pression vectors (BEV)[15], and the hormone super-expressed during infection of lepidopteran larvae to synergize the viral pathology[6]. In a preliminary experiment, *Bombyx mori* larvae infected with *B. mori* BEV transformed with a synthetic MasDH coding sequence expressed under control of the viral polyhedrin promoter resulted in a 30% decrease in hemolymph volume and larval death one day earlier than controls[16]. Although these latter effects are modest from the standpoint of insect control, they suggest that the concept of manipulating insect neurohormone levels is possible.

In conclusion, these studies provide evidence that insect water balance can be disturbed in vivo by exogenously administered diuretic peptides. The means to alter insect neurohormone titers are not presently available, but water balance and its neuroendocrine regulation are a likely focus for future research on insect pest control using advances in the peptide and genetic bioengineering technologies.

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Modulation of C2 and C3 gene expression of human peripheral blood monocytes by interleukin 1β, interferon γ, tumor necrosis factor α and lipopolysaccharide

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Abstract. The effect of interleukin 1β (IL-1β), interferon γ (IFN-γ), tumor necrosis factor α (TNFα) and lipopolysaccharide (LPS) on the expression of the C2 and C3 genes in human adherent monocytes was studied. Stimulation of monocytes with IFN-γ increased both C2 and C3 mRNA. IL-1β also increased C2 mRNA level, whereas C3 gene expression was not enhanced. TNFα failed to increase either C2 or C3 mRNA. LPS increased C2 mRNA, but suppressed C3 gene expression. These results suggest that C2 and C3 production by monocytes is regulated by IL-1β and IFN-γ in the local tissues.
Key words. IL-1β; IFN-γ; LPS; C2; C3.

Monocytes have many activities including phagocytosis, superoxide release, and production of IL-1β and TNFα, and play a central role in the inflammatory disorders[1]. Complement components are also produced by monocytes and are thought to be important in local inflammatory reactions in such diseases as rheumatoid arthritis (RA)[2, 3] and systemic lupus erythematosus (SLE)[4]. It is likely that synthesis of complement components in local tissues by monocytes/macrophages is regulated by several cytokines. Therefore, we studied the effect of IL-1β, TFNα, IFN-γ and LPS on C2 and C3 gene expression in human peripheral blood monocytes.

Materials and methods

Materials. RPMI 1640, L-glutamine and penicillin-streptomycin (Gibco, Grand Island, NY), Hanks' balanced salt solution (HBSS, Nissui, Tokyo, Japan), phosphate buffered saline (PBS, Nissui), cesium chloride (Boehringer Mannheim, Indianapolis, IN), guanidine-isothiocyanate (Fluka, Buchs, Switzerland), Hybond-N+, multiprime DNA labeling systems (Amersham, Arlington Heights, IL), [^{32}P]dCTP (ICN, Irvine, CA), ITS+ (Collaborative Research, Bedford, MA), Falcon 3803 Primaria culture dishes (Becton Dickinson Labware, Lincoln Park, NJ), and LPS (*E. coli* 026 : B6, Dif-

co, Detroit, MI), were obtained from the suppliers noted. Human recombinant IL-1β (Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan), TNFα (Dainippon Pharmaceutical Co. Ltd., Osaka, Japan) and IFN-γ (Takeda Pharmaceutical Co. Ltd., Osaka, Japan) were kindly provided by each company. cDNA probes were purchased from American Type Tissue Culture Collection (ATCC). Purified plasmid DNA, pC2-F2 (ATCC No. 59499) and PC3-AL1 (ATCC No. 59501), were rehydrated with TE and digested with restriction enzyme Pst I, and then used for labelling.

Cell separation and culture. Leukocyte-rich human plasma was obtained as a by-product of apheresis, and mononuclear cells were isolated using a Ficol-Hypaque gradient. Mononuclear cells were washed twice with PBS and resuspended at 1×10^7 cells/ml in RPMI 1640 containing 10% heat-inactivated human AB serum. The cells were cultured in Primaria dishes at 37 °C for 2 h and were allowed to adhere. Adherent monocytes were then rinsed vigorously with HBSS and were cultured for a further 24 h in serum-free RPMI 1640 containing ITS+ with or without 20 U/ml IL-1β, 1000 U/ml TNFα, 1000 U/ml IFN-γ and 5 µg/ml LPS.

Northern blot analysis. After 24 h of culture, adherent mononuclear cells were harvested. Total cellular RNA was isolated by guanidine-isothiocyanate extraction and ethanol precipitation[5]. RNA was quantified by absorbance at 260 nm (1 OD_{260} = 40 µg/ml RNA). Total cellular RNA (50 µg) from each sample was denatured with formaldehyde, subjected to agarose-gel electrophoresis and transferred to nylon membrane Hybond-N+ by blotting.

cDNA probes were labeled with [^{32}P]dCTP by multiprime DNA labeling systems. Hybridization was performed at 42 °C for 48 h. Membranes were washed three times in 0.15XSSC/0.1%SDS at 65 °C (for 15 min), and then exposed to X-ray film at − 80 °C.

Results

After 2 h of culture in human AB serum-containing medium monocytes were further cultured in serum-free RPMI 1640 supplemented with ITS+. Under these conditions, C2 and C3 were expressed without stimulation (control, figs 1 and 2). When monocytes were stimulated with IL-1β, IFN-γ and LPS, C2 gene expression was enhanced. IFN-γ was most effective on C2 gene expression. However, stimulation with TNFα failed to show any effect on C2 mRNA levels. As shown in figure 2, stimulation with IFN-γ resulted in an increase in C3 mRNA, whereas IL-1β and TNFα had no apparent effect

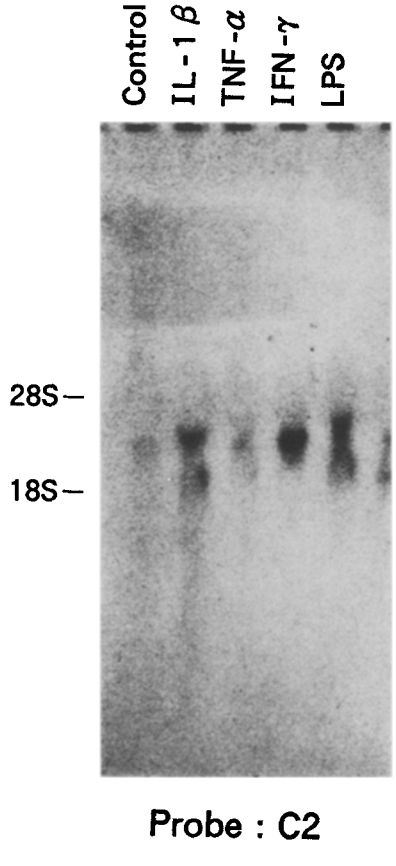

Figure 1. Northern blot analysis of C2 mRNA from cultured human peripheral blood monocytes. 50 µg of total cellular RNA from three subjects was applied in each lane.

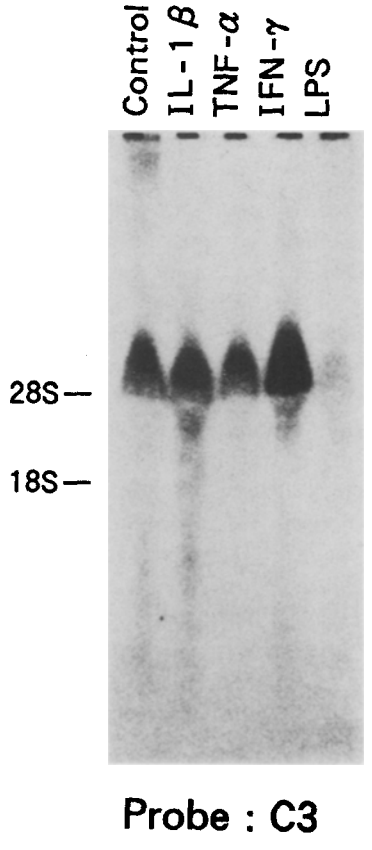

Figure 2. Northern blot analysis of C3 mRNA from cultured human peripheral blood monocytes. 50 µg of total cellular RNA from three subjects was applied in each lane.

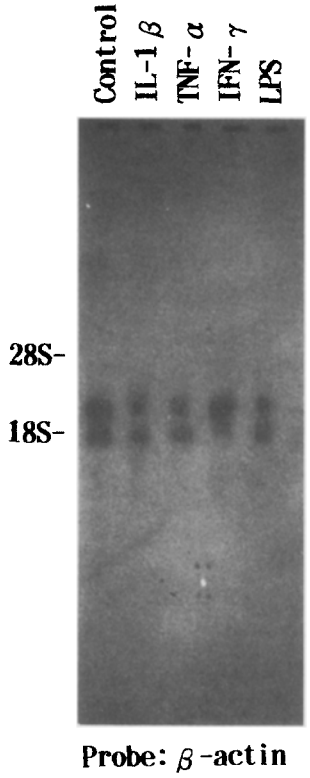

Figure 3. Northern blot analysis of β-actin mRNA of the blotted membrane as shown in figures 1 and 2.

on C3 gene expression in monocytes. LPS appeared to suppress C3 gene expression in monocytes. Expression of β-actin mRNA is shown in Figure 3 as internal control.

Discussion

Recently, several workers have shown that cytokines modify complement component production in monocytes. IFN-γ mediates a concentration- and time-dependent increase in steady-state levels of C4 mRNA[6]. C2 production by monocytes was increased by stimulation with IFN-γ and interleukin 4[7]. However, C3 production by monocytes remained to be investigated. In this study, we showed that stimulation of monocytes with IFN-γ resulted in an increase in C3 and C2 mRNA. We also studied the effect of IL-1β on C2 and C3 gene expression. Our data demonstrate that IL-1β enhanced C2 gene expression without any effect on C3 mRNA. TNFα had no effect on C2 and C3 production in spite of functional similarities to IL-1β. Thus, IFN-γ has a common stimulatory effect on complement component production in monocytes.

It is interesting to note that complement components produced by monocytes, which infiltrate local tissues, may accelerate an inflammatory process. In RA synovial fluid macrophages synthesize C2, C3, C4, C5 and factor B[2], and local production of these complement components may be important in inflammatory response. Synovial fluid macrophages synthesize more C2 than peripheral blood monocytes in RA, and RA synovial cells produce cytokines including IL-1β[8]. In murine lupus nephritis, local expression of complement genes including C2, C3, C4 and factor B was increased, and an increase in the synthesis of C3 and factor B was associated with glomerular and renal interstitial macrophage infiltration[4].

Thus, our results suggest that IL-1β produced in situ may stimulate monocytes/macrophages leading to local synthesis of complement components. IFN-γ released by T lymphocytes infiltrating the tissues may be more important than IL-1β.

LPS increased C2 mRNA levels but suppressed C3 gene expression. Increase in C2 gene expression may be attributed to IL-1β produced by monocytes stimulated with LPS. Recently, Kulics et al.[6] reported counterregulatory effects of LPS and IFN-γ on C4 gene expression and synergistic effects on factor B synthesis in mononuclear phagocytes. They proposed an interesting model in which the initial host response at local sites of bacterial infection shifted from classical complement pathway activation to alternative pathway activation. However, our data show that LPS enhanced C2 and suppressed C3 gene expression, indicating a different pathway is involved in the synthesis of each component. The reason for the decrease in C3 mRNA remains unknown.

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