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Podocyte expression of MHC class I and II and intercellular adhesion molecule-1 (ICAM-1) in experimental pauci-immune crescentic glomerulonephritis

W. COERS, E. BROUWER*, J. T. W. M. VOS, A. CHAND†, S. HUITEMA, P. HEERINGA*, C. G. M. KALLENBERG* & J. J. WEENING† *Department of Pathology, University of Groningen, *Department of Clinical Immunology, University Hospital Groningen, Groningen, and †Department of Pathology, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands*

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SUMMARY

We examined immunopathological changes of podocytes *in vivo* which, based on *in vitro* studies, are thought to be relevant for the pathogenesis of renal diseases. We investigated the alterations of podocytes in local inflammation in a recently developed model of pauci-immune necrotizing crescentic glomerulonephritis (NCGN) in the rat. Frozen and plastic embedded kidney sections at different time points of the disease were incubated with antibodies directed to MHC class I, MHC class II, ICAM-1 and to relevant cytokines. Strong glomerular expression of MHC class I, II and ICAM-1 was found within 4 days, and plastic embedded sections clearly demonstrated increased cell membrane staining of podocytes. Increased glomerular interferon-gamma (IFN-γ) was detected within 24 h of induction of NCGN, and IL-1β and tumour necrosis factor-alpha (TNF-α) were found from day 4. The potency of these cytokines to induce adhesion molecules on podocytes was investigated on rat glomerular epithelial cells *in vitro*. By using FACS analysis and electron microscopical techniques, we found that the *in vivo* expression of MHC class I, II and ICAM-1 by podocytes could *in vitro* be simulated by IFN-γ. IFN-α weakly induced MHC class I, while IL-1β and TNF-α were ineffective. We hypothesize that podocytes in this *in vivo* model are important to maintain the local inflammatory process in the glomerulus by expression of relevant adhesion molecules and MHC molecules upon stimulation with specific cytokines.

Keywords glomerular visceral epithelial cells MHC intercellular adhesion molecule-1 interferon-alpha interferon-gamma

INTRODUCTION

A model for pauci-immune necrotizing crescentic glomerulonephritis (NCGN) in the Brown Norway rat was recently developed in our department [1]. The disease is induced in myeloperoxidase (MPO)-immunized rats by unilateral kidney perfusion with proteolytic enzymes, in particular MPO with trace amounts of proteinase 3 (PR-3) and elastase, in conjunction with H_2O_2. Within 4 days the animals develop proliferative glomerulonephritis with intra- and extracapillary cell proliferation, ruptured Bowman's capsule, periglomerular granulomatous inflammation and vasculitis. The interstitial infiltrates consist of monocytes, polymorphonuclear leucocytes (PMN) and lymphocytes.

Proinflammatory cytokines, chemotactic agents and adhesion molecules probably play a pivotal role in the formation of cellular infiltrates and the development of NCGN, with involvement of glomerular endothelial and epithelial cells. Expression of ICAM-1 and MHC molecules by glomerular cells are considered to be key factors in local inflammation, by stimulation of adhesion and activation of attracted inflammatory cells [2,3]. The presence of the ICAM-1-inducing cytokines IL-1β and tumour necrosis factor-alpha (TNF-α) has been demonstrated recently in human crescentic glomerulonephritis [4]. ICAM-1 [3] and IL-1 [5] play a crucial role in the development of experimental crescentic glomerulonephritis as well through direct and indirect mediation of inflammatory cell adhesion to the glomerular endothelium.

In vitro, glomerular epithelial cells express MHC class I and MHC class II molecules and process and present antigens upon stimulation with interferon-gamma (IFN-γ) [6], similar to tubular epithelial [7,8] and other non-lymphoid cells [9,10]. IFN-γ can indirectly trigger expression of cytokines by glomerular cells as well [9,11].

Our aim was to study adhesion molecule expression of

Correspondence: Wilko Coers, University of Groningen, Department of Pathology, Oostersingel 63, 9713 EZ Groningen, The Netherlands.

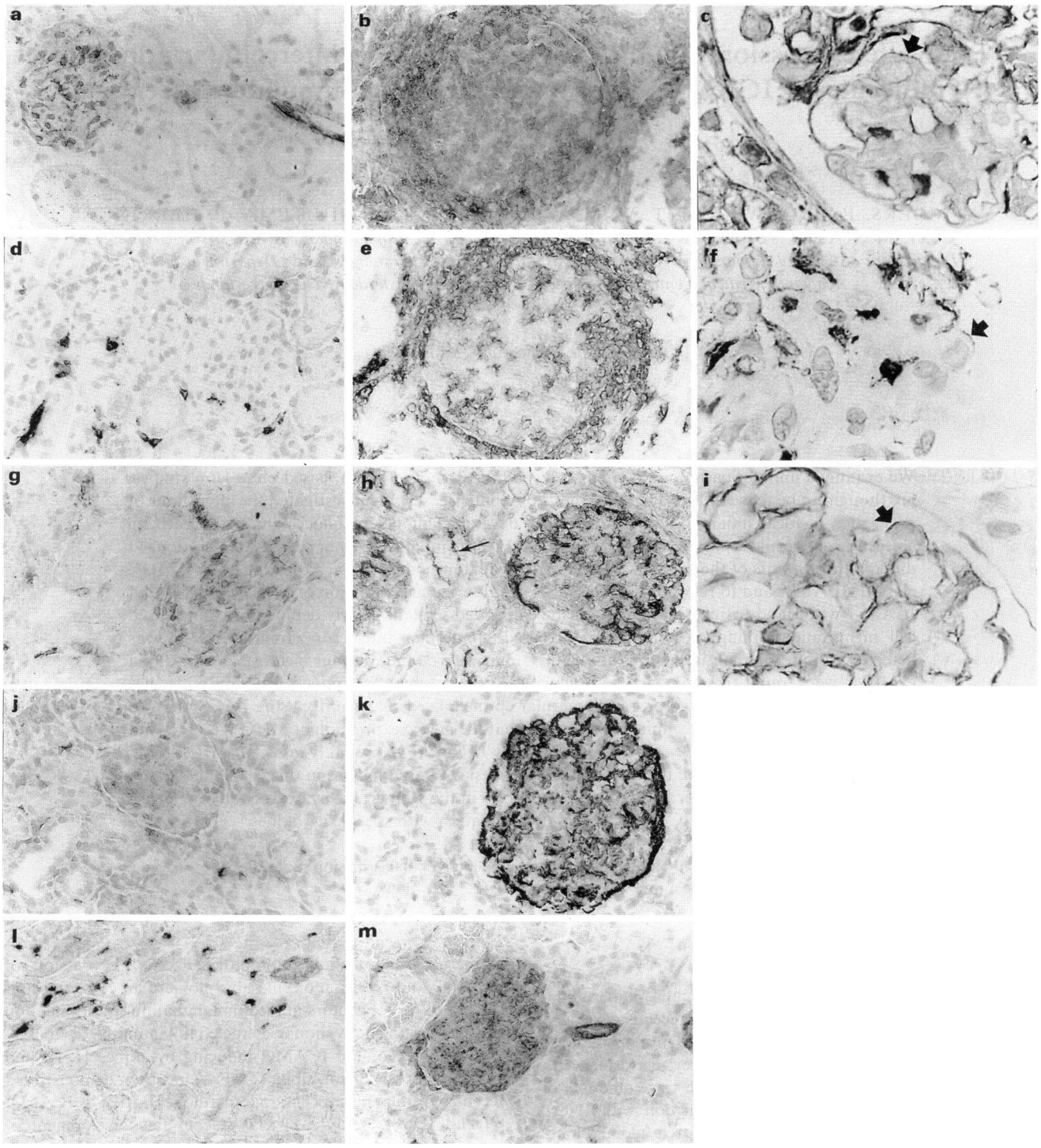

Fig. 1. Glomerular immunoreactivity of frozen kidney sections of control immunized animals (a,d,g,j,l; ×350), necrotizing crescentic glomerulonephritis (NCGN) animals (b,e,h,k,m; ×350), and plastic embedded NCGN animals (c,f,i; ×1400) all at day 4 after perfusion, except l and m, which are taken from animals 24 h after perfusion. Sections were stained with antibodies to MHC class I (a,b,c), MHC class II (d,e,f), ICAM-1 (g,h,i), IL-1β (j,k) and IFN-γ (l,m). Apical podocyte immunoreactivity is marked by an arrow (c,f,i). Apical tubular ICAM-1 immunoreactivity is marked by a thin arrow (h).

podocytes during the development of NCGN. We first investigated which changes in surface expression of molecules that could be involved in the local inflammation in our model of crescentic glomerulonephritis took place in podocytes. In addition, we tried to establish *in vivo* and *in vitro* which cytokines could be responsible for these changes. We therefore studied total glomerular and specific podocyte expression of the molecules MHC class I, MHC class II and ICAM-1 and the local expression of the cytokines IL-1β, TNF-α and IFN-γ by immunohistology. In addition, glomerular epithelial cells were exposed *in vitro* to several cytokines, and studied for the kinetics and patterns of expression of MHC class I, class II and ICAM-1.

MATERIALS AND METHODS

Induction of NCGN in BN rats

NCGN was induced in BN rats as described previously [1]. In brief, rats were immunized with human MPO in Freund's complete adjuvant (FCA) supplemented with H37Ra (Difco Labs, Detroit, MI). Humoral and cellular responses were monitored by immunoprecipitation, ELISA and skin tests. Five weeks after immunization, the kidneys of MPO-immunized rats were perfused unilaterally with a lysosomal extract of human PMN containing in particular MPO and trace amounts of PR-3 and elastase, in combination with H_2O_2 (group I; $n = 19$). Control rats were immunized with the solvent (sodium acetate buffer) without MPO in FCA, and perfused as above 5 weeks after immunization (group II; $n = 11$).

Immunohistology in vivo

Rats were killed at 4 h, 24 h, 4 days and 10 days after perfusion with lysosomal extract and H_2O_2. The kidneys were cleared from blood by perfusion with ice-cold PBS. Samples from both kidneys were snap-frozen in isopenthane at −80°C for preparation of frozen sections, or embedded in a glycol methacrylate plastic for morphological detail (see below). For immunohistochemistry we used MoAbs directed to: MHC class I (OX18; IgG1; MRC Cellular Immunology Unit, William Dunn School of Pathology, University of Oxford, Oxford, UK), MHC class II (OX6; IgG1; MRC), and ICAM-1 (1A29; IgG1; Seikagaku, Tokyo, Japan) [12]. We also used rabbit polyclonals directed to human IL-1β (Genzyme, Kent, England), human TNF-α (Genzyme), rat IFN-α (provided by Dr P. van der Meide; TNO Institute of Applied Radiobiology and Immunology, Rijswijk, The Netherlands) and rat IFN-γ [13].

Frozen sections were cut at 4 μm and fixed with 100% acetone at room temperature for 10 min. The sections were preincubated with 10% normal goat serum, followed by a specific antibody in the optimal dilution. Endogenous peroxidase reactivity was blocked with 0·05% H_2O_2 in PBS. Sections were subsequently incubated with isotype-specific affinity-purified peroxidase-conjugated goat anti-mouse IgG1 antibody (Southern Biotechnology Associates, Birmingham, AL), or with peroxidase-conjugated goat anti-rabbit antibody (Kirkegaard & Perry Labs, Gaithersburg, MD). Sections were counterstained with haematoxylin and scored blindly by two investigators in a semiquantitive way for intra- and periglomerular, tubular, vascular and interstitial reactivity. The intensity was scored as: −, absent; +, weak; + +, moderate; + + +, strong; + + + +, very strong [1]. The total glomerular score as depicted in Fig. 2 was arbitrarily calculated by summation of the intra- and periglomerular score.

Immunohistology on plastic embedded kidneys

Renal tissue was embedded in Technovit 8100 glycolmethacrylate (Kulzer GmbH, Wehrheim, Germany) for optimal morphological detection of immunoreactivity as described [14]. Briefly, 1-mm^3 pieces of renal cortex were fixed in 2% paraformaldehyde in PBS (3 h at 4°C), washed overnight with PBS containing 6% sucrose, dehydrated in 100% acetone (30 min at 4°C) and infiltrated in Technovit 8100 solution A (6–8 h at 4°C). Polymerization was started with solution B and embedding was performed according to the manufacturer's recommendations. Sections (2 μm) were stained with the antibodies and sera mentioned above using a standard plastic incubation protocol with a trypsin pretreatment and antibody incubations at 37°C [14]. Endogenous peroxidase was blocked with H_2O_2 in PBS, and bound antibodies were detected as described above. Peroxidase reactivity was developed using 3,3′-diaminobenzidine. The sections were counterstained with haematoxylin.

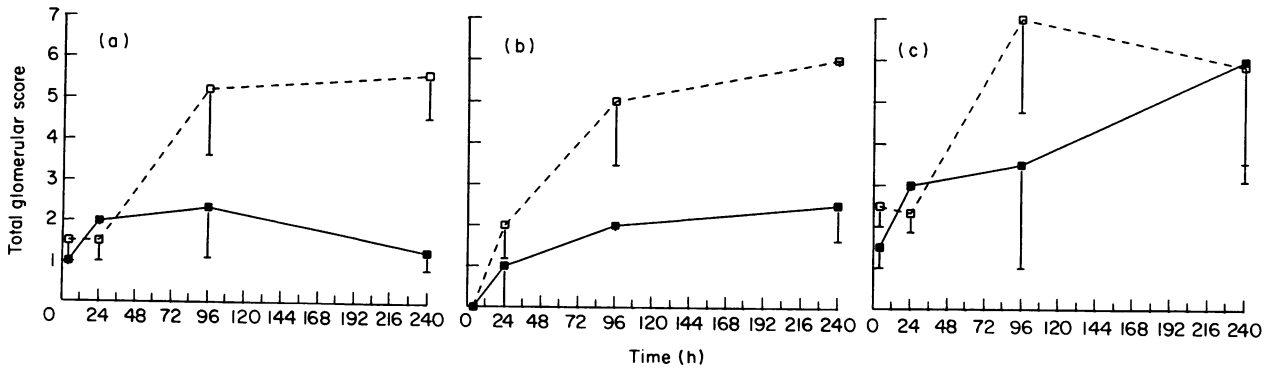

Fig. 2. Semiquantitative score of total glomerular immunoreactivity of necrotizing crescentic glomerulonephritis (NCGN) (group I; □) and control (group II; ■) animals at different time points after perfusion. Scoring was performed on frozen sections stained for MHC class I (a), MHC class II (b) and ICAM-1 (c). Each point represents the mean score of at least four animals ± s.d.

Culture of glomerular visceral epithelial cells

For this study an established line of glomerular visceral epithelial cells (GVEC) was used, derived from explants of Sprague Dawley rat glomeruli, as described elsewhere [15–17]. This cell line was found to express a ganglioside reported to be specifically expressed by visceral and not parietal epithelial cells of the glomerulus, as studied by immuno overlay experiments of isolated gangliosides and by immunoprecipitations with the 27A antibody [18] (Coers *et al.*; submitted for publication). The cell line was cultured and maintained on a collagen gel (Vitrogen 100; Collagen Corp., Palo Alto, CA) in a humidified 5% CO_2–95% air incubator. The culture medium was the generally used glomerular epithelial cell medium containing K1 hormone mix (all ingredients from Sigma, St Louis, MO), 100 U/ml penicillin (GIBCO, Grand Island, NY), 100 μg/ml streptomycin (GIBCO) and 5% NuSerum (Collaborative Research Inc., Bedford, MA), as described [15,19]. Experiments were performed with GVEC cultured on a matrix of rat collagen type I (1·5 μg/cm^2; Sigma)-coated surfaces [20]. All experiments were performed with GVEC at the generally used passage numbers 25–45 [15–17,19]. Explants and early passages from rat glomeruli were similar in their kinetics of IFN-γ-induced MHCI and II expression (not shown), and therefore the cell line was used for further experiments.

GVEC cultures were exposed during different periods of time to the following cytokines: human rIL-1β (Genzyme), rat rTNF-α, rat rIFN-α [21], rat rIFN-β [21] and rat rIFN-γ [13] (all rat cytokines were generously provided by Dr P. van der Meide). Titration experiments revealed that maximal MHC class I and II expression by IFN-γ was found at 100 U/ml in concordance with other reports [6,22]. For reasons of comparison, all subsequent experiments with cytokines were performed using the concentration of 100 U/ml. The culture media were replenished every day, to minimize autocrine effects.

Qualitative expression of MHCI, MHCII and ICAM-1 on podocytes in vitro

Changes in the expression of MHC class I, II and ICAM-1 after cytokine treatments were investigated by immunofluorescence and immunoelectron microscopy.

Immunohistology was tested on GVEC grown on collagen-coated plastic chamberslides (Nunc, Roskilde, Denmark). After washing the cultures they were fixed with 100% acetone (15 min at −20°C) and incubated using the procedure mentioned above, but bound antibodies were now detected with affinity-purified FITC-conjugated goat anti-mouse antibodies (KPL). As a negative control an isotype-matched antibody to desmin (D33; IgG1; Dakopatts A/S, Glostrup, Denmark) was used. The slides were mounted in citifluor (Citifluor Ltd, London, UK) to prevent fading of the fluorochrome and studied with a Leitz diaplan fluorescence microscope (Leica, Rijswijk, The Netherlands).

The cell membrane expression of MHC class I and class II and ICAM-1 on GVEC was investigated using a pre-embedding immunoelectron microscopy technique. For this purpose, cells were cultured on collagen-coated polycarbonate porous supports (custom-made non-tissue culture treated; surface area 1·0 cm^2; pore size 0·4 μm; thickness 10 μm; Costar Europe B.V., Badhoevedorp, The Netherlands) in 12-well plates, thus creating two separate compartments. Cytokines were added to both the basolateral and apical compartment to prevent formation of a concentration gradient or dilution upon potential disturbance of the monolayer. GVEC reached confluency after 2 days and were incubated up to 4 days, with daily replenishment of the medium. At the end of the experiment, the cells were rapidly chilled and incubated at 4°C on both sides for 2 h with the antibodies in culture medium. The cells were washed twice with PBS, 0·01% $CaCl_2$, fixed for 15 min with 2% paraformaldehyde in PBS, and washed again with PBS, all at 4°C. Bound antibodies were detected by incubating for 30 min with isotype-specific affinity-purified peroxidase-conjugated goat anti-mouse IgG1 antibody (SBA) followed by diaminobenzidine for 20 min, at room temperature. The filters were washed, fixed with 2% glutaraldehyde, cut into small strips and embedded in vials with epon using standard procedures. No counterstaining was performed, and the cells were viewed in a Philips EM201 Transmission Electron Microscope.

Quantification of MHCI, MHCII and ICAM-1 on cultured podocytes

Quantitative analysis of cell surface molecules was performed by FACS analysis. At the end of the incubation period, single-cell suspensions were prepared with trypsin/EDTA (GIBCO) and DNAse I (Boehringer, Mannheim, Germany). GVEC were incubated for 60 min at 4°C in the appropriate dilution of antibodies in 100 μl PBS containing 5% fetal calf serum (FCS; GIBCO) and NaN_3 (Sigma). Cells were washed, incubated with FITC-conjugated rabbit anti-mouse antibodies (Dakopatts) for 30 min at 4°C, and measured with a FACScan coupled to LYSIS II software (Becton Dickinson, Erembodegem-Aalst, Belgium).

RESULTS

Immunohistology in vivo

During the development of NCGN in BN rats strong changes in glomerular immunoreactivity were observed (Figs 1 and 2). Increased diffuse intra- and periglomerular staining could be observed after 24 h in three out of five NCGN rats for MHC class I, but not for MHC class II or ICAM-1. At 24 h of NCGN, increased intraglomerular MHC class II strongly positive cells were found, representing the described early monocyte influx in this model [1] (Fig. 2b). At day 4, strong intra- and periglomerular reactivity could be found for all three molecules tested (Fig. 2). MHC class I and MHC class II glomerular reactivities were strong and diffusely distributed (Fig. 1b,e), but the increased ICAM-1 reactivity at day 4 was almost entirely of the glomerular epithelial pattern (Fig. 1h). Severe glomerular destruction and large intra- and periglomerular infiltrates at later stages of the disease did not allow identification of specific cellular reactivity on frozen sections.

Fig. 3. Pre-embedding imunoelectron microscopy of glomerular visceral epithelial cells (GVEC) cultured on porous supports, treated for 4 days with 100 U/ml IFN-γ (b,d,f; ×20 000) *versus* untreated controls (a,c,e; ×20 000). Monolayers were incubated with antibodies to MHC class I (a,b), MHC class II (c,d) or ICAM-1 (e,f). Basolateral immunoreactivity is marked by an arrow, apical immunoreactivity is marked by a double arrow. Note the total absence of MHC class II immunoreactivity (c), and the very weak lateral immunoreactivity of ICAM-1 (e) in the control cells.

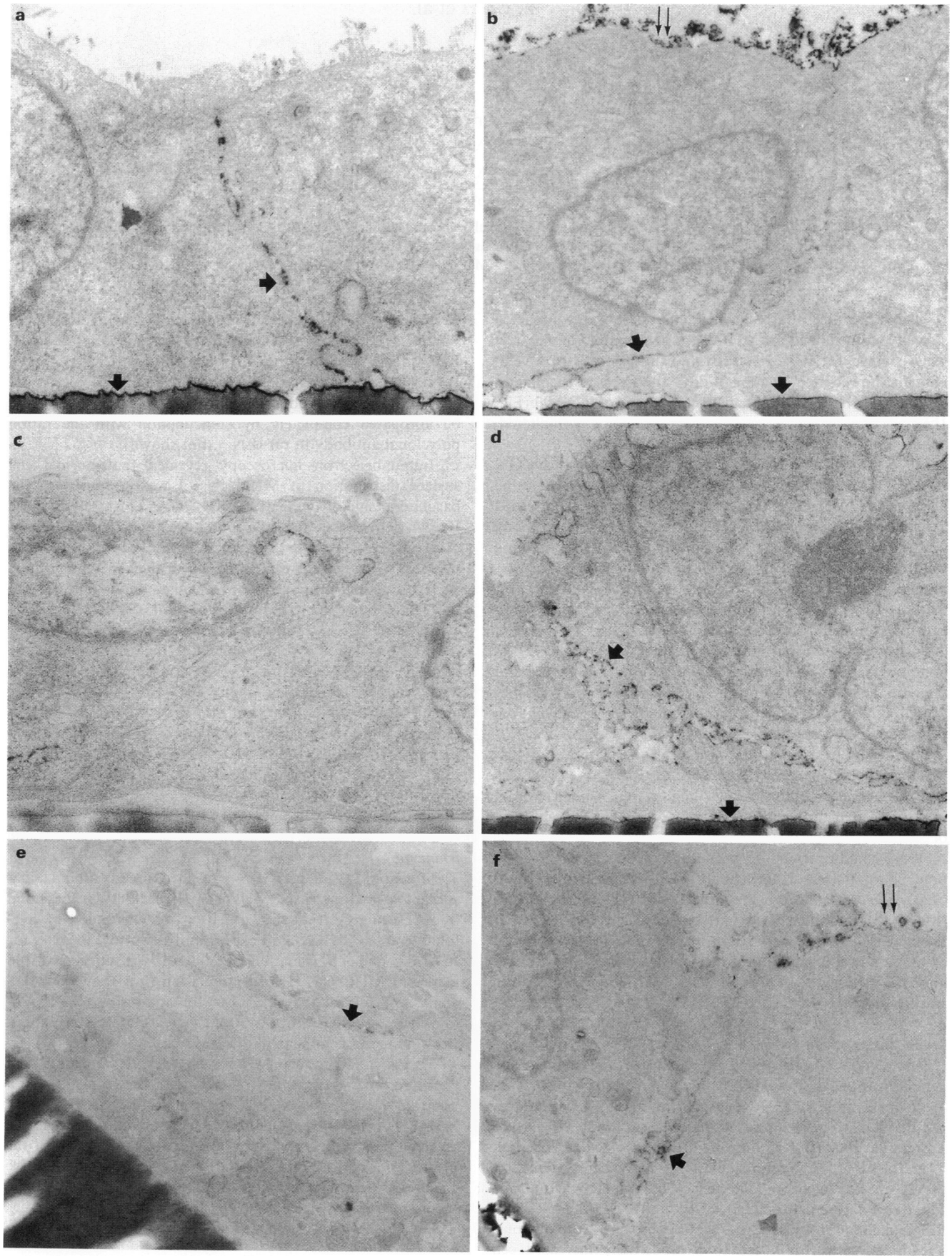

Fig. 3.

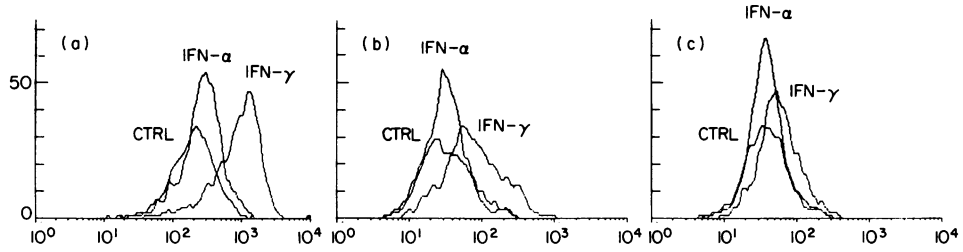

Fig. 4. Representative graphs of FACS experiments with glomerular visceral epithelial cells (GVEC) treated for 4 days with IFN-α, IFN-γ or controls. Cells were stained with antibodies to MHC class I (a), MHC class II (b), or ICAM-1 (c). Note the IFN-γ-induced strong increase in MHC class I and II and mild increase in ICAM-1, and the mild IFN-α-induced increase in MHC class I.

The large interstitial infiltrates present from day 4 reacted strongly when stained for MHC class I and II, and moderately when stained for ICAM-1. Proximal tubular cells of several animals showed at 24 h and at 4 days an increased basolateral staining of MHCI, MHCII and apical staining of ICAM-1 (Fig. 1h) [8]. The vascular immunoreactivity was mostly unaltered, apart from some perivascular infiltrates.

IFN-γ could already be found in the glomeruli of NCGN rats 24 h after perfusion, and stayed detectable throughout the disease (Fig. 1m). The IFN-γ immunoreactivity in diseased animals could be blocked by preincubation of the antibody with the rat cytokine IFN-γ (not shown). Strong IL-1β staining was found in diseased glomeruli from day 4 (Fig. 1k). Glomerular reactivity for TNF-α at day 4 was similar to IL-1β, whereas IFN-α was only found in some interstitial cells. In controls, some interstitial cells showed IL-1β and TNF-α, and glomeruli were staining diffusely positive for TNF-α. IFN-γ immunoreactivity was present in some tubular cells [23] and interstitial leucocytes in controls. Weak anti-IFN-α/β reactivity was found in the controls in mesangial cells and in the perivascular smooth muscle cells.

Immunohistology on plastic embedded sections

Plastic embedded sections allowed high resolution detection of intraglomerular distribution of immunoreactivity. From day 4 of NCGN increased expression of MHCI, MHCII and ICAM-1 could be found on glomerular epithelial cells. At that time point, immunoreactivity for all these molecules could clearly be detected on the apical side of the podocytes (Fig. 1c,f,i). Endothelial cells and mesangial cells contributed to the increased immunoreactivity for these molecules too, as well as the large cellular infiltrates present from day 4. In control animals, podocytes expressed only MHCI and sometimes weakly ICAM-1.

Immunohistology in vitro

Unstimulated (control) GVEC in culture showed moderate staining with MHCI, weak staining of some cells with ICAM-1, and no staining for MHCII. Treatment of GVEC with IFN-γ resulted in strongly increased paranuclear and cell surface expression of MHCI within 24–48 h, and of MHCII within 72 h. The MHCII immunoreactivity was first localized and later more general. Increased cell surface ICAM-1 could only be found in a diffuse pattern after 72–96 h of IFN-γ treatment. At longer incubation times, these changes became more obvious. Treatment with IFN-α led to a moderately increased cell surface, but not paranuclear expression of MHCI after 96 h, which had almost disappeared after 7 days (not shown). IL-1β, TNF-α and IFN-β did not cause changes in expression of MHCI, MHCII or ICAM-1 at any of the time points studied. Exposure to dosages of up to 1000 U/ml IL-1β, TNF-α and IFN-β did not alter the expression of these molecules either (not shown). IFN-γ-induced expression of MHCI and II could be abrogated completely by coincubation with the rabbit polyclonal antibody to rat IFN-γ (not shown).

Immunoelectron microscopy revealed that normal cell surface distribution of MHC class I was restricted to the basolateral side of the GVEC. MHC class II was absent, and ICAM-1 could sometimes be found weakly on the apical and occasionally also on the basolateral side (Fig. 3a,c,e). Treatment for 96 h with IFN-γ resulted in a strongly increased MHC class I and less prominently MHC class II staining on the basolateral but also on the apical side of the GVEC (Fig. 3b,d). This treatment also resulted in a moderate increase of ICAM-1 reactivity, which was found mainly at the apical side of the cell (Fig. 3f). IFN-α treatment resulted in a changed pattern of MHC class I expression; about 20% of the cells showed apical staining with the OX18 antibody, similar to IFN-γ-induced MHC class I expression (not shown). Treatments with IFN-β, TNF-α, and IL-1β resulted in a polarized staining similar to the controls with respect to all the surface molecules tested.

Quantitative changes of cell surface molecules on cultured podocytes

Treatment of GVEC with IFN-γ resulted already after 4 h in a subtle two-fold increased MHC class I reactivity as measured by FACS analysis (not shown). After 24 h we observed a five-fold increased MHC class I expression, a two-fold increase for ICAM-1, and the formation of moderately positive, two-fold increased MHC class II cell population (not shown). FACS analysis of cells treated with IFN-γ for 4 days showed a 10–20-fold increase of MHCI, a five-fold increase of MHCII, and a two-fold increase of ICAM-1 (Fig. 4a,b,c).

IFN-α could induce MHC class I expression after 48 h exposure time. Treatment for 4 days with IFN-α resulted in a two-fold increase of MHCI expression and no effects on MHCII or ICAM-1 (Fig. 4a), which could no longer be detected after 7 days exposure time (not shown). None of the cytokines IL-1β, TNF-α, or IFN-β induced quantitative changes as determined by FACS.

DISCUSSION

The development of NCGN in BN rats was clearly associated with increased MHC class I, MHC class II and ICAM-1

immunoreactivity. In general, increased total glomerular MHC class I expression seemed to precede the increases in MHC class II and ICAM-1 expression, although this was only observed in 60% of the NCGN rats. GVEC themselves showed increased expression of especially ICAM-1 at day 4. In plastic embedded sections, increased MHC class I and II could also be found on GVEC. Specific basolateral differentiation was hard to distinguish *in vivo*, but apical reactivity of MHC class I, MHC class II and ICAM-1 could clearly be identified on glomerular epithelial cells. However, glomerular endothelial and mesangial cells were also partly responsible for the increased glomerular immunoreactivity.

The cytokines IFN-α/β, IFN-γ, IL-1β and TNF-α as detected by immunohistochemistry *in vivo* at different time points in the NCGN lesions, have all been described to be capable of inducing adhesion molecules on parenchymal kidney cells. When macrophage-derived IFN-α is administered to mice, it strongly induces glomerular MHC class I but not MHC class II expression [21,24]. The T cell cytokine IFN-γ is well known for its capacity to induce MHC class I, MHC class II and ICAM-1 on several cell types [9,10], and it is considered a crucial mediator in the development of a variety of renal diseases [9,25,26]. We could demonstrate the presence of the ICAM-1-inducing cytokines TNF-α and IL-1β in rat NCGN at later stages, in agreement with human crescentic glomerulonephritis [4]. The importance of IL-1β in the development of rat NCGN has clearly been demonstrated by Lan *et al.* in an anti-glomerular basement membrane (GBM) model [5]. The beneficial effects of anti-ICAM-1 and LFA-1 treatment [3] in anti-GBM nephritis demonstrate the importance of these adhesion molecules in the development of NCGN.

Our cell culture experiments demonstrated that only two of the cytokines detected *in vivo* were able to alter adhesion molecule expression of cultured podocytes: IFN-α and IFN-γ. The lack of response to the other cytokines tested may be due to dosage or to the dedifferentiated state of cultured GVEC. Cell-type specific (lack of) responses to identical cytokines have also been described [9], and differences in basal or induced expression of adhesion molecules by epithelial cells have been reported as well [6,7]. A synergistic effect of the cytokines tested, or a role of other cytokines in this model can not be excluded. We also do not know whether other mediators such as oxygen radicals [27] or growth factors [23] potentiated the sensitivity of glomerular cells for cytokines in this model of NCGN. The time course of MHC and ICAM-1 expression by GVEC *in vivo* was, however, very similar to the IFN-γ-induced GVEC immunoreactivity *in vitro*. Moreover, IFN-γ was found to be increased early (24 h after perfusion) in the disease, indicating its probable role in the induction of MHC and ICAM-1 molecules *in vivo*. Apical expression of adhesion molecules on podocytes as found *in vivo* was also similar to the IFN-γ-induced apical expression of MHC class I, II and ICAM-1 on cultured podocytes. The weak response of GVEC to IFN-α *in vitro* and the fact that IFN-α is hardly detectable *in vivo*, would suggest that only IFN-γ was responsible for the *in vivo* expression of MHC and ICAM-1 on podocytes. Whether this is true can only be verified by blocking studies with anti-cytokine antibodies *in vivo*.

This study provided arguments for an active role of podocytes in the development of NCGN, probably via the cytokine IFN-γ. Since no influx of T cells in the kidney was found during the first 24 h, IFN-γ either originated from local parenchymal [9,10] or distant systemic sources. MPO-immunized animals showed systemic immune activation, as demonstrated by an increased anti-MPO titre 4 days after perfusion (not shown), probably with systemic production of cytokines. The source of IFN-γ can, however, only be verified by measurement of plasma levels of rat IFN-γ and detection of intraglomerular IFN-γ mRNA levels. Irrespective of the source of the potentially responsible cytokine, the GVEC were induced to express MHC and ICAM-1 during the development of NCGN. Altered expression of these molecules coincided with the occurrence of the histological lesions. This indicates that podocytes became activated in the process of local or systemic inflammation, to be involved at later stages of NCGN. When activated, they might play a role in maintaining the inflammatory process, by expression of adhesion molecules which may facilitate a prolonged stay of infiltrated inflammatory cells. In addition, Mendrick *et al.* have demonstrated that GVEC can actually process and present antigens *in vitro* [6]. Apical expression of adhesion molecules as we demonstrated might reflect a functional role for antigen presentation by GVEC to inflammatory cells that are already present in Bowman's space. The influx of inflammatory cells in Bowman's space in its turn may also be modulated by podocytes by production of chemotactic proteins such as IP-10 in the mouse [28] and rat [29], MCP-1 in humans [30] and rats (Coers *et al.*, unpublished observations) and other chemotactic factors [31].

In conclusion, we demonstrate that podocytes are capable of expressing MHCI, MHCII and ICAM-1 *in vivo*, and that the time course and pattern of expression can be simulated *in vitro* with IFN-γ-treated podocytes. These data suggest that podocytes are induced by cytokines, in particular IFN-γ, to play a role in maintaining the inflammatory process of NCGN, by expression of adhesion molecules and by presentation of antigens to inflammatory cells in Bowman's space.

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