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Production of tumor necrosis factor by rat mesangial cells in response to bacterial lipopolysaccharide

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Production of tumor necrosis factor by rat mesangial cells in response to bacterial lipopolysaccharide. Tumor necrosis factor (TNF) is a cytokine which is produced by mononuclear phagocytes upon activation by bacterial lipopolysaccharide (LPS) and various other stimuli. In immune-mediated glomerulonephritis, infiltration of glomeruli by monocytes-macrophages is associated with production of TNF. The purpose of the present experiments was to determine whether mesangial cells could also contribute to glomerular TNF synthesis. TNF activity has been determined in the culture medium of rat mesangial cells using a L-929 fibroblast lytic assay. This activity was detectable only when the cells were exposed to LPS (0.1 to 10 μg/ml) and for periods longer than one hour. The cytotoxic factor was identified as TNF since: (1) the lytic activity was completely inhibited by an anti-mouse TNF polyclonal antibody and was associated with suppression of lipoprotein lipase activity in adipocytes; (2) its molecular weight (110,000 daltons) corresponded to that observed for murine TNF under non-denaturing conditions; and (3) mRNA encoding TNF was expressed by mesangial cells two hours after addition of LPS. To assess the mechanisms whereby TNF production was regulated, the role of prostaglandin E_2 (PGE_2) was determined. LPS caused a dose-dependent increase of PGE_2 synthesis by mesangial cells. Treatment by indomethacin promoted a suppression of PGE_2 production together with an increase of TNF synthesis, indicating that PGE_2 acted in a negative feedback manner to regulate the production of TNF. Addition of PGE_2 (0.1 to 300 nM) or 8-bromo cyclic AMP (0.1 to 100 μM) induced similar dose-dependent reductions of TNF synthesis. Thus the inhibitory effect of PGE_2 probably required in part cyclic AMP accumulation. It is concluded that TNF is released by mesangial cells upon stimulation by LPS, and that its synthesis is regulated by both PGE_2 and cyclic AMP. Production of TNF could participate in the mechanism of endotoxin-induced glomerular injury.

Rat mesangial cells possess macrophage-like characteristics such as the capacity to secrete a wide variety of biologically active factors including reactive oxygen metabolites [1], neutral proteinases [2], eicosanoids [3], platelet activating factor [4], and cytokines [5, 6]. In immune-mediated glomerulonephritis, the mechanisms of glomerular injury involve most of these mediators which are released by mesangial cells and/or invading bone marrow-derived cells. In these models, the importance of hydrogen peroxide, proteinases and leukotrienes has been inferred from the beneficial effects of their specific pharmacological inhibitors [7–9], and the role of cytokines has been suggested by the increase in the renal levels of interleukin I (IL-1) and tumor necrosis factor (TNF) mRNA [10, 11]. If much interest has focused on IL-1, only little information on the role of TNF as a mediator of glomerular injury is presently available. Current evidence suggests that TNF could play a major role since it has been shown to affect the surface antigen expression in vascular endothelial cells, the growth of fibroblasts and different functions of both polymorphonuclear leukocytes [12] and mesangial cells [13]. Moreover, the cellular origin of TNF in the glomerulus is still unknown. If infiltrating macrophages represent a potential source of TNF [11, 14], the glomerular resident cells themselves could synthesize this cytokine as well. The current studies have been designed to examine TNF synthesis by rat cultured mesangial cells. In addition, because prostaglandin E_2 (PGE_2) is the main metabolite of the cyclooxygenase pathway in these cells [3], we also checked the role of PGE_2 and of its second messenger, cyclic adenosine monophosphate (cAMP), as an autoregulating factor of TNF synthesis. We report here that incubation of cultured rat mesangial cells with bacterial lipopolysaccharide (LPS) induces the production of TNF and that PGE_2 and cAMP act in a negative feedback manner to regulate the release of this cytokine.

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Methods

Mesangial cell culture

Mesangial cells were cultured from isolated rat glomeruli according to a method previously described [1]. Briefly, glomeruli were prepared from the renal cortices of Sprague-Dawley rats weighing 100 to 150 g by sieving techniques and differential centrifugations. The final preparation, which was resuspended in Dulbecco's phosphate-buffered saline (PBS), was checked for purity under light microscopy. Glomeruli were free of Bowman's capsule and virtually no afferent or efferent arterioles could be detected. After treatment by 300 U/ml of collagenase (300 U/mg; Sigma Chemical Co., St. Louis, Missouri, USA) during 30 minutes at 37°C, isolated glomeruli were resuspended in RPMI 1640 medium (Flow Laboratories, Irvine, UK), buffered with 20 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES) to pH 7.4, and supplemented with 10% decomplemented fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamine. Then, they were

plated onto plastic Petri dishes (Nunc, Roskilde, Denmark) and cultured at 37°C in 5% CO_2 in air. Under such conditions, mesangial cells appeared after 7 to 14 days in culture and reached confluency by day 21. Near confluent cells in primary culture or after one to two passages were used in these studies. The lack of contamination of these cells by macrophages was confirmed as previously reported [1].

Triggering TNF release

The Petri dishes were rinsed with serum-free culture medium, and the medium was replaced with 2 ml of fresh RPMI-1640 medium containing 1% fetal calf serum and bacterial LPS (from *E. coli* 026 B6; Sigma Chemical Co.) together with the drug to be tested. Cell-free supernates were collected after 1 to 48 hours of incubation and stored at −70°C until assayed for either TNF activity or cAMP and PGE_2 concentrations.

Tumor necrosis factor assay

The activity of TNF was determined in the cell culture medium using a L-929 fibroblast lytic assay as previously described [15]. Briefly, L-929 cells were grown in 96-well microplates (3 × 10^4 cells per well) at 37°C in 100 μl medium consisting of Minimum Essential Medium Eagle (Boehringer, Mannheim, FRG) supplemented with 1% glutamine, 1% non-essential amino acids (Flow Laboratories), and 10% decomplemented fetal calf serum. They were incubated overnight at 37°C in an atmosphere of 5% CO_2 in air. Serial dilutions of test samples were made in the above medium containing 1 μg/ml actinomycin D (Sigma Chemical Co.), and the cell culture medium was replaced with 100 μl volumes of these dilutions in duplicate. After 18 hours, the medium was discarded and the remaining viable adherent cells were stained with crystal violet (0.2% in 2% ethanol) for 10 to 15 minutes as previously described [15]. Microplates were rinsed with tap water, and 100 μl of 33% acetic acid were added to each well to solubilize the stained cells. The absorbance of each well was read at 540 nm with a Titertek Multiscan (Flow Laboratories). Percentage cytolysis was calculated from the formula 100(a-b)/a, where b and a are the mean absorbances of duplicate wells with or without sample dilution. One TNF unit was defined as the reciprocal of the dilution which causes 50% cytotoxicity under the conditions of the present assay. An internal standard of human recombinant TNF (2 × 10^7 units/mg; Genzyme, Boston, Massachusetts, USA) was included as a control. For neutralization studies, equal volumes of recombinant TNF or of test samples were mixed with serial dilutions of normal rabbit serum, anti-mouse TNF (10^6 neutralizing units per ml; Genzyme) or anti-human TNF (Dr. J. Wietzerbin, Institut Curie, Paris, France). Samples were incubated at 37°C for 90 minutes and then assayed in triplicate for residual cytotoxic activity. In additional experiments, the activity of TNF was identified using a 3T3-L1 lipoprotein lipase inhibition assay [16]. 3T3-L1 cells were plated in 3.5 cm plastic tissue culture dishes and grown to confluence in Dulbecco modified Eagle's medium supplemented with 10% fetal calf serum (DME-FCS). After one week, they were differentiated by incubation for 48 hours in DME-FCS medium supplemented with 10 μg/ml bovine insulin, 0.5 mM methylisobutylxanthine and 1 μM dexamethasone. The differentiated cells were used after one week of culture in DME-FCS medium supplemented with 50 ng/ml bovine insulin. At that time, they were exposed to up to 50 μl of 10-fold concentrated culture medium of mesangial cells in a final volume of 1 ml. After 16 hours, the medium was aspirated and replaced by 500 μl of DME-FCS medium containing 10 U/ml heparin. The heparin-releasable lipoprotein lipase (LPL) activity was measured after one hour incubation at 37°C according to the method of Corey and Zilversmit [17], and was expressed as mU/dish.

Sephacryl-200 chromatography

The stored frozen medium of LPS-activated mesangial cells was thawed and concentrated as well as dialyzed using a Centricon 10 microconcentrator (Amicon Corp., Danvers, Massachusetts, USA). One ml of 20-fold concentrated medium was applied to a 1.6 × 100 cm Sephacryl S-200 HR column (Pharmacia Fine Chemicals, Uppsala, Sweden) that had been calibrated with known standard molecular weight markers (Bio-Rad, Richmond, California, USA). The column was equilibrated at 4°C with 0.1 M NaCl, 50 mM Tris/HCl buffer, pH 7.4, and the sample was eluted at a flow rate of 12 ml/hr. Fractions of 1.8 ml were collected, sterilized by filtration, and assayed after ⅕ dilution for cytotoxic activity on L-929 cells.

Preparation of RNA and Northern analysis

Following incubation with or without LPS (10 μg/ml), mesangial cells were exposed to the lysing buffer [10 mM Tris/HCl, pH 7.4, containing 100 mM NaCl, 1 mM EDTA, 1% sodium dodecyl sulfate (SDS), and 250 μg/ml proteinase K (Sigma Chemical)] for 45 minutes at 37°C. Total RNA was then extracted by the phenol-chloroform method [18], precipitated with isopropanol and, after dissolution in 10 mM Tris/HCl buffer, pH 7.4, containing 1 mM EDTA, precipitated again with 3 M LiCl. After two supplementary precipitations with ethanol, RNA concentration and purity were determined by obtaining the A_{260} and A_{280} readings.

Agarose gel (0.9%) electrophoresis of total RNA (10 μg/lane) in the presence of 20% formaldehyde was followed by Northern blot transfer on Gene Screen Plus membrane (New England Nuclear, Boston, Massachusetts, USA). After prehybridization, the filters were hybridized with a ^{32}P-labeled murine TNF probe [19], from Dr. W. Fiers, Biogen, Ghent, Belgium.

Determination of PGE_2 synthesis by mesangial cells

Radioimmunoassay of PGE_2 was carried out in the culture medium of mesangial cells after extraction according to the technique of Maurin [20]. After centrifugation, supernatants (0.5 ml) were diluted in 2.5 ml of H_2O, acidified to pH 4.0 with 1 N HCl, and purified through C_{18} columns (Waters Associates, Milford, Massachusetts, USA). The columns were rinsed with 10 ml H_2O to remove polar substances and PGs were eluted with 7 ml ethylacetate. Extracts were then evaporated under nitrogen and the dry residues were resuspended in the radioimmunoassay buffer. PGE_2 was assayed at four increasing dilutions by using [^{3}H] PGE_2 from the Radiochemical Centre (Amersham, UK) and anti-PGE_2 antibody from Institut Pasteur (Paris). This antibody cross reacts only slightly with other PGs and could be considered as specific [3].

Determination of cAMP synthesis by mesangial cells

Cyclic AMP was extracted by adding 0.2 ml of culture medium to 0.4 ml of an ice-cold ethanol/formic acid mixture

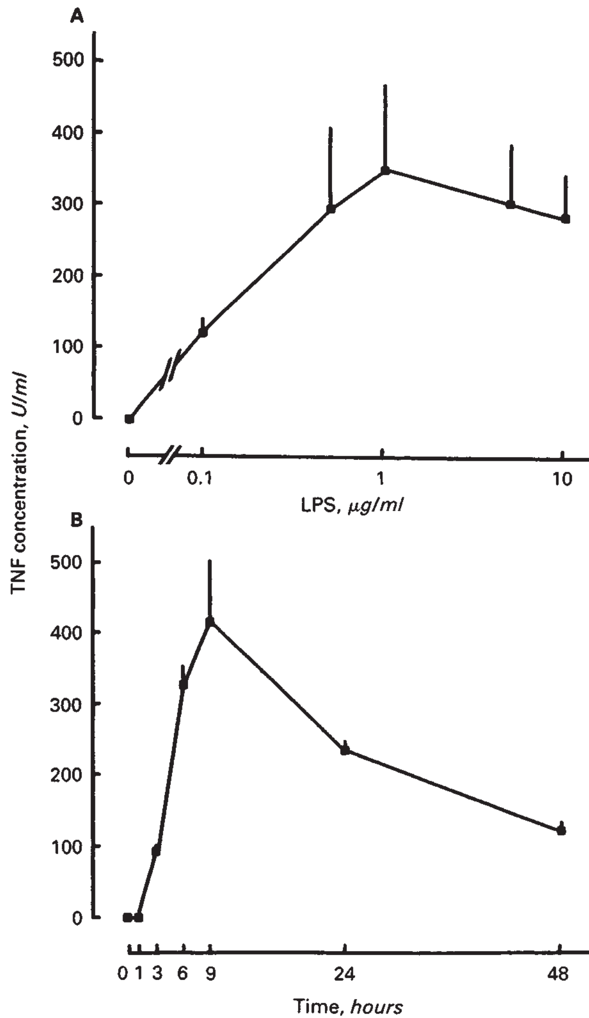

Fig. 1. *Dose-response and time-course curves of LPS-induced TNF production by cultured rat mesangial cells.* Mesangial cells were incubated for 14 hours with increasing concentrations of LPS (A) or they were incubated in the presence of 1 μg/ml of LPS and supernates were collected at the times indicated (B). TNF concentration was estimated using the cytotoxic activity assay on L-929 cells. Means and SEM of values obtained in three experiments are given.

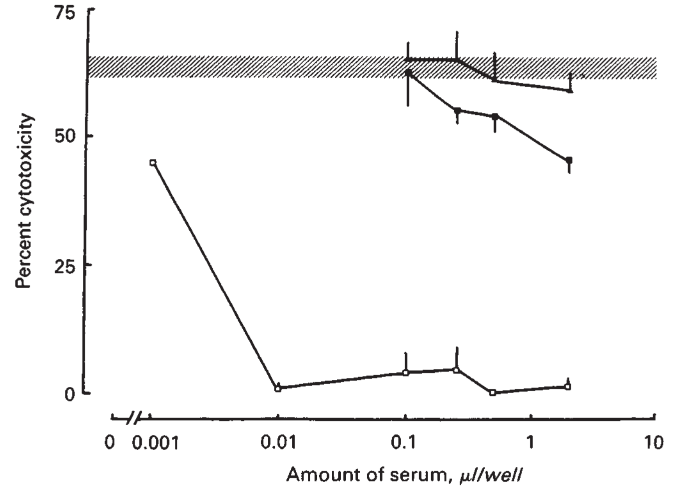

Fig. 2. *Effect of increasing amounts of normal rabbit serum (▲) and of two rabbit antiserums directed against human recombinant TNF (■) and mouse recombinant TNF (□), respectively, on the cytotoxicity of mesangial cell supernates.* Mesangial cells were incubated with 1 μg/ml LPS for 14 hours. Then, aliquotes of the incubation medium were incubated with the serums tested for 90 minutes at 37°C and the residual cytotoxicity was assayed in triplicate. Means and SEM of values obtained in three experiments are given.

(85:15, vol/vol). After 30 minutes at 4°C, extracts were evaporated under nitrogen and the dry residues were resuspended in the radioimmunoassay buffer. Radioimmunoassay of cAMP was performed after acetylation of the samples, as previously described [21].

Results

Production of TNF activity by LPS-activated mesangial cells

In the absence of exogenous stimulation, that is, other than medium and serum factors, rat mesangial cells did not spontaneously release TNF activity (Fig. 1A). In contrast, incubation of these cells with various doses of LPS for 14 hours resulted in a dose-dependent and saturable production of TNF activity. This stimulatory effect was maximal at 1 μg/ml. Higher concentrations of LPS produced no further increase in TNF activity. At these concentrations, TNF amounts reached 300 U per ml of medium, that is, about 300 U/500,000 cells. This value is close to that obtained with LPS-treated, immunologically-elicited murine macrophages [22]. It was verified that LPS by itself, even at the highest doses used, had no effect on the viability of L-929 cells. The time course for the production of TNF following LPS stimulation of mesangial cells is shown in Figure 1B. A slight TNF activity was detectable after three hours of LPS stimulation. This was followed by a major peak of activity at nine hours, whereafter activity started to decline. We also investigated whether anti-TNF antibodies could block the activity of mesangial cell-derived cytotoxic factor. Figure 2 shows that rabbit anti-human-recombinant TNF polyclonal antibody significantly reduced L-929 cell killing and that anti-mouse recombinant TNF polyclonal antibody even completely neutralized it. In contrast, normal rabbit serum that had been added at the highest dose had no effect on mesangial cell-derived cytotoxic activity. Thus, this factor appears to be antigenically related to TNF. Gel filtration on Sephacryl S-200 HR was used to determine the molecular weight of the mesangial cell-derived TNF activity (Fig. 3A). Only one peak of cytotoxic activity was observed with an apparent molecular weight of 110,000. This value is close to those reported for the molecular weight of murine TNF, (>70,000 [16] or about 150,000 [23]) when the biological activity is recovered under nondenaturing conditions. Since TNF is known to exhibit cachectin-like activity [12], it was also important to determine whether the conditioned medium of mesangial cells could inhibit the lipoprotein lipase activity of adipocytes. Cachectin bioactivity was detectable only in the conditioned medium of LPS-treated mesangial cells. Indeed, LPL activity released in the supernatants of 3T3-L1 cells was 14.88 ± 1.41, 14.07 ± 0.98 and 1.51 ± 0.17 mU/dish after exposure of these cells to 50 μl of 10-fold concentrated control medium, mesangial cell-conditioned medium and LPS-

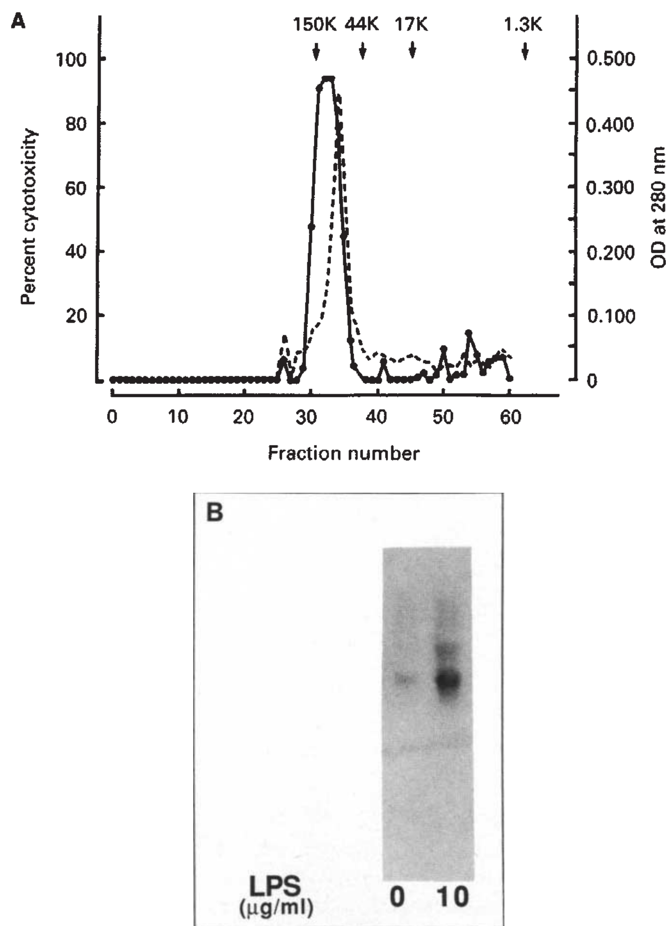

Fig. 3. A. *Gel filtration on Sephacryl S-200 HR.* Conditioned medium of LPS-activated mesangial cells was concentrated 20-fold and chromatographed on a calibrated 1.6 × 100 cm column. Fractions of 1.8 ml were assayed after ⅕ dilution for cytotoxic activity on L-929 cells. **B.** Representative Northern blot analysis of TNF mRNA derived from cultured rat mesangial cells. Mesangial cells were incubated with or without 10 μg/ml LPS for 2 hours. Total RNA was extracted, blotted and hybridized with a TNF probe. Ten μg of RNA were used in each sample.

treated mesangial cell-conditioned medium, respectively (mean ± SEM; $N = 3$).

Identification of TNF mRNA in LPS-activated mesangial cells

Mesangial cells were incubated in the absence or in the presence of LPS (10 μg/ml), and total RNA was extracted after two hours of incubation. For Northern blot analysis, equal amounts of RNA (10 μg) were used in each lane of the gel. As shown in Figure 3B, one strongly hybridizing band and a second weaker band of higher molecular weight were observed after cell treatment with LPS. In contrast, hybridization signals were barely detectable before LPS treatment. These results are consistent with the previously reported TNF mRNA expression in LPS-treated murine peritoneal macrophages [24], which is also characterized by two TNF-hybridizing bands, neither band being due to cross-hybridization with ribosomal RNA [25].

Effect of prostaglandin concentration on LPS-induced TNF synthesis

PGE_2 has been shown to reduce the LPS-stimulated TNF production by macrophages, whereas inhibition of endogenous PGE_2 synthesis by indomethacin enhances this production [22]. Because in cultured rat mesangial cells PGE_2 is the main product of the cyclooxygenase pathway [3], the effects of indomethacin on both PGE_2 and TNF synthesis by LPS-treated cells were also assessed. In the absence of cyclooxygenase inhibitor, LPS stimulated PGE_2 synthesis in a dose-dependent manner but saturation concentrations of LPS were not reached in the experiments reported (Fig. 4A). PGE_2 synthesis was almost completely blocked when indomethacin (1 μM) was added to the cell culture medium together with LPS (Fig. 4A). Under these conditions, the dose-response curve of TNF production was modified. Progressive additional increases were observed at concentrations of LPS higher than 1 μg/ml in contrast to the results obtained in the absence of indomethacin (Fig. 4B). For example, in the culture medium of cells exposed to 5 μg/ml LPS, the level of TNF reached 771 ± 128 U/ml in the presence of cyclooxygenase inhibitor, whereas it was only 306 ± 85 U/ml in control cultures ($N = 3$). To confirm the inhibitory effect of PGE_2 on TNF synthesis, mesangial cells were exposed to 10 μg/ml LPS and 1 μM indomethacin in the presence of exogenous PGE_2 (Fig. 5A). PGE_2 promoted a reduction of TNF synthesis in a dose-dependent manner between 1 and 100 nM. These concentrations are close to those of endogenous PGE_2 which were observed in the culture medium of cells exposed to LPS at doses higher than 1 μg/ml, that is, those which effectively induced a reduction of TNF synthesis (approximately 20 ng/ml, that is, 56.8 nM; Fig. 4).

Effect of cAMP concentration on LPS-induced TNF synthesis

To determine whether the inhibitory effect of PGE_2 on TNF synthesis could be attributed to an increase of cAMP, the role of exogenous PGE_2 on the cAMP content of mesangial cells exposed to 10 μg/ml LPS and 1 μM indomethacin was studied. Only a slight increase in cAMP concentration was observed at doses of PGE_2 ranging between 0.1 and 10 nM, whereas doses above 10 nM were markedly stimulatory (Fig. 5B). The inhibitory effect of cAMP was established by directly adding 8-bromo-cAMP to LPS-stimulated mesangial cells together with 1 μM indomethacin. Figure 6 shows that 8-bromo-cAMP caused a dose-dependent inhibition of TNF production.

Discussion

TNF plays a central role in a number of inflammatory processes [reviewed in 12]. This cytokine was found to be a product of mononuclear phagocytes, lymphocytes [12] and endothelial cells [26]. The observations reported here further extend the range of TNF sources to rat mesangial cells and provide evidence for the down-regulation of TNF synthesis by cAMP.

Mesangial cells produced a TNF-related factor that was cytotoxic for L-929 cells (Fig. 1). This factor was elicited only after treatment with LPS, at concentrations ranging between 0.1 and 10 μg/ml. LPS was chosen because numerous studies have established that LPS is particularly effective to induce TNF synthesis, while phagocytic stimuli, including zymosan,

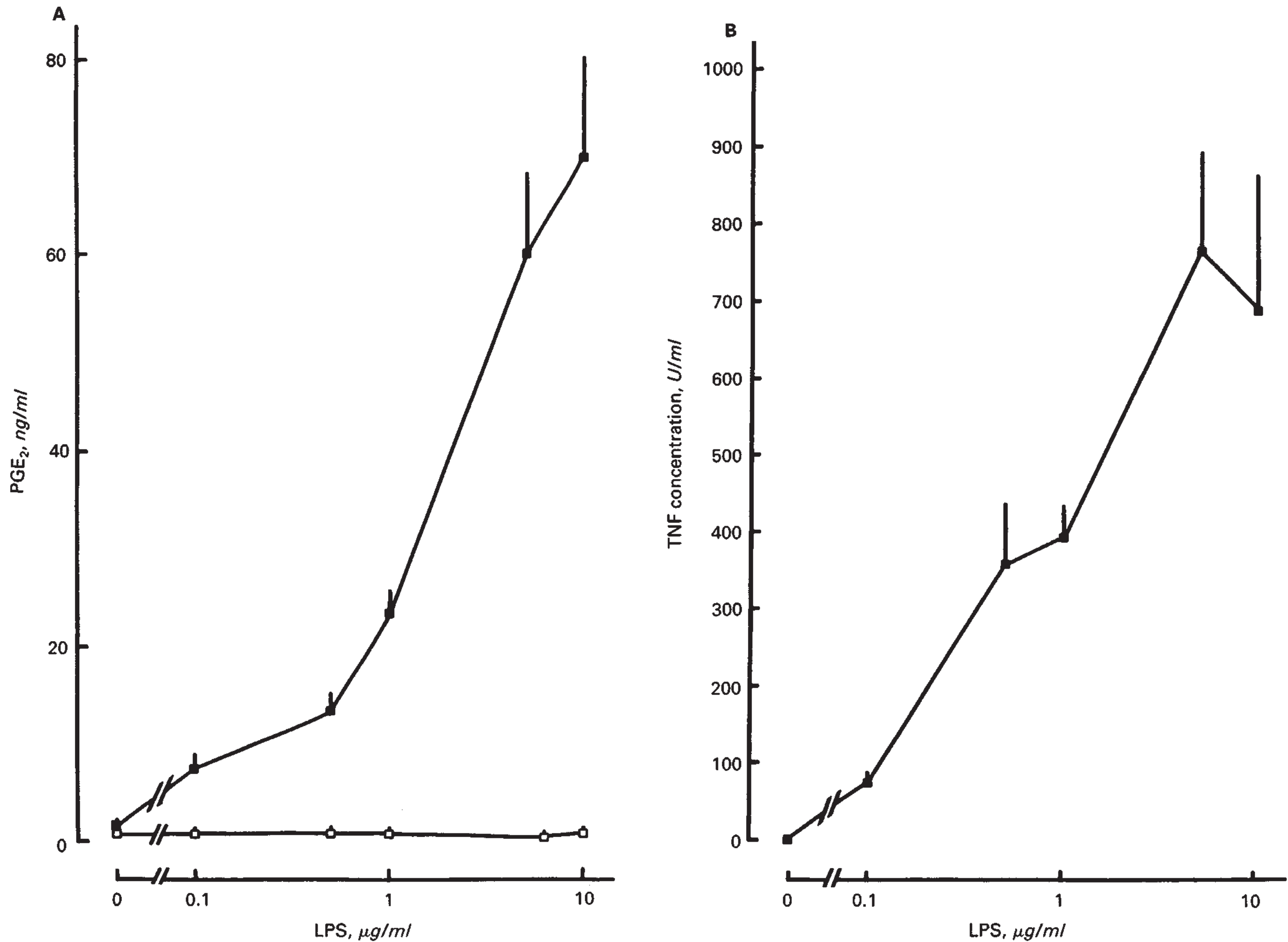

Fig. 4. *Effect of indomethacin on LPS-induced PGE_2 (A) and TNF (B) synthesis by cultured rat mesangial cells.* For PGE_2 synthesis (4 experiments), incubations were carried out during 14 hours with (□) or without (■) 1 μM indomethacin. For TNF synthesis (3 experiments), incubations were carried out over the same period in the presence of 1 μM indomethacin. Means and SEM are given. Control experiments (without indomethacin) are shown in Fig. 1A.

are inactive [22]. Moreover, glomerular cells are already known to be a target for gram-negative bacteria toxins and particularly for *Escherichia coli* toxin [27]. As shown on Figure 1B, the kinetics of LPS-induced TNF production demonstrated a progressive rise between three and nine hours, followed by an evident decay. Why TNF activity decreased at the later period was not determined, but two possibilities could be considered: First, degradation of TNF might be achieved in the presence of mesangial cell proteinases [2] as previously shown with L-929 and FS-4 cells [28]. Second, mesangial cells could release an inhibitory factor directed against TNF activity and similar to that identified in the urine of febrile patients [29]. Identification of TNF-like activity generated by LPS-activated mesangial cells was based on immunological, physical and functional similarities to recombinant TNF. Similarity in antigenicities of the mesangial cell-derived factor and TNF was indicated by the fact that anti-human and anti-mouse TNF polyclonal antibodies both reduced the cytotoxic activity of this factor (Fig. 2). Cytotoxicity was blocked only partially by anti-human TNF antibody, while anti-mouse TNF antibody completely neutralized it. These results indicate that human, mouse and rat TNF are similar in some of their antigenic epitopes but that a higher degree of homology exists in the three species between mouse and rat TNF than between human and rat TNF. Such a species specificity of anti-human and anti-mouse TNF neutralizing antibodies has been previously described [30].

Molecular weight determination of the TNF-like activity released by mesangial cells also revealed similarities with recombinant TNF. The cytotoxic activity recovered from the medium of LPS-activated mesangial cells under nondenaturing conditions exhibited an apparent molecular weight of 110,000 (Fig. 3A). This value is close to that reported for murine TNF when analyzed under identical conditions [16, 23]. Such a form of TNF could consist of either aggregates with different proteins [16] or of noncovalent multimers which are, in fact, much more active than the monomer [31]. The medium of LPS-activated mesangial cells also suppressed lipoprotein lipase activity in 3T3-L1 cells, thus exhibiting a cachectin activity similar to that of recombinant human TNF [12]. As such, it confirmed the capacity of rat mesangial cells to produce TNF-cachectin activity. Finally, TNF gene was expressed by these cells after exposure to LPS since their RNA hybridized to a murine TNF

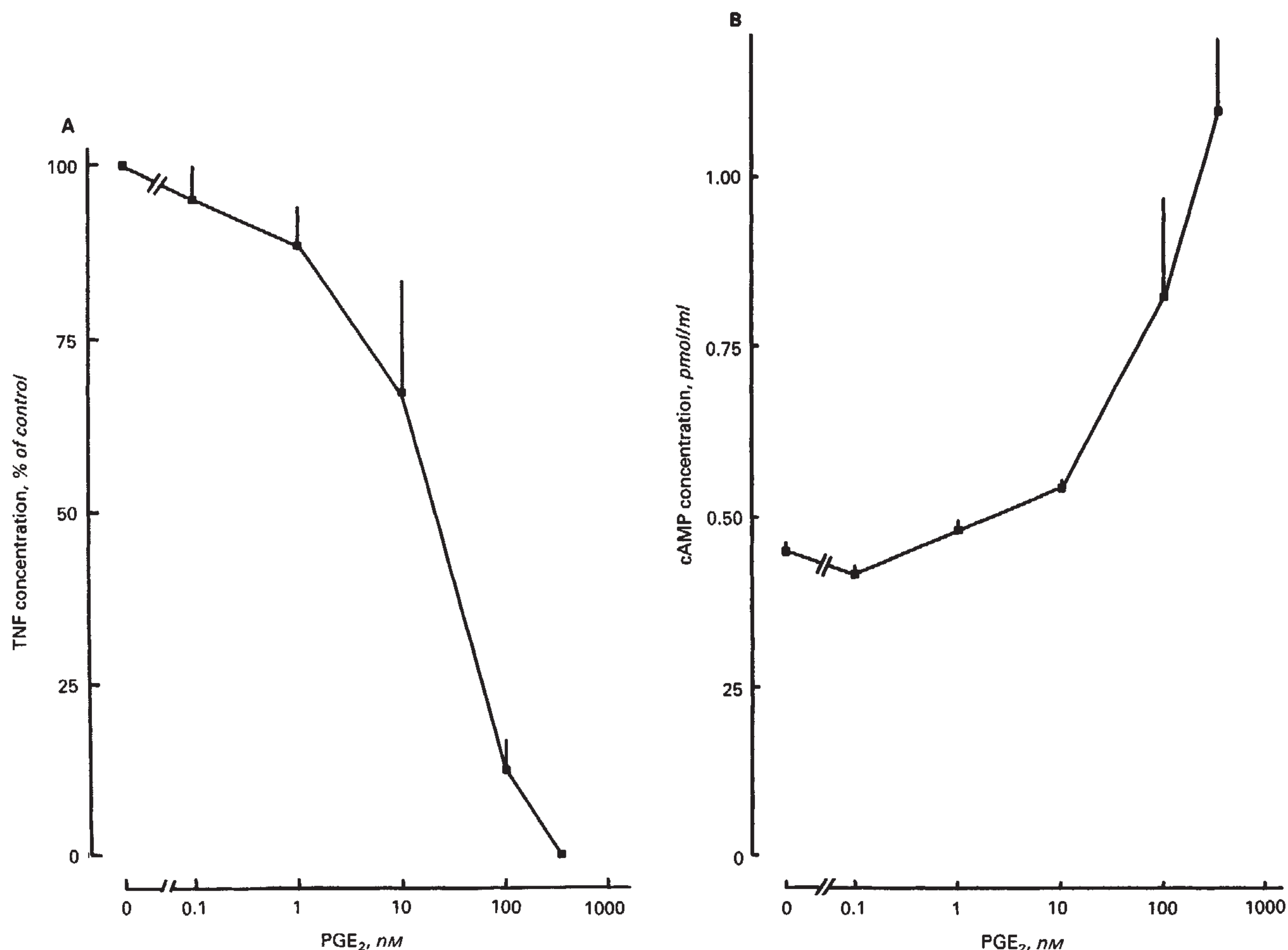

Fig. 5. *Effect of PGE_2 on LPS-induced synthesis of TNF* (**A**) *and cyclic AMP* (**B**) *by cultured rat mesangial cells.* Incubations were carried out for 14 hours with 10 μg/ml LPS and 1 μM indomethacin. Means and SEM of values obtained in 3 experiments are given.

cDNA probe in Northern blot (Fig. 3B). This probe recognized two bands as previously described for LPS-treated macrophages; in fact, the weaker of these bands could represent a precursor of the main TNF RNA [32].

After identification of TNF as a mediator released by rat mesangial cells in response to LPS challenge, the mechanisms whereby its production was regulated were also determined. We have demonstrated that both endogenous and exogenous PGE_2 down-regulate TNF production by mesangial cells (Figs. 4 and 5). Indeed, LPS induced the production of TNF as well as that of PGE_2, and treatment of the cells with indomethacin promoted both inhibition of PGE_2 synthesis and stimulation of TNF production. However, indomethacin effect on TNF production was significant only for LPS concentrations above 1 μg/ml, suggesting that PGE_2 was a potent regulator at concentrations higher than 20 ng/ml (56.8 nM). These data explain the difference in the patterns of LPS dose response curves for TNF (Fig. 1) and PGE_2 (Fig. 4A) productions. Activation of mesangial cells by LPS yielded to parallel productions of TNF and PGE_2, only when TNF synthesis was determined in the presence of indomethacin (Fig. 4B). The inhibitory effect of PGE_2 was confirmed by the reduction of TNF synthesis in the presence of exogenous PGE_2 at doses ranging between 10 and 100 nM. Our observations are in general agreement with those of other investigators who have reported that PGE_2 regulates macrophage-derived TNF gene expression [33]. They also suggest that several mediators, released in the glomerulus during inflammatory processes, could modulate local TNF production by enhancing PGE_2 production. These mediators include angiotensin II, platelet activating factor, bradykinin [34], hydrogen peroxide [35], complement components [36] and IL1 [37]. The physiological mechanisms whereby PGE_2 reduces TNF synthesis by mesangial cells are still unknown, but they probably require cAMP generation since: (1) exogenous PGE_2 induced an increase of cAMP production by mesangial cells treated by both LPS and indomethacin (Fig. 5B) and (2) 8-bromo-cAMP promoted a dose-dependent reduction of TNF synthesis under identical conditions (Fig. 6). This suggests that inflammatory mediators, such as histamine, which stimulate cAMP accumulation in mesangial cells without modifying prostaglandin production [38] could also modulate TNF synthesis.

The physiological role of TNF production by mesangial cells remains to be determined. Unlike IL-1 [5] or platelet-derived growth factor [6], TNF was not synthesized in the absence of LPS challenge. Thus, its production cannot be considered as an autocrine mechanism whereby mesangial cells in culture would

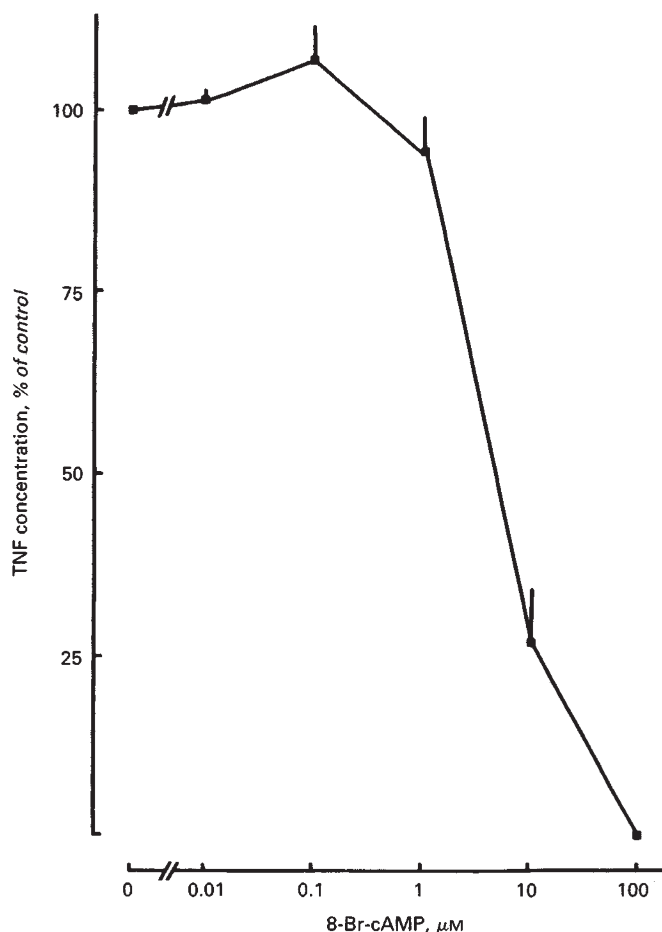

Fig. 6. *Effect of 8 bromo-cyclic AMP on LPS-induced TNF synthesis by cultured rat mesangial cells.* Incubations were carried out for 14 hours with 10 μg/ml LPS and 1 μM indomethacin. Means and SEM of values obtained in 3 experiments are given.

promote their own proliferation. In contrast, mesangial cell-derived TNF could participate in inflammatory processes within the glomerulus in several ways. It may first stimulate the synthesis of PGE_2, $PGF_{2\alpha}$ and prostacyclin by both resident glomerular cells [13] and infiltrating macrophages [39]. Moreover, because TNF has been shown to increase the cAMP content of mesangial cells by prostaglandin-dependent and independent mechanisms [13], it is likely that it induces the production of its own inhibitor. Finally, TNF, which has been released within the glomerulus either alone or together with IL-1, can also regulate several activities of endothelial cells including expression of surface antigens promoting neutrophil adherence and enhancement of procoagulant activity [12]. Whether TNF production by mesangial cells may play a role in vivo in the course of renal and, specifically, glomerular diseases remains to be determined. It is conceivable that such a local synthesis participate in the mechanisms of endotoxin-induced renal injury which have been shown to imply resident renal parenchymal cells rather than infiltrating bone marrow-derived cells [40].

Acknowledgments

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