Polymeric IgA increases the synthesis of macrophage migration inhibitory factor by human mesangial cells in IgA nephropathy


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Abstract

Background. It has been suggested that polymeric IgA (pIgA) or IgA immune complexes play a significant pathogenic role in IgA nephropathy (IgAN). Macrophage migration inhibitory factor (MIF) shares many activities with other pro-inflammatory cytokines. In human glomerulonephritis, including IgAN, glomerular expression of MIF is found to correlate with progressive renal injury. We hypothesized that deposition of pIgA within the kidney may lead to enhanced synthesis of MIF by mesangial cells.

Methods. In this study we examined the effect of pIgA and monomeric IgA (mIgA) from randomly selected patients with IgAN in clinical quiescence on the gene expression and protein synthesis of MIF in cultured human mesangial cells (HMC).

Results. Both pIgA and mIgA from IgAN patients or matched healthy controls increased MIF gene expression and protein synthesis in a dose-dependent fashion. The magnitude of MIF protein induction by pIgA (100 μg/ml) was similar to that of tumour necrosis factor-α (TNF-α) at 10 pg/ml. In all subjects, the induction of MIF was higher for pIgA when compared with mIgA (P < 0.01). Furthermore, the up-regulation of MIF synthesis by either pIgA or mIgA was significantly higher in IgAN patients than in healthy controls (P < 0.05). Similarly, pIgA and mIgA were able to induce TNF-α gene expression and protein synthesis in mesangial cells. Incubation of mesangial cells with neutralizing antibody to TNF-α reduced the MIF synthesis induced by pIgA.

Conclusion. We demonstrate that pIgA is capable of inducing MIF and TNF-α production in HMC, which may play a major pathogenic role in IgAN. Induction of MIF can be partially blocked by neutralizing antibody to TNF-α, suggesting the possibility that up-regulation of MIF synthesis in HMC is mediated via an amplifying proinflammatory loop involving TNF-α.

Keywords: IgA nephropathy; IgA1; macrophage migration inhibitory factor; mesangial cells; polymeric IgA; tumour necrosis factor-α

Introduction

IgA nephropathy (IgAN), characterized by predominant mesangial deposition of polymeric IgA1 subclass, is the most common type of glomerulonephritis worldwide, with a slowly progressive and indolent clinical course. Immunoregulatory abnormalities involving IgA1 synthesis in IgAN have been documented and these include overproduction of IgA1 by B-lymphocytes in vitro, raised serum level of IgA, IgA-containing immune complexes, and IgA of anionic nature. Recent consensus is that the hinge region of the serum IgA1 of IgAN patients showed reduction in galactose (Gal) content. Deficiency of Gal residues in the hinge region may have a profound effect on the recognition of IgA1 by IgA receptors and, hence, its catabolism. Five different IgA receptors have been identified in humans. These include the IgA Fc receptor (FcR1 or CD89), the polymeric-immunoglobulin receptor (pIgR), the asialoglycoprotein receptor (ASGPR), the FcγR and the transferrin receptor (TfR or CD71) [1]. Increased occupation and delayed kinetics of FcR1-mediated endocytosis of endogenous IgA have been documented in patients with IgAN. A recent study has suggested that soluble CD89–IgA complexes may play a central role in the pathogenesis of IgAN [2]. Furthermore, a genome-wide analysis has associated the linkage of IgAN to chromosome 6q22–23 [3]. Nonetheless, the pathogenesis of IgAN is still not completely resolved since none of these abnormalities can adequately explain how IgA1 deposits in the glomerular mesangium leading to progressive renal injury. T cells and...
Macrophage migration inhibitory factor synthesis in IgA nephropathy

Macrophages play an important and pivotal role in mediating the glomerular and interstitial injuries in IgAN [4]. CD4-positive T cells from patients with IgAN demonstrate enhanced gene expression of cytokines that induce the IgA switch and differentiation [4]. The degree of local macrophage proliferation is associated with the severity of renal injury. Recent data suggest serum IgA from patients with IgAN are different from those of healthy subjects, and can exert a pathophysiological effect on target cells [5]. Despite the plausible mechanisms in which glomerular deposits of immune complexes mediate mesangial proliferation and sclerosis, the pathogenetic role of IgA in glomerular injury remains ill defined. There are few studies on the stimulatory effect of IgA on the release of growth factors or cytokines from mesangial cells.

Macrophage migration inhibitory factor (MIF) was originally described as a product of activated T cells that inhibits the migration of macrophages. Now, however, it is evident that MIF is not exclusively produced by T cells but is also expressed by many cell types, including monocytes/macrophages, vascular endothelia and renal tubular epithelial cells [6]. Antibody-blocking studies have shown a key pathogenic role for increased MIF production in experimental endotoxaemia. Glucocorticoids at low levels up-regulate MIF expression via a counter-regulatory mechanism whereby MIF can then act to override the glucocorticoid effects [7]. MIF is positioned strategically in a key pathogenic role upstream of the pro-inflammatory cascade regulating the inflammatory and immune responses [8]. Interleukin 1 (IL-1), tumour necrosis factor-α (TNF-α), and MIF regulate activates nuclear factor-κ B (NF-κB) that affects cell growth, apoptosis, and inflammatory response [9]. Apparently, MIF can increase the NF-κB DNA binding via the antagonistic effect on glucocorticoids in the setting of active inflammation [10]. In addition, MIF induces TNF-α and interferon-γ production in macrophages via an amplifying proinflammatory loop response [6].

In this study we explore whether polymeric IgA (pIgA) isolated from patients with IgAN exerts any stimulatory effect of the synthesis of MIF from mesangial cells.

Subjects and methods

Materials

RPMI 1640 and fetal bovine serum were obtained from Life Technologies (Rockville, MD, USA). Jacalin agarose was obtained from Pierce (Rockford, IL, USA). Neutralizing goat anti-human TNF-α antibody, standard and antibodies for MIF ELISA were from R & D Systems (Minneapolis, MD, USA). Anti-CD89 blocking antibody (clone My43) was from Medarex Inc. (West Lebanon, NH, USA). Asialoorosomucoid was prepared from desialylation of human orosomucoid with neuraminidase (0.03 U/mg protein) by incubation for 8 h at 37°C in 0.1 M sodium acetate buffer pH 5.0. Superose FPLC column was obtained from Amersharm Pharmacia Biotech (Uppsala, Sweden). Consumables for electrophoresis were obtained from Bio-Rad Laboratories (Hercules, CA, USA). All other chemicals were obtained from Sigma (St Louis, MO, USA).

Patients and controls

Sixty-six Chinese patients (35 male and 27 female) with a clinical and renal immunopathological diagnosis of primary IgAN were studied. The patients’ histological diagnoses were made at least 18 months prior to the study and their serum creatinine had remained stable over the previous 12 months; proteinuria ranged from 0.4 to 2.6 g/day, and the subjects were between 18 and 45 years of age (mean ± SD, 27.1 ± 6.5 years). IgAN was diagnosed by the presence of predominant granular IgA deposits, mainly in the glomerular mesangium and occasionally along the peripheral capillary basement membrane by immunofluorescence studies, as well as mesangial electron-dense deposits in ultrastructural examination. Increasing severity of glomerular and tubulo-interstitial pathologies were classified by an independent observer into grade 1, 2 or 3 in ascending order. Systemic lupus erythematosus, Henoch–Schönlein purpura (HSP), and hepatic disease were excluded by detailed clinical history, examination, and negative laboratory testing for hypocomplementaemia, anti-DNA antibody, or hepatitis B virus surface antigen. The subjects’ endogenous creatinine clearance was > 65 ml/min/1.73 m². Seventeen patients (26%) had histories of macroscopic haematuria. Twenty millilitres of blood were collected from each patient at clinical quiescence (a period of no macroscopic haematuria or mucosal infection and urinary erythrocyte count < 10 000/ml in uncentrifuged urine). The serum was isolated and frozen at −20°C until isolation of IgA1 by a jacalin–agarose affinity column. Serum IgA levels were determined by nephelometry.

Thirty-eight healthy subjects (20 males and 18 females), comparable in age and race with no macroscopic haematuria or proteinuria, were used as controls. Patients with active IgAN (macroscopic haematuria), HSP, lupus nephritis, minimal-change nephropathy, membranous nephropathy, and healthy subjects with pharyngitis were also recruited as disease controls. Serum levels of IgA were similarly collected from these individuals.

Culture of human mesangial cell culture

Isolation and characterization of human mesangial cells (HMC) were performed as previously described [11]. Glomeruli were prepared from the cortex of human cadaveric kidney judged unsuitable for transplantation, or from the intact pole of kidneys removed for circumscribed tumours. Histological examination of these kidney samples revealed no renal pathology. Glomerular cells were grown in RPMI 1640 medium supplemented with glutamine (2 mmol/l), N-[2-hydroxyethyl]-piperazine-N’-[2-ethanesulfonic acid] (HEPES) (10 mmol/l), penicillin (50 U/ml), streptomycin (50 μg/ml) and 12% fetal calf serum in an atmosphere of 5% CO2-95% air. Mesangial cells have a stellate appearance and grow in clumps. They show a network of intracellular fibrils of myosin and they contract in the presence of 1 nmol/l of angiotensin II. Mesangial cells from a single
nephrectomy sample at fourth to seventh passage were used in our experiments.

**Purification and characterization of polymeric and monomeric IgA1 by jacalin affinity chromatography and fast protein liquid chromatography (FPLC)**

IgA1 was purified from five randomly selected patients with mild IgAN (grade 1) in clinical quiescence using a jacalin–agarose affinity column and IgA1 was fractionated at room temperature by the FPLC system (Pharmacia, Uppsala, Sweden) as described previously [11]. The subjects’ endogenous creatinine clearances were normal with proteinuria <0.5 g/day. IgA1 was also purified from five age- and sex-matched healthy controls. Monomeric IgA1 (mIgA) (fractions #34 to #50) and polymeric IgA1 (pIgA) (fractions #34 to #50) were separated by FPLC following affinity chromatography with jacalin. The content of IgG in the fraction was measured by an anti-IgG ELISA. The pooled fractions were dialysed and concentrated to 2 ml with Centriprep (Amicon, Beverly, MA, USA) and stored at −70°C until use. The purity of IgA1, fractions was confirmed by SDS-PAGE and ELISA [11]. The endotoxin content in the IgA preparations was determined using a Limulus amebocyte lysate (LAL) assay (BioWhittaker, Walkersville, MD, USA). The endotoxin level for all IgA preparation was <1 pg/mg protein.

**Treatment of HMC with different IgA preparations or TNF-α**

HMC were grown to log phase and harvested using 0.05% trypsin. The cells were allowed to recover overnight in suspension with gentle shaking. The trypsinized-recovered cells were adjusted to 5 × 10⁶/ml and 100 μl of cell suspension were used for the experiments. The cells were exposed to recombinant human TNF-α or IgA preparations at increasing concentrations (0, 0.1, 1, 10, 100, and 1000 pg/ml for TNF-α and 0, 25, 50, 100, or 200 μg/ml for IgA) for 6 h at 37°C. In order to study whether MIF synthesis by mesangial cells was also inducible via an amplifying pro-inflammatory loop involving TNF-α, similar experiments were performed in cells pre-incubated with antibody against TNF-α before stimulating with different IgA preparations or TNF-α. To explore whether the induction of MIF synthesis was through various IgA receptors, similar experiments were performed in cells pre-incubated with antibody against IgA receptor, CD89 (clone My43) or various proteins (IgM, orosomucoid, asialo-orosomucoid, IgG, transferrin) before stimulating with pIgA preparations from IgAN patients. To study whether MIF synthesis by mesangial cells was also inducible via an amplifying pro-inflammatory loop involving TNF-α, similar experiments were performed in cells pre-incubated with neutralizing antibody against TNF-α before stimulating with different IgA preparations. To study whether MIF or TNF-α were specifically induced by pIgA from IgAN patients, similar experiments were performed in cells incubated with 50 μg/ml for pIgA preparation from IgAN, normal controls and different disease groups. The culture supernatant was collected for measurement of MIF and TNF-α by ELISA. Preliminary experiments were conducted showing incubation for 6 h was convenient and was associated with optimal synthesis of MIF or TNF-α.

**RNA extraction, cDNA synthesis and MIF/TNF gene expression in cultured mesangial cells**

Specific primers for MIF, TNF-α and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) were designed from known GenBank sequences (MIF, XM_009875; TNF-α NM_011402; GAPDH, J04038). The sequences of each primer were as follows: (i) MIF, sense primer 5’-CTCTCC-GAGCTCACCACGACG-3’ and anti-sense primer 5’-CGCGTTGATG-CGTAATAGTT-3’; (ii) TNF-α, sense primer 5’-CGGGAACGTGGAGCTGGCCGAGGAG-3’ and anti-sense primer 5’-CACCCGCTGTATCCTCAAGCTC-3’; and (iii) GAPDH, sense primer 5’-TGAAGGTCG-GAGTCACCGATTTG-3’ and anti-sense primer 5’-CATGTGG-GCCAATGAGGTCCACAC-3’. We performed reverse transcription and polymerase chain reaction (PCR) using the following profile: first cycle, 94°C for 3 min, 55°C for 1 min, 72°C for 1 min; 2nd to 30th cycles, 95°C for 45 s, 55°C for 40 s and 72°C for 45 s. The final cycle was 94°C for 1 min and 72°C for 10 min. The PCR products from MIF or TNF-α and control (GAPDH) amplicons were mixed and separated by 1.5% wt/vol agarose gels, stained with ethidium bromide and the gel image was captured and analysed using the Gel Doc 1000 Gel Documentation System and Quantity One software (Bio-Rad, Hercules, CA, USA). We expressed the result of MIF or TNF-α mRNA yield as a ratio of MIF or TNF-α amplicon to GAPDH amplicon. All necessary steps were taken cautiously to ensure the validity of the results.

**ELISA of MIF and TNF-α in culture supernatants**

Immunoreactive MIF in cell culture supernatants were quantitated by a sandwich ELISA. All incubation was carried out at room temperature. Briefly, plates (Immunon 2, Dynatech, Marnes LaCoquette, France) were coated with 100 μl of anti-human MIF monoclonal antibody (3 μg/ml) in 0.1 M carbonate–bicarbonate buffer pH 9.8 overnight. After washing with wash buffer [0.01% BSA, 0.05% Tween 20 in TBS (20 mM Tris, 150 mM NaCl) pH 7.3], the plate was blocked with 3% BSA in TBS and were incubated for 2 h. After further washing, 100 μl of sample or recombinant human MIF standards were added and incubated overnight. After washing, 100 μl of biotinylated polyclonal rabbit anti-human MIF antibody (0.2 μg/ml) was added and the plates were incubated for 2 h. The plates were washed again and 100 μl of 1:3000 streptavidin alkaline phosphatase (Dako, Copenhagen, Denmark) was added and incubated for a further 1 h. After washing, 100 μl of Fast p-nitrophenyl phosphate (Sigma) was added and the reaction was stopped after 30 min with 100 μl of 2 M NaOH. The absorbances were measured at 405 nm. MIF levels in the culture supernatants were determined by extrapolating from the standard curve. The sensitivity of the assay was 150 pg/ml MIF with an intra-batch CV of ± 2.3%.

A sandwich ELISA performed as modified from the manufacturer’s protocol (Alexis Corporation, San Diego, CA, USA) was used to quantitate the level of immunoreactive TNF-α in cell culture supernatants. The sensitivity of the assay was 5 pg/ml and the intra-batch CV was ± 6.8%.

**Statistics**

All data (from patients or cell culture experiments) were expressed as mean ± SD. Inter-group differences for continuous
variables were assessed by the unpaired $t$-test. The MIF/TNF-$\alpha$ mRNA expression or MIF/TNF-$\alpha$ protein synthesis in cultured cells following exposure to different concentrations of IgA preparations or TNF-$\alpha$ were analysed with multivariate ANOVA for repeated measures. All $P$ values quoted are two-tailed and significance is defined as $P<0.05$.

## Results

The serum IgA level in patients with IgAN (2.94 ± 1.44 g/l) was significantly higher than that of healthy controls (1.88 ± 0.89 g/l, $P<0.001$). No IgG or IgM was detected in the mIgA$_1$ fractions and IgG represented 0.1% of total protein in pIgA$_1$ fractions as measured by ELISA. Studies of IgA$_1$ in FPLC fractions by Ig ELISA showed that mIgA$_1$ amounted to 92% of total IgA$_1$ from either healthy controls or patients (data not shown). Similarly, pIgA$_1$ represented 8% of total IgA$_1$ from either group of subjects. The ratio of monomeric to polymeric IgA$_1$ did not differ between patients and controls.

The serum level of MIF in 66 patients with IgAN at clinical quiescence not different from that of healthy controls (277.9 ± 204.2 vs 277.3 ± 139.7 pg/ml). The serum level of MIF in patients with grade 3 glomerular pathology was not different from that of grade 2 or grade 1 (303.6 ± 146.9 vs 290.7 ± 198.7 and 260.8 ± 146.9 pg/ml). Similarly, there was no correlation between the tubulointerstitial changes and the serum MIF concentration. In two patients who subsequently developed synpharyngitic haematuria, 3- and 4-fold increases in serum MIF level were observed when compared with their baseline values in clinical quiescence. There was no significant correlation between the serum level of MIF and urinary protein excretion, serum creatinine or endogenous creatinine clearance. The serum concentrations of MIF in patients with histories of macroscopic haematuria did not differ from those in patients with no macroscopic haematuria (301.7 ± 188.6 vs 264.2 ± 154.7 pg/ml, $P>0.05$).

pIgA$_1$ from patients with IgAN or healthy controls up-regulated significant MIF gene expression in a dose-dependent manner, reaching a 107 and 76% increase, respectively, at a concentration of 100 µg/ml (Figure 1A). The up-regulation of MIF gene expression by pIgA$_1$ was accompanied by a parallel increase of MIF protein release exceeding 110 and 83% of baseline values at a concentration of 100 µg/ml of pIgA$_1$ from patients and controls, respectively (Figure 1B). The magnitude of the up-regulatory effect by pIgA$_1$ was comparable to that of TNF-$\alpha$ at a concentration of 10 pg/ml (Figure 2A and B). At any concentration, the up-regulatory effect of pIgA$_1$ on MIF gene expression and protein synthesis was significantly higher in preparations from patients with IgAN as compared with healthy controls ($P<0.025$). Monomeric IgA$_1$ from patients with IgAN or healthy controls exerted a smaller up-regulatory effect on TNF-$\alpha$ gene synthesis in a dose-dependent manner. At concentrations of 100 and 200 µg/ml, the up-regulatory effect of mIgA$_1$ from patients with IgAN on MIF gene expression and protein synthesis was significantly higher than that from controls ($P<0.05$).

We then investigated the potential effects of a neutralizing anti-TNF-$\alpha$ antibody in down-regulating the MIF inflammatory response induced by pIgA$_1$ in cultured mesangial cells. Anti-TNF-$\alpha$ at a concentration of 0.1 µg/ml effectively down-regulated MIF gene expression and protein synthesis by 40.5 and 39.2%, respectively, in HMC stimulated with pIgA$_1$ from patients with IgAN at a concentration of 100 µg/ml (Figure 4A and B).

The effect of blocking the binding of IgA to IgA receptors on MIF synthesis induced by pIgA$_1$ was tested using specific blocking antibody (anti-CD89; clone My43) or ligands to IgA receptors (IgM, transferrin, asialo-orosomucoid). The results are presented in Table 1. None of these agents blocked the release of MIF by pIgA$_1$.

HMC were incubated with pIgA$_1$ from various disease groups to explore whether these pIgA$_1$ preparations also up-regulated MIF or TNF-$\alpha$ production by HMC. As shown in Figure 5A and B, there was significant increases in MIF and TNF-$\alpha$ production only reaching a 38 and 49% increase, respectively, even at a concentration of 200 µg/ml (Figure 1A).

Similarly, there was a parallel increase of MIF protein release only exceeding 45 and 53% of baseline values at maximal dose of mIgA$_1$ (200 µg/ml) from controls or patients, respectively (Figure 1B). No statistical difference was demonstrated in the up-regulatory effect of mIgA$_1$ on MIF gene expression and protein synthesis between preparations from patients with IgAN and healthy controls. At any concentration, the up-regulatory effect of pIgA$_1$ on MIF gene expression and protein synthesis was significantly higher than mIgA$_1$ irrespectively in patients or healthy controls ($P<0.01$). Polymyxin B at 10 µg/ml did not block the pIgA$_1$-induced MIF production, suggesting that the MIF production by IgA$_1$ preparation was not due to LPS contamination (MIF level in HMC culture supernatant activated with 100 µg/ml pIgA$_1$ in the presence or absence of polymyxin B (1182 ± 62 vs 1151 ± 77 pg/ml)).

The effects of pIgA$_1$ on the synthesis of TNF-$\alpha$ by HMC were also studied. As observed in MIF, pIgA$_1$ from patients with IgAN or healthy controls up-regulated significant TNF-$\alpha$ gene expression in a dose-dependent manner reaching a 139 and 87% increase, respectively, at a concentration of 100 µg/ml (Figure 3A). Similarly, there was a parallel increase of TNF-$\alpha$ protein release in HMC cultured with pIgA$_1$ (Figure 3B). Again, at any concentration, the up-regulatory effect of pIgA$_1$ on TNF-$\alpha$ gene expression and protein synthesis was significantly higher in preparations from patients with IgAN as compared with healthy controls ($P<0.025$). Monomeric IgA$_1$ from patients with IgAN or healthy controls exerted a smaller up-regulatory effect on TNF-$\alpha$ synthesis in a dose-dependent manner. At concentrations of 100 and 200 µg/ml, the up-regulatory effect of mIgA$_1$ from patients with IgAN on MIF gene expression and protein synthesis was significantly higher than that from controls ($P<0.05$).
after culturing HMC with pIgA preparations from patients with quiescent IgAN, active IgAN, HSP or lupus nephritis as compared with normal controls ($P < 0.0001$). There was no difference in the level of MIF or TNF-α when HMC were cultured with pIgA from subjects with minimal-change nephropathy, membranous nephropathy, or pharyngitis as compared with normal controls. The degree of MIF or TNF-α induction was higher with pIgA preparations from patients with quiescent or active IgAN than that of HSP or lupus nephritis.

**Discussion**

The pathological hallmark of IgAN is the predominant mesangial deposition of pIgA₁ subclass. The glomerulopathy usually runs an indolent but slowly progressive course leading to end-stage renal failure in 20–50% of patients over 30 years [12]. The symptoms and prevalence vary between regions because of ethnic differences and differences of biopsy criteria. With more frequent renal biopsy in patients with asymptomatic proteinuria and/or haematuria, the prevalence is higher in Asia and Australasia. Similarly, the percentage of patients in these regions with macroscopic haematuria is lower than that of North America (28–35 vs 58%) [12]. Hence, despite the earlier report of an association of macroscopic haematuria episodes with increased production of pIgA and disease activity, a large proportion of patients run a progressive renal deterioration despite the lack of apparent disease exacerbation detected by episodes of macroscopic haematuria. The exact pathological events leading to renal fibrosis following the mesangial deposition of IgA remain unclear. Few studies have addressed the

![Fig. 1. Up-regulation of (A) MIF mRNA and (B) MIF protein synthesis in cultured HMC incubated with pIgA or mIgA from IgAN patients ($n=15$) or healthy controls ($n=15$) ($P < 0.0001$ by MANOVA). The values in cells incubated with pIgA from patients were higher than those incubated with same concentration of pIgA from controls ($P < 0.05$). The values in cells incubated with pIgA were always higher than those incubated with the same concentration of mIgA isolated from the same subject ($P < 0.01$). The results represent the mean ± SD.](https://academic.oup.com/ndt/article-abstract/18/1/36/1809073)
pathophysiological effect of polymeric IgA from patients with IgAN on target cells such as the mesangial or tubular epithelial cells. Gomez-Guerrero et al. [5] demonstrated that mesangial cells incubated with aggregated IgA elicited a dose-dependent increase in cytosolic calcium followed initially by calcium mobilization from inositol triphosphate-sensitive intracellular stores, while sustained levels were maintained through extracellular calcium flux.

Other in vitro studies revealing binding of IgA to mesangial cells led to increased expression of the nuclear transcriptional factor (NF-κB) [13], c-jun [14], interleukin 6 [15], interleukin 8 [13], monocyte chemoattractant-protein 1 [13] and TNF-α [14]. The observed enhancement of production of chemoattractant interleukin 8 may lead to the accumulation of neutrophils in the kidney of patients with IgAN. Recently, Peruzzi et al. [16] studied the in vitro integrin expression in cultured mesangial cells pre-conditioned with IgA. Mesangial cells conditioned with pIgA or aggregated IgA expressed more αv receptor per cell than those incubated with unconditioned medium, suggesting that IgA may play a role in modulating the cell–matrix interaction in IgAN.

In this study we explored the effect of pIgA on the synthesis of MIF from mesangial cells in IgAN. MIF plays a key pathogenic role in the upstream position for different inflammatory responses. Interleukin 1, TNF-α and MIF regulate NF-κB that affects cell growth, apoptosis and inflammatory response [9]. Apparently MIF can increase the NF-κB DNA binding via the antagonistic effect on glucocorticoids in the setting of active inflammation [10]. Elevated serum levels of MIF and glomerular localization of MIF have been demonstrated in a small group of patients with various types of glomerulonephritides including IgAN, especially during disease exacerbation [17]. In our patients, studied during clinical quiescence, their serum levels of MIF were not different from those of healthy controls. The findings are not surprising for three reasons. First, the serum level of MIF reflects a systemic response to acute inflammatory and immune reactions, and hence the serum levels may be elevated during clinical exacerbation associated with macroscopic haematuria often accompanying mucosal infection. We have observed similar behaviour in other acute inflammatory mediators such as vascular cell adhesion molecule-1 and E selectin whose levels rose.

![Fig. 2. Up-regulation of (A) MIF mRNA and (B) MIF protein synthesis in cultured HMC incubated with TNF-α (P < 0.0001 by MANOVA). The results represent the mean ± SD of five separate experiments.](https://academic.oup.com/ndt/article-abstract/18/1/36/1809073)
during clinical exacerbation in IgAN [18]. Secondly, mesangial cells produce MIF in vitro and in vivo, and the MIF production by mesangial cells correlates with macrophage accumulation within segmental proliferative lesions in rat anti-Thy-1 nephritis. De novo MIF expression was evident in glomerular endothelial cells and mesangial cells in proliferative forms of glomerulonephritis including IgAN, and this correlated with leukocyte infiltration, histological damage and renal function impairment [19]. Thirdly, a large proportion of our patients do not have history of macroscopic haematuria yet develop an indolent but slowly progressive disease [12]. The effect of MIF in these patients is likely to be localized in target organs (i.e. kidneys) rather than systemic. This is supported by the detection of MIF in glomerular and tubular cells in the renal biopsies of our patients (data not shown)—findings similar to a previous report [19].

We examined instead the effect of pIgA and mIgA from patients with asymptomatic IgAN on the gene expression and synthesis of MIF in cultured HMC. Their histological examination revealed mild glomerular pathology with no tubulointerstitial pathology. Our in vitro studies showed that the gene expression and synthesis of MIF was up-regulated by pIgA from IgAN patients or healthy controls in a dose-dependent fashion. There was a smaller magnitude of MIF induction with mIgA. In all circumstances, the induction of MIF was significantly higher for IgA preparations from patients with IgAN when compared with equivalent protein concentration of IgA from healthy controls. Our experiments also showed that pIgA from patients with IgAN up-regulated the gene expression and synthesis of TNF-α in manner similar to that of MIF. More intriguing is the down-regulation of pIgA-induced MIF synthesis by a neutralizing...
anti-TNF-α antibody. These findings support the notion that MIF and TNF-α production in macrophages are induced via an amplifying pro-inflammatory loop response [6]. Blocking one of these cytokines may effectively down-regulate the production of the others in macrophages. Failure to suppress IgA-induced MIF synthesis by blocking IgA receptors using specific antibody (anti-CD89; clone My43) or various ligands to IgA receptors (IgM, transferrin, asialo-orosomucoid) suggested that induction of MIF production by pIgA was through other unidentified IgA receptors or through a receptor-independent mechanism. Our result also showed that pIgA preparation from patients with active IgAN (macroscopic haematuria) had a more profound effect on MIF production by HMC than IgA isolated from IgAN patients during clinical quiescence. Indeed, glomerular MIF expression was previously found to correlate with macrophage and T-cell infiltration, the severity of graft rejection and the loss of renal function [19]. All these findings suggest that MIF is a key mediator in human immunological renal injury. It is likely that MIF and TNF-α induced by pIgA from patients with IgAN sustain a continuous inflammatory process in the kidney despite apparent clinical quiescence, hence leading to a relentless but slowly progressive clinical course. The increased MIF/TNF-α production by HMC during clinical exacerbation is likely to exert further deleterious effects on kidney injury.

Our present findings have interesting bearing on the therapeutic approach of acute exacerbation of IgAN. Mononuclear cell (including macrophages) infiltration

![Graph showing MIF and TNF-α production](Fig. 4. A neutralizing anti-TNF-α antibody at a concentration of 0.1 μg/ml effectively down-regulated (A) MIF mRNA and (B) MIF protein synthesis in cultured HMC incubated with pIgA or mIgA (at a concentration of 100 μg/ml) from IgAN patients or healthy controls. Asterisks signify a reduction following treatment with anti-TNF-α antibody at a *P* value of <0.001. The results represent the mean ± SD of five separate experiments.)

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<th>MIF (pg/ml)</th>
<th>TNF-α (pg/ml)</th>
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<tr>
<td>pIgA 0.1 mg/ml</td>
<td>1182 ± 62</td>
<td>597 ± 25</td>
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<td>Pre-incubation with following proteins before pIgA incubation</td>
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<tr>
<td>Orosomucoid 5 mg/ml</td>
<td>1120 ± 95</td>
<td>598 ± 24</td>
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<tr>
<td>Asialo-orosomucoid 5 mg/ml</td>
<td>1122 ± 123</td>
<td>613 ± 40</td>
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<td>IgM 5 mg/ml</td>
<td>1161 ± 92</td>
<td>601 ± 27</td>
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<td>My43 1 mg/ml</td>
<td>1202 ± 73</td>
<td>602 ± 20</td>
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<tr>
<td>Transferrin 5 mg/ml</td>
<td>1159 ± 118</td>
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The results represent the mean ± SD of five separate experiments.
is detected in acute exacerbation of IgAN with crescent formation. This is frequently associated with acute deterioration of renal function. The activation of macrophage leads to MIF production by infiltrating mononuclear cells and residential glomerular cells, resulting in active inflammatory injury. Reducing the synthesis and release of MIF can alleviate the acute inflammatory injury. This may be achieved by pharmacological blocking of the pro-inflammatory loop involving MIF, TNF-α and interferon-γ as shown in our in vitro experiments. Yang et al. [20] reported reversal of established rat crescentic glomerulonephritis by blockade of MIF. Alternatively, a short-term administration of glucocorticoids is plausible with the unique counter-regulatory system between MIF and glucocorticoids that functions to control inflammatory and immune responses [7]. For those patients with an indolent and progressive disease, the search for new pharmaceutical agents that could provide a long-term modulation of MIF synthesis is required.

In conclusion, pIgA from patients with IgAN is capable of inducing MIF and TNF-α synthesis in HMC, which may play a significant pathogenetic role in the inflammatory injury in IgAN.

Acknowledgements. This study was supported by the Research Grant Committee (Hong Kong SAR) grant number 7329/00M.

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Received for publication: 18.10.01
Accepted in revised form: 29.8.02