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Participation of Cytokines in Proliferation of Mesangial Cells

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Summary. **Immunohistochemistry using monoclonal antibody to rat macrophage identified infiltrating macrophages in mesangial cell proliferative glomerular lesions induced by intravenous administration of an antibody against mesangial cell and suggested a role for macrophage-derived cytokines on the proliferation of mesangial cells. Production of tumor necrosis factor (TNF) and interleukin 1 (IL-1) was enhanced in culture medium of the glomeruli isolated from rat kidneys with the mesangial cell proliferative lesions. Further, recombinant TNF, IL-1 and culture medium of lipopolysaccharide-silica-stimulated rat macrophages promoted a proliferative response in quiescent rat mesangial cells in culture especially when fetal bovine serum was concomitantly present. However, the gel chromatographic profile of the stimulated macrophage culture medium demonstrated a different peak and therefore, different molecular weight of mesangial cell proliferation-promoting activity compared to the peaks of TNF and IL-1 activities.**

These findings suggested that TNF and IL-1 as well as other factor(s) derived from macrophages enhanced proliferation of mesangial cells in the glomerulus.

INTRODUCTION

Anti-thymocyte serum (ATS) was shown to react with mesangial cells and induce injury and proliferation of these cells when given intravenously.[1] However, the mechanism of the mesangial cell proliferation is still unknown. Since participation of cytokines in the development of glomerulonephritis has been suggested in previous observations,[2] we examined the presence of macrophages in the glomeruli of rats with ATS-induced mesangial cell proliferation, production of tumor necrosis factor (TNF) and interleukin 1 (IL-1) in glomeruli, and the effects of these cytokines on the proliferation of rat mesangial cells in culture.

MATERIALS AND METHODS

Mesangial cell proliferative glomerulonephritis models

ATS raised in rabbits by the method described previously[3] was given intravenously in Lewis rats (0.5 ml/100 g body weight) to induce mesangial cell proliferative glomerular lesions and the kidneys were obtained after 7 days for histology and isolation of glomeruli. As a control, normal rabbit serum (NRS) was given intravenously.

The kidneys were fixed in 10% buffered formalin overnight, embedded in paraffin, and sectioned. The sections were deparaffinized, hydrated and incubated with monoclonal antibody against rat macrophage (ED1, Serotec, Bioproducts for Science, Inc, Indianapolis, USA) for identification of macrophages in the tissue by the immunoperoxidase technique.

The glomeruli were isolated by a standard sieving method, suspended at a density of 5×10^3 glomeruli/ml in RPMI 1640 medium containing 1% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin (1% FBS medium) and cultured for 24 h in a CO_2 incubator at 37℃. The culture medium was used for evaluation of TNF and IL-1 activity by L929 cytotoxicity and thymocyte-costimulation assay, respectively. As a standard, human recombinant TNF alpha (rTNF, 3×10^6 U/mg protein) supplied through the kindness of Dinippon Pharmaceutical Co. (Osaka, Japan) and human recombinant IL-1 beta (rIL-1, 2×10^7 U/mg protein) from Otsuka Pharmaceutical Co. (Tokushima, Japan) were used.

Macrophage-derived cytokines

Rat peritoneal macrophages were obtained from the

peritoneal cavity of Lewis rats, each of which received 20 ml of thioglycolate medium intraperitoneally 4 days earlier. After washing with RPMI 1640 medium, the cell density of the exudate cells was adjusted to 5×10^6 /ml in 1% FBS medium and the cells were divided into two groups. One group of cells was stimulated with 10 μg/ml lipopolysaccharide (LPS, E. Coli, 055: B5, Sigma Chemical Co, St. Louis, USA) and 50 μg/ml silica (0.014 μm, Sigma) for varying time periods in culture dishes while the other group was not stimulated. The culture supernatants were collected and used for measurement of IL-1 and TNF activities and for evaluation of their effects on mesangial cell proliferation in culture.

The culture medium of macrophages stimulated for 24 h was concentrated 10 times by ultrafiltration using a YM 15 membrane (Amicon Corp, Lexington, USA) and fractionated by Sephadex G-100 gel chromatography. The effect of each fraction as well as the effect of rIL-1 and rTNF on the proliferation of cultured mesangial cells was examined as described below.

Assay for mesangial cell proliferation

The cultured rat mesangial cells (3-10th passage)[4] were suspended at a concentration of 5×10^4/ml in 10% FBS medium and 100 μl of each cell suspension was placed in a well of plastic 96-well culture plates. After overnight culture in a CO_2 incubator, the culture medium was replaced with 0.5% FBS medium (100 μl/well) to achieve quiescence of the cells (serum-deprivation arrest). Six days later, the medium was replaced with 50 μl of RPMI 1640 medium supplemented with or without 5% FBS. Then, the test samples (50 μl/well) were added and 24 h later tritiated thymidine (0.5 μCi/well) was pulsed for 24 h. The mesangial cells were trypsinized and harvested on glass filter and the radioactivity was counted in a scintillation counter.

RESULTS AND DISCUSSION

By the immunoperoxidase technique, infiltration of macrophages was visualized in the mesangial cell proliferative glomerular lesions induced by intravenous administration of ATS in Lewis rats (Fig. 1). The accumulation of macrophages in mesangial hypercellular lesions was previously speculated by the presence of leukocyte common antigen-positive or Ia antigen-positive cells or mononuclear leukocytes in association with Thy-1- and desmin-positive mesangial cells in the glomeruli.[5]

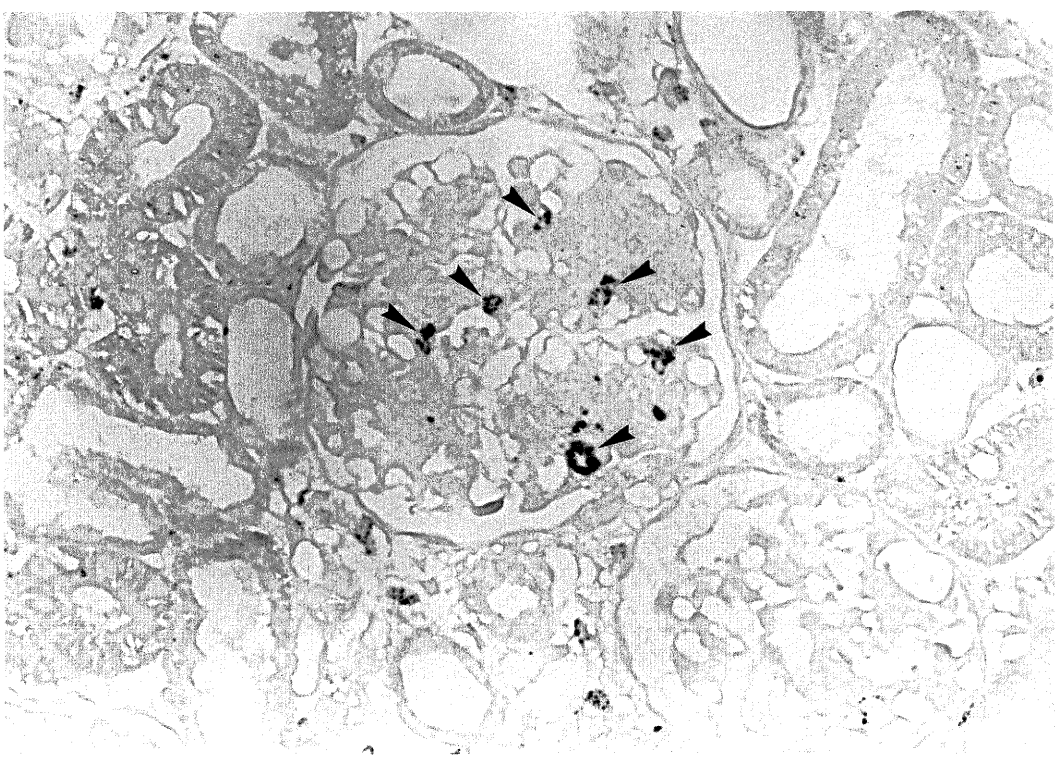

Fig. 1. Immunoperoxidase staining shows several cells labeled with a monoclonal antibody against rat macrophages (arrowheads) in the glomerulus of a kidney with ATS-induced mesangial cell proliferative glomerular lesion (×400).

As shown in Fig. 2, both TNF and IL-1 activities were elevated in the culture supernatant of the nephritic glomeruli in comparison with those of control glomeruli isolated from NRS-administered rats. However, activities of both cytokines were smaller than those in the LPS-silica-stimulated macrophage culture medium. Generation of cytokines has been assumed to be related to inflammatory processes in glomeruli. The present results support this assumption. Macrophages have been shown to produce many kinds of cytokine in response to various stimuli. We examined the kinetics of TNF and IL-1 released from rat peritoneal macrophages stimulated with LPS and silica and the effect of macrophage-derived factors on the proliferation of rat mesangial cells in culture. The release of IL-1 in the culture supernatant by the stimulated macrophages increased gradually and reached a plateau at 24 h, whereas TNF was released immediately with a peak at 4 h as shown in Fig. 3. However, no or minimal production of these cytokines was observed when macrophages were not stimulated.

The effect of factors derived from macrophages stimulated with LPS and silica for 24 h on the proliferation of rat cultured mesangial cells was examined and compared to the effect of rTNF or rIL-1. No stimulatory or inhibitory proliferative responses of quiescent rat mesangial cells were elicited by culture supernatant of the stimulated or unstimulated macrophages alone. This held true for rTNF or rIL-1 as well (Fig. 4). However, proliferation of the quiescent mesangial cells was significantly enhanced when the macrophage culture supernatant or cytokines were supplemented with 5% FBS. Stimulation of cultured mesangial cell proliferation by IL-1 in the presennce of FBS has been shown previously.[6] We demonstrated here that TNF also enhanced mesangial cell proliferation in the presence of FBS. Although the culture supernatant of the stimulated macrophage facilitated the proliferative response more than that of unstimulated macrophage, the latter still had significant potential for mesangial cell proliferation. This finding suggested that macrophages released factor(s) other than TNF or IL-1 which stimulated mesangial cell proliferation. As shown in Fig. 5, one major peak of stimulatory activity of mesangial cell proliferation was revealed in the fractionated culture medium of stimulated macrophages. This peak (approximately 10 KDa) did not correspond to TNF or IL-1 activity. Further study is necessary to con-

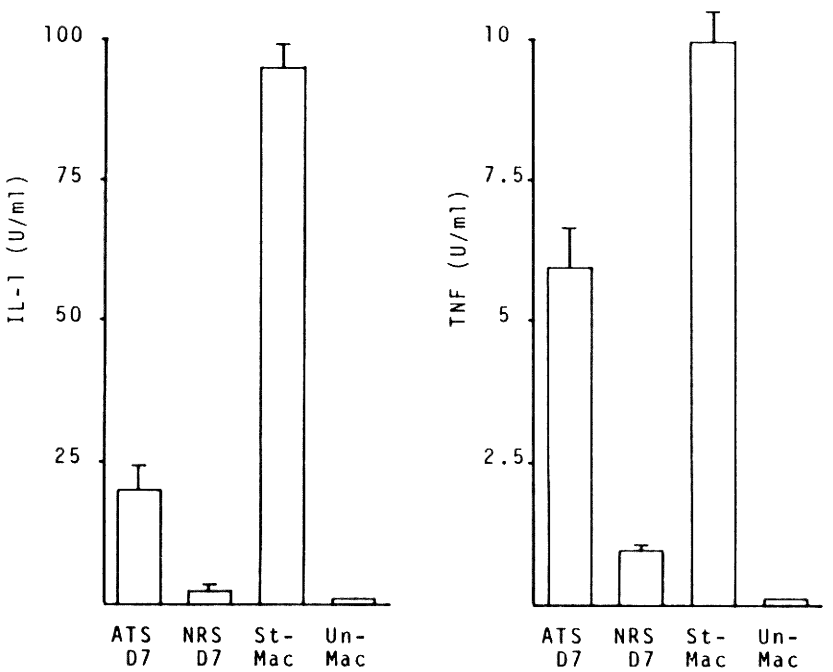

Fig. 2. IL-1 and TNF activities in the 24-h culture medium (1:10 dilution for IL-1 assay and neat medium for TNF assay) of the glomeruli (5×10^3/ml) isolated from rats given ATS (ATS D7) or NRS 7 days earlier (NRS D7), and of macrophages stimulated (ST-Mac) or unstimulated (Un-Mac) with LPS and silica for 24 h (1:625 dilution for IL-1 assay and 1:10 dilution for TNF assay).

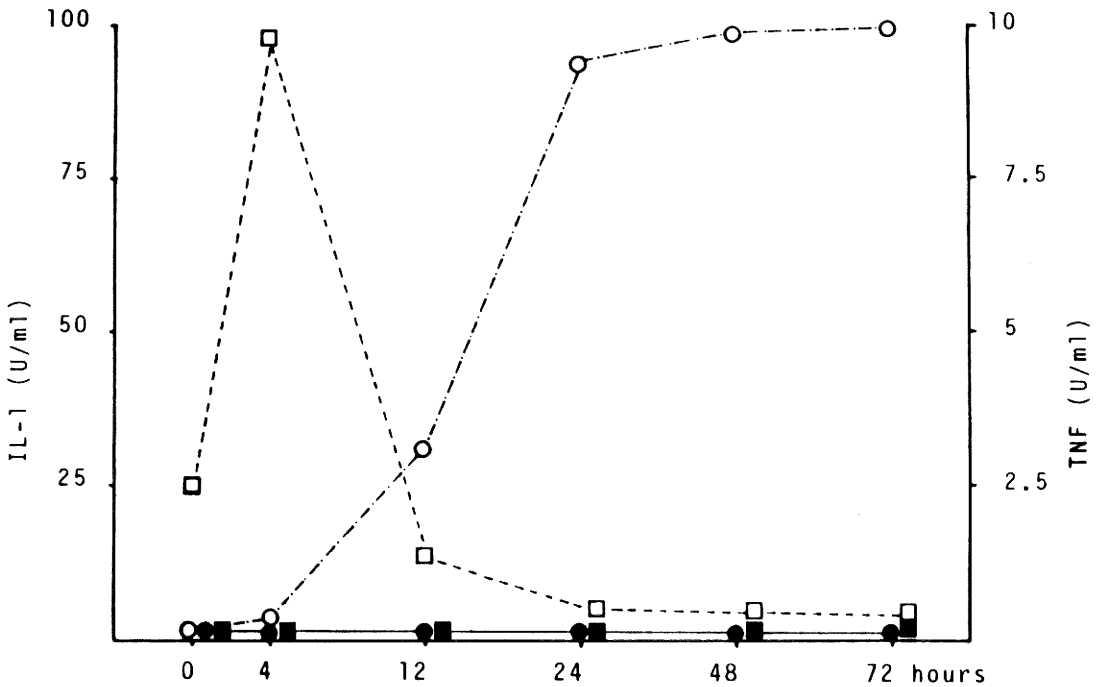

Fig. 3. Kinetics of TNF (□) and IL-1 (○) activities released during varying time periods from stimulated (open symbol) or unstimulated (closed symbol) macrophages.

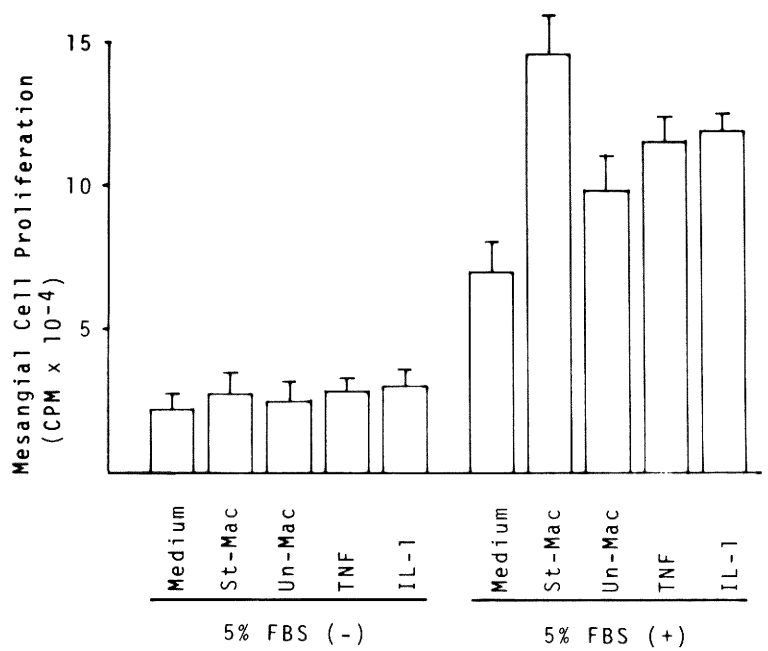

Fig. 4. Effect of culture medium (1:20 dilution) of stimulated (St-Mac) or unstimulated (Un-Mac) macrophages on proliferation of quiescent cultured rat mesangial cells compared to the effect of rTNF or rIL-1 (50 U/ml each) in the absence or presence of 5% FBS.

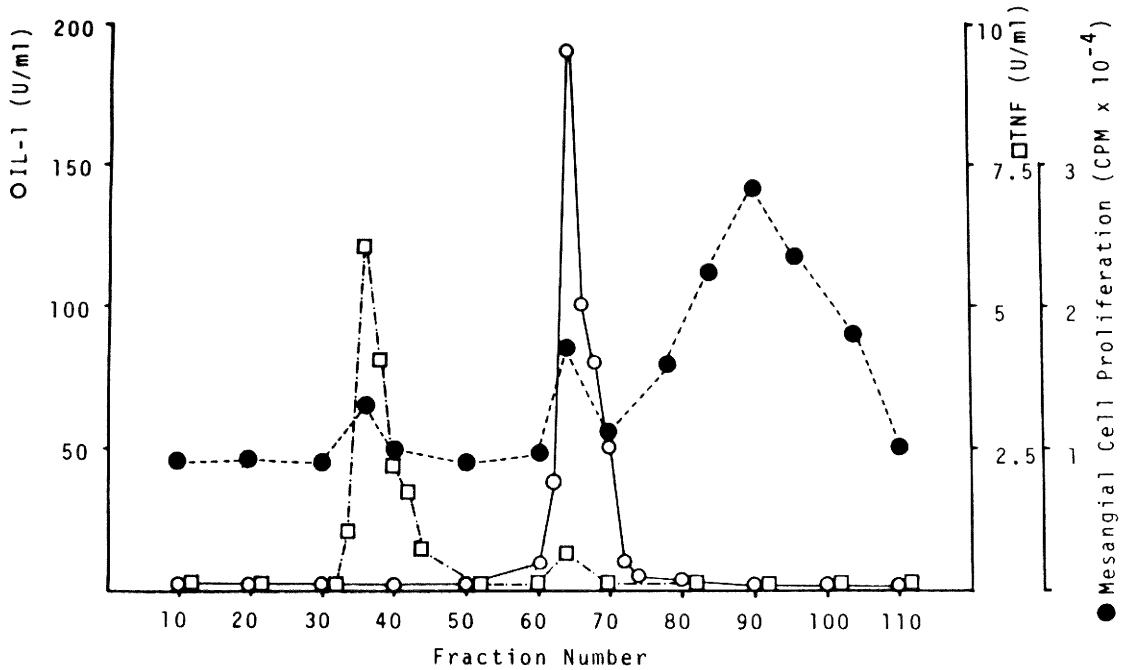

Fig. 5. TNF (□), IL-1 (○) and mesangial cell proliferation-promoting activities (●) in the fractions of the LPS-silica-stimulated macrophage culture medium separated through Sephadex G-100.

clude whether this peak contains new factors or breakdown products of TNF or IL-1.

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