macrophage inflammatory protein-2 (MIP-2) in vitro and in vivo. (abstract) *J Am Soc Nephrol* 4:637, 1993

41. ZOJA C, WANG JM, BETTONI S, SIRONI M, RENZI D, CHIAFFRARINO F, ABBOUD HE, DAMME JV, MANTOVANI A, REMUZZI G, RAMBALDI A: Interleukin-1β and tumor necrosis factor-α induce gene expression and production of leukocyte chemotactic factors, colony-stimulating factors, and interleukin-6 in human mesangial cells. *Am J Pathol* 138:991–1003, 1991

42. SATRIANO JA, HORA K, STANLEY ER, MORI T, SCHLONDORFF D: Regulation of monocyte chemoattractant protein-1 and macrophage colony-stimulating factor-1 by INF-γ, tumor necrosis factor-α, IgG1 aggregates, and cAMP in mouse mesangial cells. *J Immunol* 150:1971–1978, 1993

43. LARGEN PJ, TAM FWK, REES AJ, CATTELL V: Rat mesangial cells have a selective role in macrophage recruitment and activation. *Exp Nephrol* 3:34–39, 1995

44. SOZZANI S, RIEPPI M, LOCATI M, ZHOU D, BUSSOLINO F, PROOST P, DAMME JV, MANTOVANI A: Synergism between platelet activating factor and C-C chemokines for arachidonate release in human monocytes. *Biochem Biophys Res Commun* 199:761–766, 1994

45. WU X, WITTWER AJ, CARR LS, CRIPPES BA, DELARCO JE, LEFKNOWITH JB: Cytokine-induced neutrophil chemoattractant mediates neutrophil influx in immune complex glomerulonephritis in rat. *J Clin Invest* 94:337–344, 1994