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Secretion of interleukin-6 (IL-6) by human monocytes stimulated by muramyl dipeptide and tumour necrosis factor alpha

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SUMMARY

We studied IL-6 gene expression in human monocytes stimulated by muramyl dipeptide (MDP), a synthetic immunomodulator derived from mycobacterial cell walls. In control monocytes, two IL-6 transcripts of 3·4 kb and 1·6 kb were easily detected at 2·5 hr of culture and remained stable until 18 hr. In MDP-treated monocytes, three IL-6 RNA species displayed different kinetics of accumulation: a 3·4 kb RNA whose expression already reached its maximum after 2·5 hr exposure to MDP; a 1·6 kb RNA whose expression peaked at 5 hr; and a new RNA species of 1·4 kb which was transiently induced in early time of cell stimulation. TNF-α co-operated with MDP to increase IL-6 gene expression and secretion of biological active protein (measured by the hybridoma plasmacytoma growth factor assay). MDP exhibits a broad spectrum of immunomodulation properties such as adjuvant activity, enhancement of macrophage cytotoxicity against tumour and induction of non-specific resistance to intracellular agents. The results reported here suggest that these properties might be linked to the stimulation by MDP of genes coding for key cytokines such as IL-6, TNF and IL-1.

INTRODUCTION

We recently showed that the HuIFN-β2 gene is spontaneously expressed in human monocytes (Sancéau *et al.*,1988).This was done using a HuIFN-β2 probe derived from super-induced human fibroblasts (Zilberstein *et al.*,1986). IFN-β2 cDNA is now known to be identical to the cDNA of the 26,000 MW molecule described by Haegeman *et al.* (1986) and to that of the B-cell stimulating factor 2 (BSF-2) which induces terminal differentiation of B cells to produce immunoglobulins (Hirano *et al.*, 1985) and which was recently cloned by Hirano *et al.* (1986). IFN-β2 cDNA is also identical to the hybridoma/plasmacytoma growth factor (Bradkenhoff *et al.*,1987; Van Damme *et al.*, 1987) and to the hepatocyte stimulating factor (Gauldie *et al.*, 1987). This cytokine is now nominated interleukin-6 (IL-6).

Since IL-6 is expressed in human monocytes, it was of interest to study the effects on its expression of monocyte exposure to adjuvant molecules such as MDP known to amplify the antibody response (Lederer, 1980; Adam, 1985; Leclerc & Chedid, 1986). MDP is the minimal adjuvant active structure (Ellouz *et al.*, 1974) derived from mycobacterial cell walls (Wietzerbin *et al.*, 1974). It also stimulates non-specific resistance to bacterial and parasite infections *in vivo* and *in vitro* (Lederer, 1980; Leclerc *et al.*, 1986; Parant, 1987). However, very little is known about the intracellular events elicited by MDP in immunocompetent cells.

The major target cell for MDP was identified as the monocyte/macrophage (Leclerc *et al.*, 1986). We therefore decided to study the possible relationship between the biological properties of MDP and the expression of the IL-6 gene in monocytes. We also studied the effect of simultaneous treatment of human monocytes with MDP and TNF-α, because MDP was recently reported to synergize with TNF for the induction of mouse resistance to intracellular agents such as *Candida albicans* (Parant, 1987). MDP was also shown to induce IL-1 and TNF-α activities in macrophage cultures (Oppenheim *et al.*, 1980; Parant, 1987; Vermeulen *et al.*, 1987). Since in other systems these cytokines were reported to be implicated in IL-6 gene expression (Content *et al.*, 1985; Kohase *et al.*, 1986; Defilippi *et al.*,1987; Kohase *et al.*, 1987; Van Damme *et al.*, 1987), we analysed the effect of MDP on the induction of IL-1 mRNA and TNF-α mRNA.

Abbreviations: BSF-2, B-cell stimulatory factor 2; HGF, hybridoma/plasmacytoma growth factor; HuIL-1β, human interleukin-1β; HuTNF-α, human tumour necrosis factor α; IL-6, interleukin-6; MDP, muramyl dipeptide; PBS, phosphate-buffered saline.

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MATERIALS AND METHODS

Cells

Monocytes were separated from human peripheral blood mononuclear cells freshly obtained by cytapheresis from healthy

donors (Centre de Transfusion Sanguine de l'Hôtel-Dieu, Paris, France) by centrifugation on a Percoll gradient (Wright & Silverstein, 1982). Briefly, cells were pelleted at 300 *g* for 10 min at 4°, washed twice in cold phosphate buffer and adjusted to $40–50 \times 10^6$ cells/ml in RPMI-1640 culture medium (Boehringer, France) containing 10% normal human AB serum. Five millilitres of mononuclear cell suspensions were layered on the preformed Percoll gradient, which was immediately centrifuged for 25 min at 4° in a swinging bucket rotor at 1500 *g*. Monocytes were recovered (top band: specific gravity 1055 g/cm^3) and washed twice in cold complete medium. Enriched monocyte suspensions (>95%), as monitored by naphtyl esterase (Sigma Diagnostics) were adjusted to 10^6 cells/ml in RPMI-1640 medium supplemented with 2 mM L-glutamine, 40 μg/ml gentamycin (Unicet Laboratories, France), 15 mM Tricin, pH 7·4, and 10% endotoxin-free fetal calf serum (Gibco BRL, France).

Monocyte stimulation

Monocytes were incubated for the indicated times at 37° (5% CO_2) without or with MDP, 50 μg/ml (Choay, France) and/or 4 ng/ml human recombinant TNF-α, specific activity: 5×10^7 U/mg protein, produced by Genentech and provided by Boëhringer Ingelheim (Vienna, Austria). Cells from each donor were processed separately.

RNA preparation and Northern blotting

Total RNAs were extracted by the guanidinium isothiocyanate method and Northern blots were performed, as previously described (Vaquero *et al.*,1982). Blotted RNAs were hybridized with the various nick-translated probes. Human IFN-β2 cDNA (IL-6 cDNA) (1·3 kb) was a gift from M. Revel (Rehovot, Israel) (Zilberstein *et al.*, 1986) and human IL-1β cDNA (0·9 kb) was a gift from Upjohn Company (Kalamazoo, U.S.A.).

TNF assay

TNF activity in monocyte supernatants was measured using actinomycin D-treated L929 targets (Sugarman *et al.*, 1985).

IL-1 assay

IL-1 activity was measured in the C3H/HeJ mouse thymocyte comitogenic assay as described by Mizel *et al.* (1978).

HGF assay

The assay for HGF (hybridoma/plasmacytoma growth factor) was performed as described by Van Snick *et al.* (1986) by incubating factor dependent hybridoma cells (Mouse-mouse hybrid 7TD.1) with serial dilutions of monocyte supernatants. After 4 days, the number of cells was evaluated by colorimetric determination of hexosaminidase levels. A dilution of recombinant IL-6 (1000 U/ml) produced in our laboratory was used as an internal standard in all HGF assays.

RESULTS

Enhancement of IL-6 RNA levels in monocytes by MDP and TNF

Human monocytes were treated with MDP and/or TNF-α for the different periods of time and their total RNA were extracted and submitted to Northern blot analysis. A typical experiment is shown in Fig. 1. As we have already reported (Sancéau *et al.*, 1988) in RNA from freshly harvested cells, a 1·6 kb faint band was detected by hybridization with a IL-6 probe (not shown). In control untreated cultured monocytes, two IL-6 transcripts of 3·4 and 1·6 kb were detected, reached their almost maximal level at 2·5 hr and then remained stable until 18 hr.

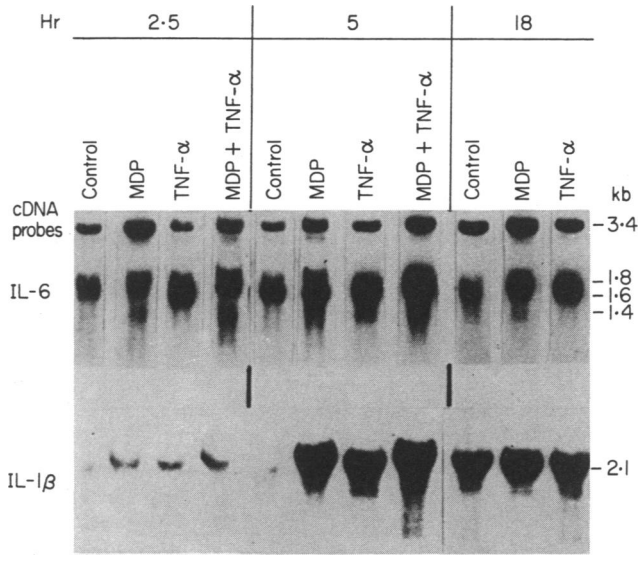

Figure 1. Kinetics of IL-6 and IL-1β RNA accumulation in human monocytes treated with MDP and/or TNF-α. Total RNA (10 μg), extrated at the indicated times, was run on agarose gel, transferred to Gene Screen membrane (NEN), and probed with sequences of ^{32}P-IL-6 (top panel) or ^{32}P-IL-1β (bottom panel). The positions of the specific RNA are indicated on the right of the radiogram. Exposure time was 24 hr.

In MDP-treated monocytes, three RNA species, which displayed different kinetics, hybridized with the IL-6 probe: a 3·4 kb RNA whose expression already reached its maximum after 2·5 hr exposure to MDP; a 1·6 kb RNA whose expression peaked at 5 hr and remained stable until 18 hr, and a new RNA species of 1·4 kb which was transiently induced, in early time of cell stimulation. Other groups reported a single 1·3 kb transcript as corresponding to the IL-6 mRNA in fibroblasts (Content *et al.*, 1985; Hirano *et al.*, 1986; Zilberstein *et al.*, 1986; Defilippi *et al.*, 1987; Kohase *et al.*, 1987). No significant differences in stimulated or unstimulated cells were detected when the same blot was rehybridized with a probe of a constitutively expressed gene: β-actin (not shown).

TNF treatment alone induced slightly the 1·4 kb RNA species, only after 5 hr of culture, which was undetectable thereafter. Its level was enhanced (compared to treatment with MDP alone) when monocytes were simultaneously treated with TNF and MDP. This possible synergistic action was observed at 2·5 hr and more clearly at 5 hr. For the 1·6 kb mRNA, an additive effect was observed at 5 hr.

Hybridization of the same total monocyte RNAs with an IL-1β probe showed that MDP and TNF treatment induced IL-1β gene expression whose mRNA (2·1 kb) was detectable after 5 hr of culture (Fig. 1). This messenger was not detectable in control untreated monocytes at 2·5 hr or 5 hr, but was induced by longer periods of culture. As LPS was shown to induce IL-1β mRNA very rapidly (Vermeulen *et al.*, 1987), the above negative results enable the presence of LPS in our culture medium to be

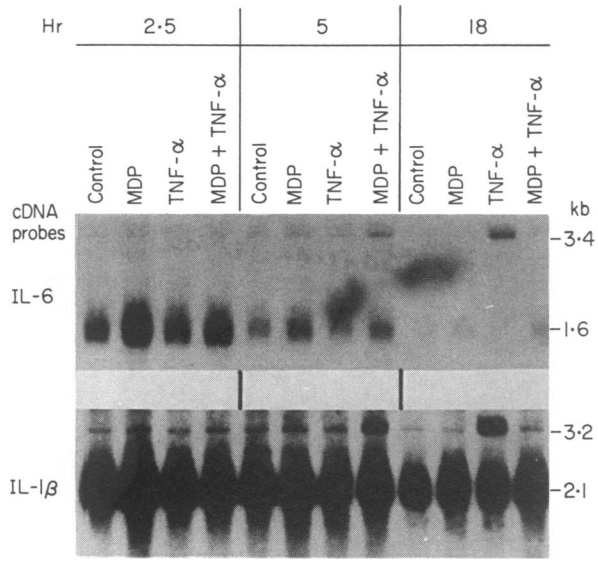

Figure 2. Kinetics of IL-6 and IL-1β RNA accumulation in human monocytes obtained from different donors and treated with MDP and/or TNF-α Total RNA (10 μg), extracted at the indicated times, was analysed by Northern blot hybridization with the labelled IL-6 and IL-1β probe, as in Fig. 1. The positions of specific RNA are indicated on the right of the radiogram. Exposure time was 10 hr.

ruled out. In addition, less than 1 U/ml of IL-1 and TNF activities were detected in supernatants from control cultures at 2·5 hr. Moreover, polymyxin B, which blocks LPS action, had no effect on the MDP or TNF-mediated stimulation of IL-6 expression (not shown).

In monocytes from certain donors, kinetics of RNA accumulation appeared to be different after MDP treatment, since the maximal level of IL-6 RNA was already observed at 2·5 hr (Fig. 2). In monocytes from these donors, IL-1β messenger was already detected in control untreated cells at 2·5 hr culture. These observations may be due to preactivation of these donors' monocytes, as testified by the detection of 40 U/ml of IL-1 and 200 U/ml of TNF in the 2·5 hr control culture supernatant. Both these cytokines, acting by an autocrine pathway, might be responsible for the high level of IL-6 RNAs detected in control monocytes, since it was previously shown in other systems that they are very powerfull IL-6 inducers (Content *et al.*, 1985; Kohase *et al.*, 1986; Defilippi *et al.*, 1987; Kohase *et al.*, 1987; Van Damme, 1987).

Accumulation of hybridoma growth factor in monocyte supernatants

Recently, Bradkenhoff *et al.* (1987) have shown after sequence analysis that the hybridoma growth factor cDNA is identical to the IL-6 cDNA. Thus, we tested for the presence of HGF in supernatants of MDP- and/or TNF-stimulated monocytes from both groups of donors analysed above (Figs 1 and 2).

As shown in Table 1, in the first group of donors, significant HGF secretion was detected in supernatant of control monocytes already after 2·5 hr of incubation (100 U/ml) and maximal yield was reached after 5 hr (4000 U/ml). MDP or TNF alone were able to increase the secretion of HGF, whose level reached 32000 U/ml at 18 hr. Stimulation of monocyte by MDP associated with TNF resulted in a higher yield (100000 U/ml) than by either cytokine alone.

Table 1. Accumulation of HGF in supernatants of monocytes after induction with MDP and/or TNF-α

	HGF activity (U/ml)*					
	First group†			Second group		
	2·5 hr	5 hr	18 hr	2·5 hr	5 hr	18 hr
Control	100	4000	4000	2000	16,000	16,000
MDP‡	2000	8000	32,000	8000	64,000	32,000
TNF-α‡	1000	4000	32,000	2000	16,000	32,000
MDP+TNF-α	2000	16,000	100,000	16,000	32,000	120,000

*HGF activity was determined in supernatants of non-stimulated or stimulated monocytes, as described in the Materials and Methods.

†First group of donors correspond to those analysed in experiment shown in Fig. 1 and second group to those analysed in experiment shown in Fig. 2.

‡MDP or TNF-α alone did not show any significant HGF activity (< 10 U/ml) in direct assay on 7 TD.1 cells.

Similar results were obtained with monocyte supernatants corresponding to the group of donors analysed in the experiment shown in Fig. 2. Although the HGF level in control monocyte supernatant in this group was higher than in the first group, MDP still enhanced HGF secretion.

DISCUSSION

The results reported here provide new information about the molecular events involved in the MDP-mediated immune response. They clearly showed that MDP increased the expression of the IL-6 gene in human monocytes. We also demonstrated that the expression of this gene is augmented by TNF treatment.

In untreated monocytes, variations in the basal level of IL-6 RNA were observed depending on the donor. Nevertheless, MDP enhanced IL-6 expression irrespective of this level.

The pattern of IL-6 RNAs in human monocytes appears to be more complex than that previously described for human fibroblast. In fact, human fibroblasts express a 1·3 kb transcript (Content *et al.*, 1985; Haegeman *et al.*, 1986; Zilberstein *et al.*,1986; Kohase *et al.*, 1987) which codes for the IL-6 protein, whereas, in human monocytes, three RNA species (1·4 kb, 1·6 kb and 3·4 kb) were detected after MDP and/or TNF treatment, suggesting a more complicated gene regulation than in fibroblasts. The nature of the three RNA detected in human monocytes remains to be determined, and more work is required to establish whether all of them code for specific proteins.

In this regard, Stevenson *et al.* (1985), who studied the production of IFN-α in human monocytes after exposure to MDP and poly (I):poly (C), found that the monocytes activated by poly (I):poly (C) expressed the 1·1 kb mRNA normally associated with secreted IFN-α. In addition, two RNA species with higher molecular weights (2·8 kb and 5·5 kb) were also detected. According to these authors, monocytes activated with MDP only contained a 2·8 kb RNA which appeared to be associated with the presence of intracellular IFN-α activity.

Thus, the induction of different molecular weight RNA species depends on the inducers.

Here, we found that TNF co-operates with MDP to enhance IL-6 gene expression, and this finding might at least partially explain their synergistic biological activities, recently reported by Parant (1987). MDP also induced the IL-1β and TNF secretion. Although both cytokines were reported to be IL-6 inducers (Content *et al.*, 1985; Kohase *et al.*,1986, Defilippi *et al.*,1987; Kohase *et al.*, 1987; Van Damme *et al.*, 1987), MDP nevertheless enhanced IL-6 gene expression in monocytes already producing these cytokines before their stimulation with MDP (Fig. 2). This suggests that MDP induced the expression of IL-6 gene by a pathway which may not involve IL-1 or TNF.

Of particular interest is the fact that the HGF accumulated in monocyte supernatants correlates with the level of IL-6 RNA. Thus, TNF-α and MDP are not only able to enhance IL-6 gene expression but also to induce synthesis and secretion of biological active protein.

The observation that MDP triggered the expression of IL-6 gene may be of physiological relevance and might explain its adjuvant activity, since this gene codes for a cytokine which plays an important role in regulating the antibody response. In addition, MDP exhibits a broad spectrum of immunomodulation properties such as enhancement of macrophage cytotoxicity against tumour cells (Saiki *et al.*, 1985) and non-specific resistance against intracellular parasites such as *Trypanosoma cruzi* (Kierzenbaum & Ferraresi, 1979). Our results suggest that these properties could be linked to the stimulation by MDP of genes coding for key cytokines such as the IL-6, TNF-α and IL-1, responsible, in turn, for monocyte activation.

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This paper is dedicated in memory of Professor Edgar Lederer.

REFERENCES

ADAM A. (1985) *Synthetic Adjuvants: Modern Concepts in Immunology* (ed. C.A. Bona), John Wiley & Sons Interscience, New York.

BRADKENHOFF J.P.J., DE GROOT E.R., EVERS R.F., PANNEKOEK H. & AARDERN L.A. (1987) Molecular cloning and expression of hybridoma growth factor in *Escherichia coli*. *J. Immunol.* **139,** 4116.

CONTENT J., DE WIT L., POUPART P., OPDENAKKER G., VAN DAMME J. & BILLIAU A. (1985) Induction of a 26 kDa protein mRNA in human cells treated with an interleukin-1-related leukocyte-derived factor. *Eur. J. Biochem*, **152,** 253.

DEFILIPPI P., POUPART P., TAVERNIER J., FIERS W. & CONTENT J. (1987) Induction and regulation of mRNA encoding 26 kDa protein in human cell lines treated with recombinant human tumor necrosis factor. *Proc. natl. Acad. Sci. U.S.A.* **84,** 4557.

ELLOUZ F., ADAM A., CIORBARU R. & LEDERER E. (1974) Minimal structure requirement for adjuvant activity of bacterial peptidoglycan derivates. *Biochem. Biophys. Res. Commun*, **59,** 1317.

GAULDIE J., RICHARDS C., HARNISH D., LANSDORP P. & BAUMANN H. (1987) Interferon-β2/B cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. *Proc. natl. Acad. Sci. U.S.A.* **84,** 7251.

HAEGEMAN G., CONTENT J., VOLCKAERT G., DERYNCK R., TAVERNIER J. & FIERS W. (1986) Structural analysis of the sequence coding for an inducible 26 kDa protein in human fibroblasts. *Eur. J. Biochem.* **159,** 625.

HIRANO T., TAGA T., NAKANO N., YASUKAWA K., KASHIWAMURA S., SHIMIZU K., NAKAJIMA K., PYUN K. & KISHIMOTO T. (1985) Purification to homogeneity and characterization of human B-cell differentiation factor (BCDF or BSF-2). *Proc. natl. Acad. Sci. U.S.A.* **82,** 5490.

HIRANO T., YASUKAWA K., HARADA H., TAGA T., WATANABE Y., MATSUDA T., KASHIWAMURA S., NAKAJIMA K., KOYAMA K., IWAMATSU A., TSUNASAWA S., SAKIYAMA F., MATSUI H., TAKAHARA F.Y. TANIGUCHI T. & KISHIMOTO T. (1986) Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. *Nature (Lond.)*, **324,** 73.

KIERZENBAUM K. & FERRARESI R.W. (1979) Enhancement of host resistance against Trypanosoma cruzi infection by the immunoregulatory agent muramyl dipeptide. *Infect. Immun.* **25,** 273.

KOHASE M., DE STEFANO D.H., MAY L.T., VILCEK J. & SEGHAL P.B. (1986) Induction of β2-interferon by tumor necrosis factor: a hemostatic mechanism in the control of cell proliferation. *Cell*, **45,** 659.

KOHASE M., MAY L.T., TAMM I., VILCEK J. & SEHGAL P.B.A. (1987) A cytokine network in human diploid fibroblasts: interactions of β-interferons, tumor necrosis factor, platelet-derived growth factor and interleukin-1. *Mol. Cell Biol.* **7,** 273.

LECLERC C. & CHEDID L. (1986) Are there receptors for MDP at the macrophage surface. *Int. J. Immunother.* **2,** 109.

LEDERER E. (1980) Synthetic immunostimulants derived from the bacterial cell wall. *J. Medicin. Chem.* **23,** 819.

MIZEL S.B., OPPENHEIM J.J. & ROSENSTREICH D.L. (1978) Characterization of lymphocyte-activating factor (LAF) produced by the macrophage cell line, P388D1. *J. Immunol.* **120,** 1497.

OPPENHEIM J.J., TOGAWA A., CHEDID L. & MIZEL S. (1980) Components of mycobacterial and muramyl dipeptide with adjuvant activity induce lymphocyte activating factor. *Cell. Immunol.* **50,** 71.

PARANT M.A. (1987) Muramyl peptides as enhancers of host resistance to bacterial infections. In: *Immunopharmacology of Infectious Diseases: Vaccine Adjuvants and Modulators of Non-specific Resistance* (ed. J.A. Madge), p. 7. Alan Liss Inc., New York.

SAIKI I., SONE S., FOGLER. W.E., KLEINERMAN E. S., LOPEZ-BERESTEIN G. & FIDLER I.J. (1985) Syngerism between human recombinant gamma interferon and muramyl dipeptide encapsulated in liposomes for activation of antitumor properties in human blood monocytes. *Cancer Res.* **45,** 6188.

SANCÉAU J., FALCOFF R., ZILBERSTEIN A., BÉRANGER F., LEBEAU J., REVEL M. & VAQUERO C. (1988) Interferon-β2 (BSF-2) mRNA is expressed in human monocytes. *J. Interferon Res.* **8,** 473.

STEVENSON H.C., DEKABAN G.A., MILLER P.J., BENYAJATI C. & PEARSON M.L. (1985) Analysis of human blood monocytes activation at the level of gene expression. Expression of alpha interferon genes during activation of human monocytes by poly IC/IC and muramyl-dipeptide. *J. exp. Med.* **161,** 503.

SUGARMAN B.J., AGGARWAL B.B., HARS P.E., FIGARI I.S., PALLADINO M.A. & SHEPARD M.S. (1985) Recombinant tumor necrosis factor: effects on proliferation of normal and transformed cells *in vitro*. *Science*, **230,** 943.

VAQUERO C., SANCÉAU J., CATINOT L., ANDREU G., FALCOFF E. & FALCOFF R. (1982) Translation of mRNA from phytohaemagglutinin-stimulated human lymphocytes: characterization of interferon mRNAs. *J. Interferon Res.* **2,** 217.

VAN DAMME J., OPDENAKKER G., SIMPSON R.J., RUBIRA M.R., CAYPHAS S., VINK A., BILLIAU A. & VAN SNICK J. (1987) Identification of the

human 25 kD protein, interferon beta-2 as hybridoma/plasmacytoma growth factor induced by interleukin-1 and tumor necrosis factor. *J. exp. Med.* **165,** 914.

VAN SNICK J., CAYPHAS S., VINK A., UYTTENHOVE C.,COULIE P.G., RUBIRA M. & SIMPSON R.J. (1986) Purification and NH2 terminal amino acid sequence of a T-cell-derived lymphokine with growth factor activity for B cell hybridomas. *Proc natl. Acad. Sci. U.S.A.* **83,** 9679.

VERMEULEN M.W., DAVID J.R. & REMOLD H.G. (1987) Differential mRNA responses in human macrophages activated by interferon-γ and muramyl dipeptide. *J. Immunol.* **139,** 7.

WIETZERBIN J., DAS B.C., PETIT J.F., LEDERER E., LEYH-BOUILLE M. & GHUYSEN J.M. (1974) Occurrence of D-alanyl-D-meso-diaminopimelic acid and meso-diaminopimelyl-meso-diaminopimelic acid, interpeptide linkages in the peptidoglycan of mycobacteria. *Biochemistry,* **13,** 3471.

WRIGHT G. & SILVERSTEIN S. (1982) Tumor promoting phorbol esters stimulate C3b and C3b' receptor-mediated phagocytosis in cultured human monocytes. *J. exp. Med.* **156,** 1149.

ZILBERSTEIN A., RUGGIERI R., KORN J.H. & REVEL M. (1986) Structure and expression of cDNA and genes for human interferon beta-2, a distinct species inducible by growth-stimulatory cytokines. *Embo J.* **10,** 2529.