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Regulation of C3 and factor H synthesis of human glomerular mesangial cells by IL-1 and interferon-gamma

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SUMMARY

Previous reports have shown production of complement components C4, C2 and factor B by renal tissue. We have shown recently that human proximal tubular epithelial cells (PTEC) synthesize C3 *in vitro*, and that IL-2 enhances this production. In the present study we demonstrate that human mesangial cells (MC) in culture produce factor H and that supernatants of activated peripheral blood mononuclear cells (T cell growth factor (TCGF)) induce C3 production and enhance factor H synthesis in both a time- and dose-dependent manner. To investigate whether certain defined cytokines from TCGF were responsible for the observed effect, we tested various cytokines for their effect on complement production by MC. It is shown that IL-1 induces C3 synthesis whereas factor H production is up-regulated by IFN-γ, in both a dose- and time-dependent manner. Antibody blocking experiments revealed that C3 synthesis induced by both TCGF and IL-1 could be blocked with antibodies specific for IL-1, and also that TCGF and IFN-γ enhanced factor H synthesis could both be blocked with antibodies specific for IFN-γ. Cycloheximide was able to inhibit C3 and factor H production, suggesting *de novo* synthesis of the proteins. mRNA-polymerase chain reaction (PCR) analysis revealed mRNA encoding for C3 after stimulation with TCGF and IL-1. Factor H genes are constitutively expressed in cultured mesangial cells and its expression is up-regulated by TCGF and IFN-γ. Northern blot analysis with specific probes for C3 and factor H revealed bands which support the results obtained by PCR analysis.

Keywords mesangial cells C3 factor H IL-1 interferon-gamma

INTRODUCTION

The complement system comprises a large number of components which may be activated via either the classical or the alternative pathway. The third component of complement (C3) occupies a central position in the complement cascade because it is involved in both pathways [1]. C3 is a 185-kD glycoprotein which is synthesized as a single chain precursor and is cleaved post-transcriptionally into a 110-kD α-chain and a 75-kD β-chain [2]. The degree of activation of C3 is directly or indirectly regulated in the fluid phase by a number of inhibitors of complement such as C1 inhibitor (C1inh), factor I, C4 binding protein (C4bp) and factor H.

Factor H is a 150-kD regulatory protein of the alternative pathway of complement. It is a cofactor for factor I which cleaves C3b, resulting in the formation of iC3b, binds to C3b in the convertases and competes for the binding of factor B to C3b [3-5], thereby regulating the generation of the amplification convertase C3bBb, which in its turn determines further activation of C3 and generation of the C5b-C9 complex.

The majority of complement components are produced in hepatocytes, but other cells like monocytes/macrophages [6], fibroblasts [7], endothelial [8,9] and epithelial cells [10] can also produce complement proteins. The extrahepatic synthesis in tissues may play an important role in local inflammatory processes. The presence of C4-, C3- and factor B mRNA transcripts has been demonstrated in normal human kidneys [11,12]. In particular, tubular epithelial cells are able to produce C3 and C4 *in vitro* and *in vivo* [13,14].

Mesangial cells (MC) contribute to the structure and function of the normal glomerulus, and participate in glomerular injury. Complement depositions are frequently found in different forms of glomerulonephritis [15]. Classical pathway activation of complement occurs in immune complex mediated disease such as type 1 membranoproliferative glomerulonephritis (MPGN) or anti-glomerular basement membrane nephritis. Alternative pathway activation of complement is seen in patients with type 2 MPGN or IgA nephropathy; here C3, properdin (P) and factor H can be found in the affected glomeruli [16].

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Until now it was not known whether MC can contribute to a possible immune reaction by local synthesis of complement components in the mesangial area. In the present study, we have therefore investigated whether MC are able to produce two important components of the complement cascade and how these components are regulated by different cytokines.

MATERIALS AND METHODS

Reagents

Trypsin, fungizone, collagenase type IA, FITC-phalloidin (Sigma Chemical Co., St Louis, MO), DMEM (Seromed, Biochrom K.G., Berlin, Germany), FCS (Hyclone Laboratories Inc., Logan, UT), α-^{32}P-dCTP (3000 Ci/mmol) (Amersham Corp. Arlington Heights, IL) were obtained as indicated. TCGF was prepared from mitogen-stimulated PBMC as described [17]. Recombinant IL-1α (Hoffman-La Roche Inc., Nutley, NJ), IL-2, IL-6, IFN-γ and TNF-α were obtained from Sanbio (Uden, The Netherlands). Normal human serum (NHS) from healthy donors was aliquoted in portions of 0·5 ml and stored at -70°C. Monoclonal anti-human vimentin was obtained from Eurodiagnostics (Apeldoorn, The Netherlands). Specific rabbit polyclonal antibodies to rIL-1α were a kind gift from Dr S. Gillis (Immunex Research and Development Corporation, Seattle, WA) and MoAbs to IFN-γ were obtained from Dr P. van der Meide (TNO, Rijswijk, The Netherlands). M-MLV reverse transcriptase, oligo (dT) 12–18 primer (GIBCO BRL, Gaithersburg, MD), Taq DNA polymerase (Boehringer Mannheim, Mannheim, Germany) and dNTP (Pharmacia, LKB, Uppsala, Sweden) were used. Human factor H cDNA probe R2a was generously supplied by Dr R. B. Sim (MRC Immunochemistry Unit, University of Oxford, UK) and human C3 cDNA probe C3A1 was a kind gift from Dr B. F. Tack (Research Institute of Scripps Clinic, La Jolla, CA).

Mesangial cell cultures

Glomeruli were isolated from human kidneys which could not be used for transplants for surgical reasons, or from the unaffected pole of kidneys removed from patients with renal carcinoma. Mechanical dissociation and sequential sieving were performed essentially as described [18,19]. Glomerular epithelial cells were removed by digestion with type IA collagenase for 20 min at 37°C. The resulting glomerular suspensions were resuspended in DMEM supplemented with 20% heat-inactivated FCS, 100 U/ml penicillin and 100 μg/ml streptomycin. The glomerular cores were plated onto plastic culture flasks (T75, Greiner) and incubated at 37°C in 5% CO_2. After outgrowth of MC, which generally occurred within 20–30 days after seeding, the hillocks formed were lifted off the culture vessel using a glass pipette and explanted into 24-well plates in fresh culture medium. MC growing out of the hillocks were washed, trypsinized (0·05% trypsin/0·02% EDTA) and subcultured in T25 and T75 flasks. As already described [18], MC represent an apparent uniform cell population, as evaluated by the following criteria: (a) morphology, (b) uniform fluorescence with FITC-phalloidin for actin and positive staining with MoAbs directed against vimentin, and (c) absence of immunofluorescence using factor VIII antibodies or antibodies for cytokeratin.

For all experimental purposes, MC between subcultures 2 and 8 were trypsinized and $1–2 \times 10^4$ cells were seeded in 48-well tissue culture plates (Costar) in DMEM containing 5% FCS and cultured for an additional 48 h. Thereafter, all cells were rinsed with PBS, three wells were trypsinized and the cells counted in a Coulter Counter (Coulter Electronics, Mijdrecht, The Netherlands) to determine the number of cells present at the beginning of the assay. The cells in the 45 remaining wells were cultured further for the desired time period to assess C3 or factor H production in 500 μl medium, either alone or containing various additives.

Sandwich ELISA for measurement of C3 and factor H protein

For quantification of C3 and factor H, sandwich ELISAs were used as described [9]. The batch of FCS used in this study did not have C3 or factor H activity.

Haemolytic assays for C3 and factor H

Fresh supernatant of MC cultured in medium containing 100 pg/ml IL-1 were diluted in 100 μl $DGVB^{2+}$. The diluted supernatants were added, together with sheep erythrocytes (E^s) sensitized with rabbit anti-E^s antibodies (1×10^7/100 μl in $DGVB^{2+}$), to a 1:50 dilution of C3-deficient human serum. After incubation for 60 min at 37°C in a shaking waterbath, haemolysis was measured spectrophotometrically. The amount of haemolytically active C3 from MC was expressed in units per μg protein and compared with the amount of C3 present in NHS.

In fresh supernatant of MC cultured in medium containing IFN-γ (1000 U/ml), factor H functional activity was assessed by determination of its decay-accelerating capabilities using E-C3b generated as described [20,21]. The intermediates were prepared so that the E-C3b carried approximately 3600 molecules for C3b per intermediate. To generate intermediates bearing cell-bound C3bBbP, limited B, 100 ng of D and 3·0 μg of P were used per 1×10^8 cells bearing C3b and incubated for 30 min at 30°C in 1 ml $DGVB^{2+}$, washed and used for the experiment. The number of convertase sites (Z) generated were developed by further incubation with 0·3 ml rat serum diluted 1:15 in veronal-buffered saline with 0·1% gelatin $(GVB)^-$-EDTA for another 60 min at 37°C, calculated and expressed as units/ml (Z) using a reagent blank without B and otherwise identical conditions. The measurement of the capacity of factor H to inhibit the C3bBb formation was performed at an amount of B which permitted approximately 63% haemolysis ($Z=1$) in the absence of inhibitor. The amount of functionally active factor H from MC was expressed in ng factor H/ml, which gave a 50% inhibition of haemolysis and compared with the amount of factor H isolated from NHS which gave a 50% inhibition of haemolysis.

Measurement of gene expression using the mRNA–PCR method

Total RNA was isolated from human MC grown in T75 bottles in medium alone or supplemented with IL-1 (100 pg/ml), IFN-γ (1000 U/ml) or TCGF (5%) for 24 h according to the method of Chomczynski [22], and reverse-transcribed into cDNA. Each sample contained 1 μg of total cellular RNA, 25 mM Tris–HCl pH 8·3, 36·5 mM KCl, 5 mM dithiothreitol, 1·5 mM $MgCl_2$ 0·5 mM of each of dNTP and 5 μg acetylated BSA, 400 U of reverse transcriptase and 0·5 ng oligo(dT)12–18 primer in a final volume of 50 μl. After incubation at 42°C for 15 min, the samples were heated for 5 min at 94°C to terminate the reaction.

Oligonucleotide primers were constructed from the published cDNA sequences of C3, factor H and β-actin [23–26]. The

primers were synthesized on a DNA synthesizer (Cyclone, Millipore, Bedford, MA) by the deoxynucleoside phosphoramidite method [27]. The sequence of the C3 primers was (a) 5′-TCGGATGACAAGGTCACCCT-3′ (coding sense) corresponding to bases 4627–4646 of the cloned full-length sequence and (b) 5′GACAACCATGCTCTCGGTGA-3′ (anti-coding sense) which anneals to bases 5015–5034. The factor H primers were (a) 5′-CAGAGACACCTCCTGTGTGA-3′ corresponding to bases 3202–3221 and (b) 5′-TGCA-CAAGTTGGATACTCCA-3′ which anneals to bases 3741–3760 of the cloned cDNA. The β-actin primers were (a) 5′-GTGGGGCGCCCCAGGCACCA-3′ corresponding to bases 144–163 and (b) 5′-GAAATCGTGCGTGACATTAAGGAG-3′ which anneals to bases 660–684. The predicted sizes of the amplified C3, factor H and β-actin cDNA amplicons were 408, 559 and 540 bp, respectively. The enzymatic amplification of cDNA by PCR was performed by a modified procedure of Saiki [28]. To a final volume of 100 μl amplification solution (50 mM KCl, 10 mM Tris–HCl pH 8·3, 2·0 mM $MgCl_2$, 2 mg/ml BSA, 0·25 mM of each dNTP), 1 μl cDNA, 50 pmol of each primer and 2 U Taq DNA polymerase were added. The mixture was heated at 95°C for 3·5 min followed by 27 cycles of incubation, each consisting of 2·5 min at 95°C, 1·5 min at 55°C and 1·0 min at 72°C. The reaction was terminated by chilling at 4°C.

Northern blot analysis

Total cellular RNA was isolated as described above. Twenty micrograms of total RNA were separated on a formaldehyde-containing agarose gel and blotted onto nitrocellulose filters (Gene Screen Plus, DuPont, Wilmington, DE). Standard gel electrophoresis, RNA transfer and hybridization were done [29]. The cDNA probes used were radiolabelled with ^{32}P by random primed labelling [30]. The intensity and area of the bands on the autoradiograms were determined with an Ultroscan XL (LKB, Woerden, The Netherlands). The cDNA probes utilized in this study are R2a, that provides the sequence of the C-terminal 653 amino acid residues of factor H [25], and C3A1, that encodes 75% of the C3α chain of C3 [23]. To quantify the amount of RNA loaded per lane, the blot was washed and rehybridized with ^{32}P-labelled chicken β-actin cDNA probe [31].

Statistical analysis

Statistical analysis was performed by Student's *t*-test for unpaired samples and a *P* value of 0·05 to determine significance.

RESULTS

MC (50 000 cells/well) were cultured in 500 μl medium in the presence of increasing concentrations of TCGF for 24 h and supernatants were assessed for C3 and factor H. In this study, MC cell lines obtained from six kidneys were analysed, and for the six cell lines tested it was found that in the absence of TCGF there was 0 ± 1 ng C3 and 50 ± 2 ng of factor H production per 10^6 cells/24 h. In all cell lines, increasing concentrations of TCGF induced a significant increase in production ($P < 0·005$) of C3 in a dose-dependent fashion; the production of factor H was also significant ($P < 0·001$), enhanced between 2·9 and 4 times at a dose of 5% TCGF (data not shown). After 24 h of culture with TCGF, there was a 13 ± 7% decrease in the amount of cells present per well ($P > 0·05$); all data were expressed as ng protein/10^6 cells. No differences in production of C3 and factor H were seen between cells in passages 2 to 8. No significant differences were observed with MC grown from normal kidneys or from unaffected kidney material removed from patients with renal carcinoma.

TCGF contains a number of growth substances such as IL-1, IL-2, IL-6, TNF-α and IFN-γ, so we tested different known

Table 1. The effect of various cytokines on the synthesis of C3 and factor H by human mesangial cells (mean ± s.d. of four experiments in triplicate culture)

Agent	Dose tested	C3*	H*
DMEM		0 (1)	50 (2)
TCGF	5 (%)	24 (2)	175 (11)
IL-1	100 (pg/ml)	25 (3)	50 (2)
IL-2	1000 (U/ml)	0 (1)	50 (2)
IL-6	1000 (U/ml)	0 (1)	50 (2)
IFN-γ	1000 (U/ml)	0 (1)	180 (14)
TNF	1000 (U/ml)	0 (1)	50 (2)

* ng/10^6 cells/24 h.

Figures in parentheses show s.d.

MC were incubated with various cytokines in different concentrations. After 24 h incubation, supernatants were harvested and assessed for C3 and factor H by ELISA.

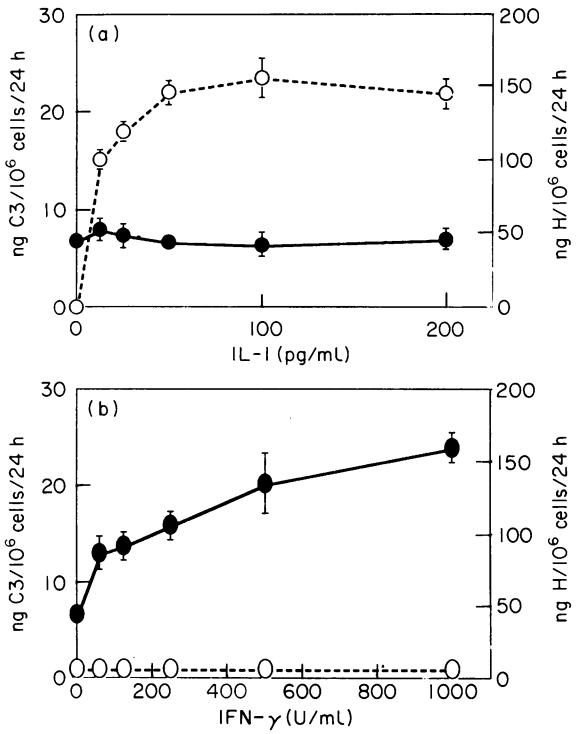

Fig. 1. Dose response of IL-1 and IFN-γ on the production of C3 (○) and factor H (●) by MC. MC were incubated with increasing concentrations of IL-1 (a) or IFN-γ (b). After 24 h of incubation, supernatants were harvested and assessed for C3 and factor H by ELISA. The results are expressed as mean ± s.d. of four different experiments in triplicate culture.

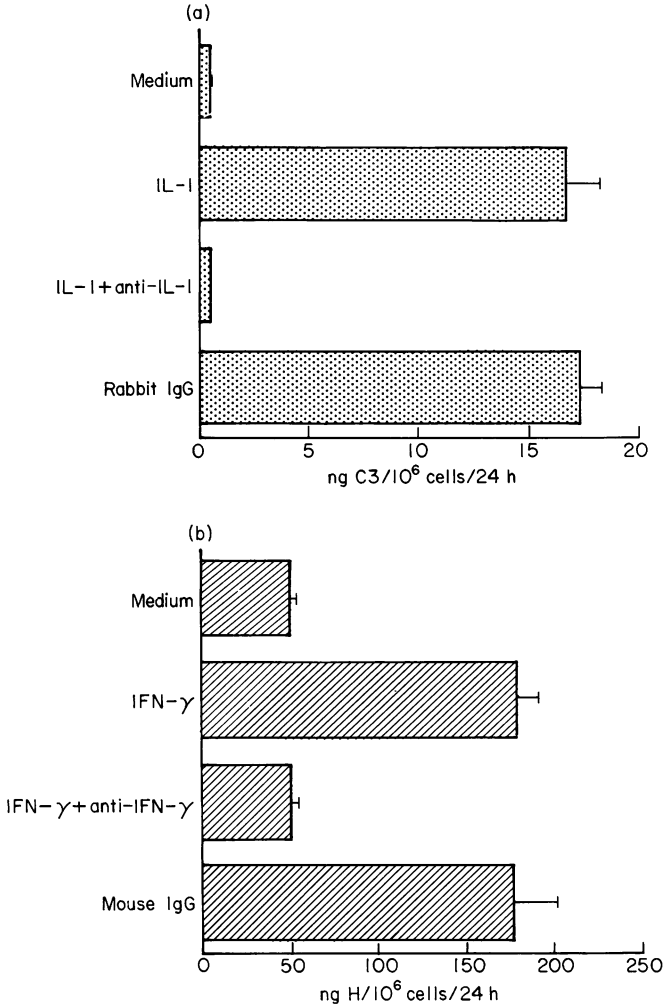

Fig. 2. Effect of rabbit anti-human-IL-1 and mouse anti-human-IFN-γ on IL-1- and IFN-γ-mediated C3 and factor H synthesis by MC. MC were incubated with IL-1 (100 pg/ml) or IFN-γ (1000 U/ml) alone, together with specific antibodies or control antibodies. After 24 h of incubation, supernatants were harvested and assessed for C3 (a) and factor H (b). The results are expressed as mean $\pm$ s.d. of four different experiments in triplicate culture.

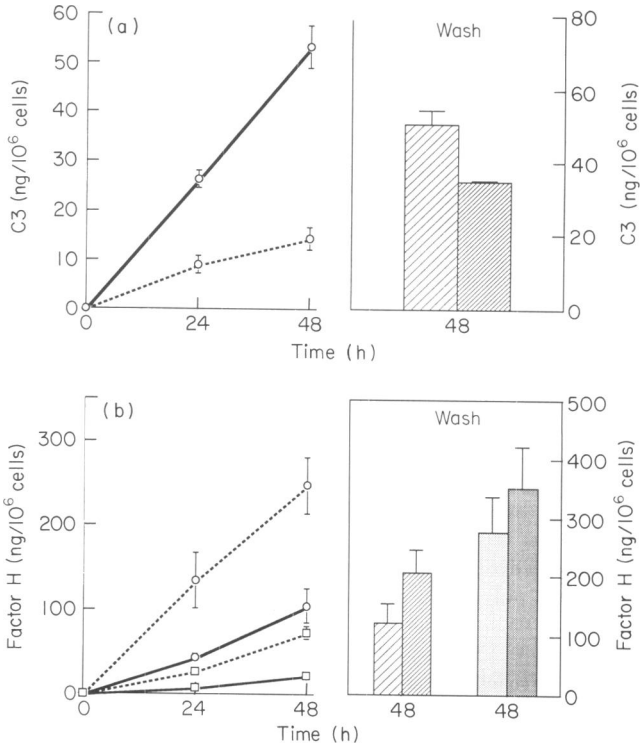

Fig. 3. The effect of cycloheximide on the protein synthesis of C3 (a) and factor H (b) by MC. Cells were incubated in medium alone, medium containing IFN-γ (1000 U/ml), IL-1 (100 pg/ml) or medium containing cycloheximide (1 μg/ml) alone or with stimuli. After 24 and 48 h supernatants were harvested and assessed for C3 and factor H (left panels). To investigate reversibility, cells were incubated for 48 h with cycloheximide, washed, cultured for another 48 h in medium alone and assessed for C3 and factor H (right panels). The results are expressed as mean $\pm$ s.d. of four different experiments in triplicate culture. (a) ——, +IL-1; - - -, +IL-1+CH; ▨, +IL-1; ▩, +IL-1+CH; (b) o——o, medium; □——□, +CH; o- - -o, +IFN-γ; □- - -□, +IFN-γ+CH; ▨, medium; ▩, +CH; ▨, +IFN-γ; ▩, +IFN-γ+CH.

cytokines to find out which were able to mimic the effect seen with TCGF. Like TCGF, IL-1 induced the production of C3 but had no effect on the amount of cells present per well after culture in the presence of IL-1. On the other hand, IL-2, IL-6, IFN-γ and TNF-α had no detectable effect on C3 production (Table 1). There was a basal production of factor H of 50 ± 2 ng per 10^6 cells/24 h and both TCGF and IFN-γ enhanced the production of factor H to the same extent, while IL-1, IL-2, IL-6 and TNF-α had no detectable effect. The culture with IFN-γ resulted in a $12 \pm 7\%$ decrease in the amount of cells present per well ($P > 0.05$).

To determine the optimal concentration for the induction and enhancement of C3 and factor H respectively, MC were cultured for 24 h in medium in the absence and presence of increasing concentrations of IL-1 and IFN-γ. IL-1 induced a dose-dependent increase in C3 production which reached optimal values at an input of 100 pg/ml of IL-1 ($P < 0{\cdot}01$) (Fig. 1a). On the other hand, there was no detectable change in the amount of factor H produced. IFN-γ enhanced factor H production in a dose-dependent fashion and was optimal at a concentration of 1000 U/ml ($P < 0{\cdot}005$) (Fig. 1b). IFN-γ had no detectable effect on C3 production.

MC were cultured in medium alone or medium containing either TCGF (5%), IFN-γ (1000 U/ml) or IL-1 (100 pg/ml) for various times. C3 production was detectable after 24 h only when TCGF or IL-1 was added, and after 72 h the production was 17·5 times higher than the production by cells in the presence of medium alone ($P < 0{\cdot}001$) (data not shown). A linear increase of factor H production was observed after a lag period of 12 h. After treatment with TCGF and IFN-γ, the amount of factor H was significantly increased by a factor 1·7 and 2 with TCGF and IFN-γ respectively ($P < 0{\cdot}01$) (data not shown).

To confirm that the regulation of C3 or factor H production by human MC is a specific property of IL-1 or IFN-γ, antibody blocking experiments were performed. MC were cultured for 24 h in medium alone or medium containing IL-1 or IFN-γ in the presence or absence of antibodies to IL-1 (50 μg/ml), IFN-γ (10 μg/ml) or control rabbit (50 μg/ml) or mouse IgG (10 μg/ml). The results show that neutralization of IL-1 with anti-IL-1 ($P < 0{\cdot}05$) (Fig. 2a) or IFN-γ with anti-IFN-γ ($P < 0{\cdot}01$) (Fig. 2b)

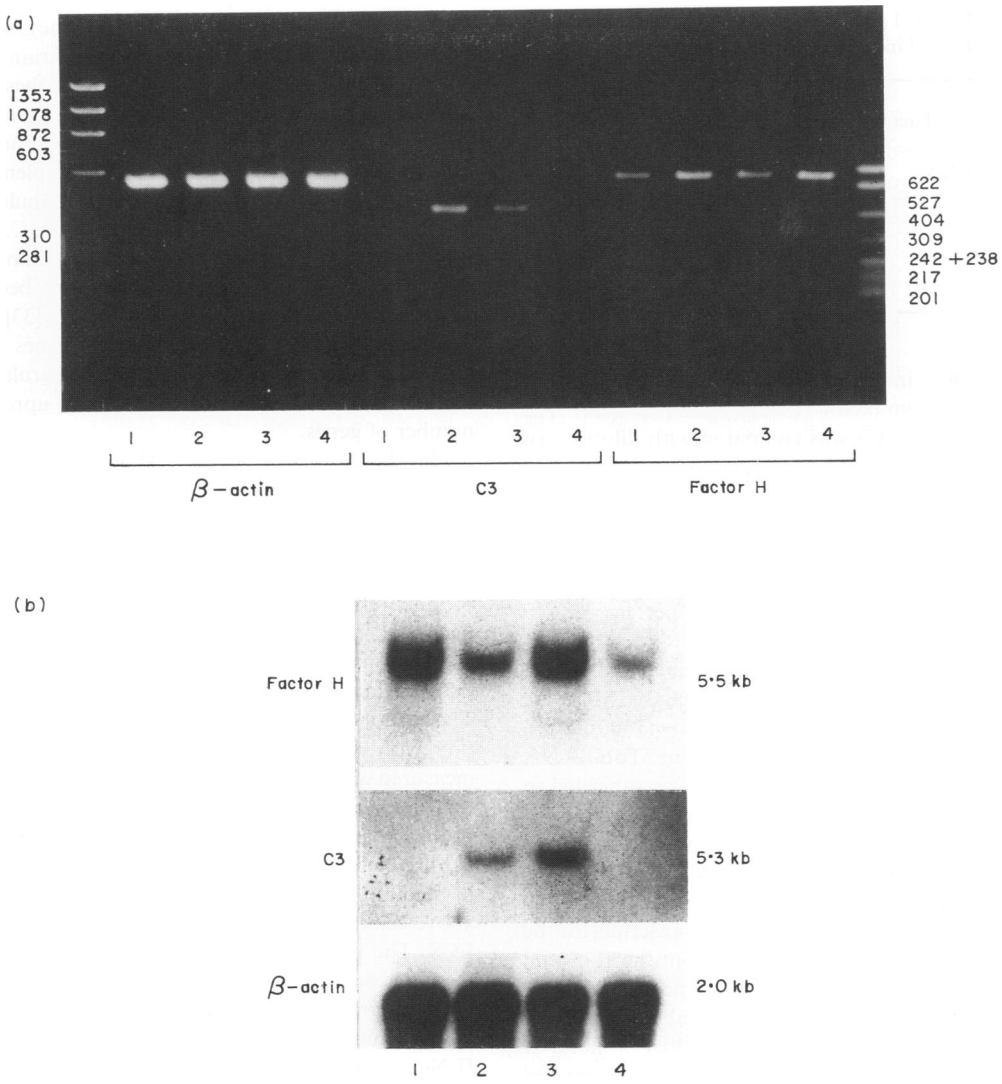

Fig. 4. (a) Total RNA was isolated from MC cultured for 24 h in medium alone (1), medium containing 5% TCGF (2), 100 pg/ml IL-1 (3) or 1000 U/ml IFN-γ (4) and 1 μg RNA was reversed transcribed into cDNA. Sequences were amplified with specific primer sets. Numbers on the left and right are markers in bp. (b) Total RNA was isolated from MC cultured for 24 h in medium containing 1000 U/ml IFN-γ (1), 100 pg/ml IL-1 (2), 5% TCGF (3) or medium alone (4). RNA was electrophoresed, blotted to nitrocellulose and detected with specific probes for C3, factor H and β-actin. Numbers on the right are molecular weights in kb.

abolished the induction of C3 production or enhancement of factor H, respectively; control rabbit or mouse IgG had no effect. The TCGF-induced C3 production could also be inhibited for 80±3·4% by antibodies against IL-1 (50 μg/ml) and TCGF-enhanced factor H production could be reduced with 94±8·3% after treatment with anti-IFN-γ antibodies (10 μg/ml); control antibodies had no effect.

Evidence for *de novo* synthesis was obtained by blocking the translation with cycloheximide. MC were grown in medium alone or stimulated with IFN-γ and IL-1 with or without cycloheximide. At timed intervals, supernatants were removed for C3 and factor H analysis by ELISA and the wells harvested for the assessment of the number of cells. The results were calculated and expressed on a 10^6 cell basis. Another set of MC-containing wells were kept either for 48 h in medium alone or in medium containing 1·0 μg/ml cycloheximide. Then all wells were rinsed thoroughly and further incubated in medium alone. Again, at timed intervals, supernatants and cells were harvested and used to assess C3 and factor H as described above. These experiments revealed that cycloheximide inhibited the IL-1 induced C3 production after 24 h ($P<0·005$) and 48 h ($P<0·005$) (Fig. 3a, left panel) and the basal- and IFN-γ enhanced factor H synthesis after 24 and 48 h (all $P<0·005$) (Fig. 3b, left panel). All effects of cycloheximide were reversible at 48 h following cycloheximide treatment, suggesting that C3 and factor H are synthesized *de novo* and that no cytotoxicity had occurred (Fig. 3, right panels).

Supernatants of MC obtained after 48 h in culture were assessed for antigenic and functional C3 and factor H activities respectively. Under the conditions used, a dose-dependent inhibition of C3bBb formation was observed by both plasma factor H and MC factor H. Plasma factor H induced a 50% inhibition of haemolytic activity at a concentration of 56·9±9 ng/ml. In the supernatants of MC, an amount of 76±8 ng/ml

Table 2. Effect of TCGF, IL-1 and IFN-γ on factor H and C3 expression (x-fold increase over control)

	Factor H		C3	
Treatment	mRNA	Protein	mRNA	Protein
TCGF	6·3	1·9	10·3	20
IL-1	1·1	1·7	4·3	15
IFN-γ	4·3	1·3	0·7	1

factor H induced a 50% inhibition of haemolysis which was not significantly different from factor H from NHS ($P > 0{\cdot}05$). The functional activity of MC C3 was compared with dilutions of NHS with the same amounts of C3 in a haemolytic C3 titration assay. The titration curves were linear and showed no significant differences between the functional activities of C3 present in NHS and C3 present in culture supernatant of MC. Plasma C3 had a functional activity of 342 ± 25 units per μg protein and C3 from MC had an activity of 405 ± 40 units per μg protein ($P > 0{\cdot}05$).

C3 and factor H mRNA transcripts were analysed by the mRNA–PCR method and by Northern blot analysis. MC were incubated for 24 h in medium containing IFN-γ (1000 U/ml), IL-1 (100 pg/ml), TCGF (5%) or medium alone. Total RNA was isolated from each culture; 1 μg of RNA was subjected to first strand cDNA synthesis and amplified for specific C3, factor H and β-actin sequences. Aliquots of cDNA which yield a 540 bp β-actin PCR product with similar intensity (Fig. 4a) were used for C3 and factor H amplification. Amplification with the C3 specific primer set revealed the presence of transcripts for the 408 bp C3 product in the cDNA obtained from the IL-1 and TCGF-stimulated human MC. Cells grown in medium alone or stimulated with IFN-γ did not express detectable C3 mRNA (Fig. 4a). mRNA encoding for the 559 bp product of factor H was found at a basal level in cells cultured in medium alone and in IL-1 stimulated cells. Factor H mRNA was found to be increased with TCGF and IFN-γ stimulation (Fig. 4a). When RNA was separated according to size on a formaldehyde-containing agarose gel, blotted to nitrocellulose and hybridized with specific C3 and factor H cDNA probes, the data agreed with the results obtained by the mRNA–PCR method (Fig. 4b). Densitometric analysis revealed a 10·3-fold and 4·3-fold increase of the ratio of C3/β-actin message after stimulation with TCGF and IL-1 respectively (Table 2). The ratios of factor H/β-actin were 6·3-fold and 4·3-fold enhanced after stimulation of MC with TCGF and IFN-γ, respectively (Table 2). These data suggest a regulation of C3 production by IL-1 and of factor H by IFN-γ at a pretranslational level.

DISCUSSION

In several renal diseases, deposits of complement together with immunoglobulins are found in the kidney. Complement components can also be found without deposition of immunoglobulins, suggesting a possible local production of complement in the kidney. Until now, little was known about the biosynthesis of complement components by human glomerular cells. It has been demonstrated that C4 mRNA is expressed in normal human kidney [11]. Passwell *et al.* [12] observed a higher expression of C2, C3, C4 and factor B mRNA in the kidney of a systemic lupus erythematosus (SLE) sensitive mouse strain compared with normal mice. The degree of expression of these components in SLE-prone mice was correlated with the degree of macrophage influx in the kidney. In these studies it remained unclear which cell type was responsible for the local complement production. It has been shown that human proximal tubular epithelial cells (PTEC) produce C3 and C4 and that C3 production is upregulated by IL-2 [13] and C4 production is enhanced by IFN-γ [32]; also C4 gene expression has been shown to be localized in tubular epithelial cells [14]. We [33], and others [34–37], recently reported that C3 and C4 genes are expressed in human glomerular MC [33–35] and glomerular epithelial cells [36,37] and that various cytokines may upregulate a certain number of genes.

In the present study, we show that human glomerular mesangial cells produce reasonable amounts of factor H, but that C3 production, compared with factor H, is very low or undetectable [33]. Stimulation of C3 production is at least dependent on IL-1 (Fig. 1a), whereas it does not influence the production of factor H. On the other hand, IFN-γ enhances factor H production but has no detectable effect on C3 production (Fig. 1b). In another study reported in abstract form [35], synthesis of 100 ng C3/ml was observed after 72 h culture. The authors also observed an increase of 3·3-fold after stimulation with 1000 U IL-1β. In this study the amounts of C3 measured were not corrected for the number of cells present and the time period of culture. The difference in amounts of C3 produced by MC in culture may also be dependent on the culture medium used. In our study, MC are originally cultured in medium containing 5% FCS, while in the study reported above cells were cultured for 72 h in medium containing 0·5% FCS. The production of very low amounts of C3 by MC in medium containing 5% FCS is supported by the observation of very low amounts of mRNA for C3 by PCR and Northern blots.

Blocking studies with neutralizing antibodies to IL-1 and IFN-γ demonstrated that the effects were indeed mediated by IL-1 and IFN-γ (Fig. 2), which was confirmed by the antibody blocking experiments with TCGF. These results demonstrated that the TCGF-driven synthesis of C3 and factor H were mediated by the single cytokines IL-1 and IFN-γ respectively. These studies indicate that IL-1 and IFN-γ have differential effects on C3 and factor H production by human MC. Comparable effects of IL-1 and IFN-γ on C3 and factor H production were seen in human umbilical vein endothelial cells (HUVEC) [38], whereas it has been shown [8] that IL-1 had no effect on the production of C3 by endothelial cells which could be caused by a different activation stage of the cells. C3 production by PTEC cells was between 89 and 210 ng/10^6 cells/24 h and could be upregulated with IL-2 [13], suggesting cell specific regulation mechanism of the complement components. After treatment with cycloheximide, treated cells produced more factor H than untreated cells. This could be caused by an overshoot phenomenon of the cycloheximide-treated cells.

The mechanisms underlying the effects described above, as reflected in the relationships between rates of protein production and levels of mRNA, were also studied. As shown in Fig. 4, the level of C3 mRNA was detectable in TCGF and IL-1 treated cells. The amount of C3 mRNA after stimulation with TCGF was 10·3-fold increased, whereas after IL-1 stimulation it was 4·3-fold. The amounts of protein produced were also higher

after stimulation with TCGF; differences between batches of TCGF could be responsible for the observed effects. Sacks [34] showed the presence of C3, and to a lesser extent C4, gene expression in unstimulated MC; IFN-γ could increase the C4 but not the C3 transcripts in MC. It is possible that the cells used by Sacks were in a different state of activation since the cells were cultured in medium containing 20% FCS. C3 and C4 gene expression was also shown in unstimulated human glomerular epithelial cells and could be increased after incubation with IFN-γ and not by TNF-α [35,36], suggesting again cell-specific regulation mechanisms of complement components. Katz [39] showed the effects of IL-1 and TNF on the message of factor B and C3 in human skin fibroblasts. TNF could also enhance factor H production, although IFN-γ was more effective than TNF. We could not show effects of TNF on C3 or factor H production. Factor H mRNA was increased in TCGF and IFN-γ treated cells to comparable amounts. The observed size of factor H mRNA was larger than the expected size of 4·7–4·8 kb [25]. A species of 5·7–5·8 kb has also been described to occur in human liver [40]. Different forms of factor H mRNA could be present in different cell types; whether this also results in production of factor H of different sizes is currently under investigation. These results indicate a pretranslational control of C3 and factor H by the cytokines in MC.

One can imagine that during an immune response *in vivo*, cytokines that are produced locally may determine which complement component is produced preferentially. However, it is still unclear whether these amounts contribute to the local immune reaction in comparison with the amounts of C3 (1·3 mg/ml) and factor H (400 μg/ml) present in plasma. Fenestrated endothelium is the only barrier between the blood and MC, so these plasma proteins could potentially reach the mesangial area easily. The basic local production of factor H, together with the presence of DAF [41], MCP [42] and HRF [43], on MC could protect them against complement-mediated damage, whereas local C3 production could activate the immune response.

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