Modulation of monocyte complement synthesis by interferons

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Recombinant *Escherichia coli*-derived γ -interferon has been shown to stimulate synthesis of the second component of complement (C2), factor B and C1 inhibitor, but to inhibit synthesis of the third component (C3). α - and β -interferons stimulate synthesis of factor B and C3 inhibitor, inhibit C5 synthesis but do not alter synthesis of C2. α - and β -interferons act synergistically with γ -interferon to enhance both factor B and C1-inhibitor synthesis.

INTRODUCTION

Human monocytes in culture synthesize a number of complement components including C1 subcomponents (Müller *et al.*, 1978), C2 (Einstein *et al.*, 1976), C3 (Strunk *et al.*, 1983), factors B and D and properdin (Whaley, 1980) and C1 inhibitor (Yeung-Laiweh *et al.*, 1985). Macrophages isolated from the joints of patients with rheumatoid arthritis synthesize greater quantities of these components than do monocytes (De Ceulaer *et al.*, 1980).

The factors which regulate the synthesis of complement components by macrophages at such sites of chronic inflammation are incompletely understood, but it is known that the addition of antigen-stimulated lymphocyte culture supernatants to monocyte cultures increases C2 synthesis (Littman & Ruddy, 1977). The lymphocyte product which was responsible for this finding has not been identified, but recent studies have shown that γ -interferon is responsible for much of the macrophage activation which occurs in response to lymphokines (Nathan *et al.*, 1983; Schreiber *et al.*, 1983; Mokoena & Gordon, 1985). In the present study we have shown that recombinant-DNA-derived γ -interferon selectively stimulates synthesis of C2, factor B and C1 inhibitor, but decreases synthesis of C3 and acts synergistically with α - and β -interferons to stimulate synthesis of C1 inhibitor and factor B.

EXPERIMENTAL

Materials

HBSS, RPMI (Flow Laboratories, Irvine, Ayrshire, Scotland, U.K.), DMEM without methionine, FCS, L-glutamine, antibiotic/antimycotic solution containing penicillin, streptomycin and fungizone (Gibco-Biocult, Paisley, Renfrewshire, Scotland, U.K.), [³⁵S]methionine (New England Nuclear), rabbit antiserum to factor B, the dye H33258 (Hoechst Pharmaceuticals, Frankfurt/Main, Germany), heat-killed formalin-fixed Cowan 1 strain of

Staphylococcus aureus, and purified lysozyme and lipopolysaccharide (*Escherichia coli*) (Sigma Chemical Co., Poole, Dorset, U.K.), [¹⁴C]methylated protein mixture [CFA/626; Amersham International, Amersham, Bucks., U.K.; containing myosin (*M*₂, 200 000), phosphorylase *b* (92 500), bovine serum albumin (69 000), ovalbumin (46 000), carbonic anhydrase (30 000) and lysozyme (14 300)] were obtained from the sources cited. Freeze-dried *E. coli*-derived recombinant γ -interferon (Immuneron, BG8201, lot no. 10M05) was a gift from Biogen SA, Geneva, Switzerland. This preparation had a specific activity of 14 IRU/ng and a pyrogen concentration of 0.4 ng/dosage unit (100 μ g). The freeze-dried material was stored at 2 °C and reconstituted with sterile water to give a concentration of 100 μ g (1.3 $\times$ 10⁶ units)/ml before use. Purified freeze-dried lymphoblastoid interferon (α -interferon, in TNE buffer, pH 7.2; lot no. 85053; Stratech Scientific, London N.1, U.K.) was stored at –20 °C until required. Immediately before use it was reconstituted in sterile deionized water to give an activity of 2.3 $\times$ 10⁵ IRU/ml (0.21 IRU/ng of protein). Purified freeze-dried fibroblast interferon (β -interferon in PBS, pH 7.4; lot no. 84032; Stratech Scientific) was stored and reconstituted in the same way as α -interferon. The activity of the β -interferon solution was 2.2 $\times$ 10⁵ IRU/ml (0.47 IRU/ng of protein). Antiserum to C1 inhibitor and C3 were produced by immunizing rabbits with the purified proteins (Naama *et al.*, 1985; Yeung-Laiweh *et al.*, 1985) and antiserum to human C2 was a gift from Dr. Michael Kerr (Department of Pathology, Ninewells Hospital, Dundee, Scotland, U.K.). A monoclonal antibody to recombinant γ -interferon was a gift from Dr. Chi-Ming Liang (Biogen Research Corp., Cambridge, MA, U.S.A.). This IgG₁ antibody had a neutralization titre of 1:100 000. A murine IgG₁ monoclonal anti-dinitrophenol antibody was a gift from Professor Michael Steward (London School of Hygiene and Tropical Medicine, Keppel Street, London W.C.1, U.K.). Human ABS was supplied by the Scottish Blood Transfusion Service (Law Hospital, Carlisle, Lanarkshire, Scotland, U.K.) and was heat-inactivated for 2 h at 56 °C.

Abbreviations used: ABS, AB serum heat-inactivated for 2 h at 56 °C; DMEM, bicarbonate-buffered Dulbecco's minimal essential medium; FCS, foetal-calf serum heat-inactivated for 2 h at 56 °C; HBSS, Hanks buffered salt solution; LPS, lipopolysaccharide from *Escherichia coli*; PBS, phosphate-buffered saline (144 mM-NaCl/2.8 mM-KCl/10 mM-Na₂HPO₄/1.5 mM-NaH₂PO₄, pH 7.4); RPMI, RPMI-1640/Hepes buffer; RPMI.ABS, RPMI containing 10% (v/v) AB serum; RPMI.FCS, RPMI containing 20% (v/v) heat-inactivated (2 h at 56 °C) foetal-calf serum; SR, synthesis rate (molecules/h per cell); TNE buffer, 100 mM-NaCl/10 mM-Tris/HCl/1 mM-Na₂EDTA, pH 7.2; IRU, international reference unit.

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Monocyte culture

Monocyte monolayers were prepared from the discarded buffy coats of blood donations, in 24-well Linbro tissue-culture plates (Lappin *et al.*, 1984). The cells were cultured in RPMI.FCS at 37 °C in a humidified atmosphere.

To study the kinetics of synthesis of complement components, the supernatants were sampled on days 1, 3, 5 and 7. After centrifugation to remove non-adherent cells the samples were stored at -70 °C. The monolayers and the non-adherent cells were each lysed in 200 μ l of SDS (0.05%) and the DNA content measured by spectrofluorimetry (Yeung-Laiweh *et al.*, 1985). The sum of the contents of the adherent- and non-adherent cell lysates was taken as the DNA content of each well. The kinetics of synthesis of complement components by control cultures were compared with cultures to which γ -interferon (0.1–10 000 ng/ml) had been added on day 0. The effect of the period of exposure of monocytes to γ -interferon was investigated by adding a fixed dose of γ -interferon (100 ng/ml) to a series of monocyte cultures. After each of the following time intervals (5, 15 and 30 min) the supernatants were removed from three cultures, the monocytes were washed and the culture continued in RPMI.FCS for a further 3 days. Concentrations of complement components and lysozyme were measured on day 3.

The effects of α - and β -interferons at concentrations of 10, 100 and 1000 IRU/ml were added to monocyte cultures on day 0 with or without a fixed dose of γ -interferon (100 ng/ml; 1400 units/ml). After 24 h the culture supernatants were assayed for their content of C1 inhibitor, C2, factor B, C3 and lysozyme.

Secretion rates of complement components in control and γ -interferon-treated monocyte cultures were investigated by internally labelling newly synthesized proteins with [³⁵S]methionine during a 1 h pulse period. Monocytes were cultured with and without γ -interferon (1000 ng/ml) for 3 days in RPMI containing 10% ABS. The supernatants were removed, the cells washed with warm (37 °C) DMEM and then cultured in DMEM (330 μ l/well) at 37 °C containing [³⁵S]methionine (500 μ Ci/ml) in a humidified atmosphere consisting of CO₂/air (1:19). After 1 h the supernatants were removed, the monolayers were washed once in warm (37 °C) RPMI and then culture was continued in RPMI at 37 °C in air. After various periods (0.25, 0.5, 1, 2, 4 and 8 h) three cultures were terminated by the removal of the supernatants, which were pooled and centrifuged (2000 g for 5 min at room temperature) to remove non-adherent cells and debris before storage at -70 °C. The three washed monolayers were each lysed in 330 μ l of Tris buffer (50 mmol/litre, pH 7.4) containing NaCl (50 mmol/litre), KCl (100 mmol/litre), EDTA (5 mmol/litre), bovine serum albumin (0.5% w/v), Triton X-100 (1% v/v), sodium deoxycholate (0.5% w/v), phenylmethanesulphonyl fluoride (5 mmol/litre) and NaN₃ (0.02% w/v). After pooling and centrifugation (5000 g for 5 min at room temperature) the lysates were stored at -70 °C until required.

Immunoprecipitation of complement components

C2, C3, factor B and C1 inhibitor were immunoprecipitated from the lysates and supernatants by the sequential addition of the appropriate antiserum (5 μ l of

antiserum diluted 1:10 for each 1 ml of supernatant or lysate). Immune complexes were isolated by binding to *Staph. aureus* (20 μ l of packed organisms/ml of supernatant or lysate) during a 30 min incubation at room temperature and subsequent centrifugation (Beckman Mirofuge; 10 000 g; 5 min) (Yeung-Laiwah *et al.*, 1985). Between each immunoprecipitation step the supernatants and lysates were adsorbed with *Staph. aureus* (20 μ l of packed organisms/ml of supernatant or lysate) to remove any persisting antibody or antigen-antibody complexes. After centrifugation the bacteria were washed four times in PBS containing Triton X-100 (1%, v/v) and then the complexes were eluted by the addition of 50 μ l of Tris buffer (63.8 mmol/litre, pH 6.8), containing SDS (3%, w/v), 2-mercaptoethanol (5%, v/v) and glycerol (10%, v/v). The eluates were boiled for 2 min and then subjected to SDS/polyacrylamide-gel electrophoresis in 7.5%-(w/v)-polyacrylamide slab gels (Laemmli, 1970), after which the gels were analysed by fluorography (Laskey & Mills, 1975). The fluorographs were analysed visually, by laser densitometry (LKB 2202 Ultro Scan laser densitometer with an LKB 2220 recording integrator), and by cutting out the bands and counting them for radioactivity in a liquid-scintillation spectrometer. The M_r markers, which were included in each run,

Fig. 1. Kinetics of synthesis of C1 inhibitor, Factor B, C2 and C3 by control monocytes (●) and monocytes treated with 100 ng/ml of γ -interferon (○).

Each point represents the mean $\pm$ S.E.M. for three cultures.

Fig. 2. Dose-response curves for the effect of γ -interferon on synthesis of C1 inhibitor (□), factor B (Δ), C2 (○) and C3 (●)

Concentrations were measured on day 3 of culture and expressed as relative amounts of those present in the control cultures, which were given an arbitrary value of 1. Each point represents the mean $\pm$ S.E.M. for three cultures.

were those included in the mixture of [¹⁴C]methylated proteins described under 'Materials' in the Experimental section.

Assays for complement components and lysozyme

C1 inhibitor was measured using a double-antibody radioimmunoassay technique (Yeung-Laiweh *et al.*, 1985), and C3, C2 and factor B were measured by using a 'sandwich' enzyme-linked immunoabsorbent assay (Lappin *et al.*, 1986). The C1-inhibitor assay could detect 1 ng/ml, whereas the assays for C2, C3 and factor B were capable of detecting 2 ng/ml. Lysozyme was measured by the lysis of *Micrococcus lysodeikticus*, 1 unit of activity being that which produces a ΔA_{450} of 0.001/min at pH 6.4 and 25 °C with a suspension of *M. lysodeikticus* (0.2 mg/ml) as substrate in a 2.6 ml reaction volume and a light path of 1 cm (Strunk *et al.*, 1980). Lysozyme levels were converted into ng/ml by comparison with a purified standard of known specific activity.

RESULTS

Effect of γ -interferon on complement synthesis

In control cultures monocytes did not synthesize C2 or C1 inhibitor before day 3 (Fig. 1). In contrast, C3 was synthesized on the first day of culture and, in the experiment shown, factor B synthesis was also detected

Table 1. Effect of γ -interferon on complement synthesis rates

This table shows the maximal and minimal effects of γ -interferon (γ -Inf) on the SRs of C1 inhibitor, factor B, C2 and C3 measured between days 1 and 3 and converted into molecules produced/h per cell and also expressed as a percentage of the number of molecules of lysozyme produced/h per cell (2804860 $\pm$ 415192) ('% lysozyme'). Lysozyme synthesis was unaffected by γ -interferon and was not significantly different in either experiment.

	C1 inhibitor*				B*				C2*				C3*			
	[γ -Inf] (ng/ml)†	SR (molecules/h per cell)	% Lysozyme	[γ -Inf] (ng/ml)†	SR (molecules/h per cell)	% Lysozyme	[γ -Inf] (ng/ml)†	SR (molecules/h per cell)	% Lysozyme	[γ -Inf] (ng/ml)†	SR (molecules/h per cell)	% Lysozyme	[γ -Inf] (ng/ml)†	SR (molecules/h per cell)	% Lysozyme	[γ -Inf] (ng/ml)†
Control	-	695 $\pm$ 219†	0.22 $\pm$ 0.007	-	600 $\pm$ 25	0.021 $\pm$ 0.001	-	3986 $\pm$ 100	0.127 $\pm$ 0.003	-	31227 $\pm$ 6722	1.460 $\pm$ 0.314	-	31227 $\pm$ 6722	1.460 $\pm$ 0.314	-
Maximum effect	$\geq$ 10000	109138 $\pm$ 5069	3.707 $\pm$ 0.183	100	18881 $\pm$ 6254	0.883 $\pm$ 0.092	100-1000	13434 $\pm$ 1977	0.477 $\pm$ 0.086	10-100	9881 $\pm$ 933	0.462 $\pm$ 0.044	10-100	9881 $\pm$ 933	0.462 $\pm$ 0.044	10-100
Minimum effect	$\leq$ 0.01	3188 $\pm$ 419	0.085 $\pm$ 0.008	1-10	7829 $\pm$ 1447	0.333 $\pm$ 0.061	0.01-1	8222 $\pm$ 409	0.263 $\pm$ 0.013	0.1-1	16527 $\pm$ 4569	0.698 $\pm$ 0.192	0.1-1	16527 $\pm$ 4569	0.698 $\pm$ 0.192	0.1-1

* C1 inhibitor and C2 were measured in a separate experiment from factor B and C3.

† This column shows the dose range at which the maximal or minimal effect was achieved in three different experiments for factor B and four different experiments for C1 inhibitor, C2 and C3.

‡ Mean $\pm$ S.E.M.

Fig. 3. Fluorographs of C1 inhibitor (a), C2 (b) and factor B (c and d) immunoprecipitated from monocytes treated with 1000 ng of γ -interferon/ml (c and left side of a and b) and from untreated monocytes (d and right side of a and b)

The control for non-specific binding is shown in track C of each autoradiograph. C1 inhibitor and C2 were precipitated sequentially from the same culture 'chased' for 4 h, factor B from a separate culture 'chased' for 8 h. The gels were run under reducing conditions.

at this time. The addition of γ -interferon (100 ng/ml) to the cultures had three obvious effects (Fig. 1): (1) no delay in the onset of C2 and C1-inhibitor synthesis was observed; (2) synthesis of C2, factor B and C1 inhibitor was increased; (3) synthesis of C3 was reduced. Fig. 2 shows the effects of γ -interferon on monocytes over the dose range 10000–0.1 ng/ml. C1-inhibitor synthesis was enhanced over the whole range and showed no signs of reaching a plateau. In other experiments doses as low as 0.01 ng/ml still caused enhancement. C2 synthesis was stimulated by γ -interferon concentration of 0.1 ng/ml and above, and maximal stimulation was achieved at a concentration of 100 ng/ml. The minimum concentration of γ -interferon which stimulated synthesis of factor B was 1 ng/ml (although in some cultures synthesis of B was not increased until a concentration of 10 ng/ml was achieved), and maximal stimulation was achieved by a concentration of 100 ng/ml. C1-inhibitor production was also enhanced by γ -interferon to a far greater extent than was that of either C2 or factor B. C3 production was inhibited by γ -interferon at doses of 1 ng/ml and above. Lysozyme synthesis was unaffected by γ -interferon (results not shown).

In another series of experiments the SRs of all four

components were measured between days 1 and 3 and expressed as a percentage of the lysozyme SR (Table 1). In control cultures the SR of C1 inhibitor was 0.02%, of factor B, 0.02%, of C2, 0.13% and of C3, 1.46%. The maximal γ -interferon effect increased the C1-inhibitor SR to 3.71%, that of factor B to 0.8% and that of C2 to 0.48%, whereas that of C3 was reduced to 0.46%. The lowest effective dose of γ -interferon increased the C1-inhibitor SR to 0.09%, that of factor B to 0.3% and that of C2 to 0.26%, whereas that of C3 was reduced to 0.7%. The maximum and minimum doses of γ -interferon which caused enhancement or inhibition of the production of a component in a number of different cultures is shown in Table 1. C1-inhibitor production was affected by the widest dose range. The other components showed a much more restricted dose response.

In order to show that these effects were due to interferon and not due to LPS contamination of the preparation, 10 ng of γ -interferon were added to monocyte cultures in the presence or absence of a monoclonal antibody to γ -interferon (which was used at a final dilution of 1:100 in the culture medium). SRs of C2, C3, B and C1 inhibitor were the same in the cultures containing the antibody alone, the antibody with

Fig. 4. Effect of incubating monocytes with γ -interferon (1000 ng/ml) for different times on production of C1 inhibitor, factor B, C2 and C3

The levels were measured 3 days after the removal of γ -interferon. Each bar represents the mean $\pm$ S.E.M. for three cultures.

γ -interferon and untreated control cultures (results not shown). A control murine monoclonal IgG₁ anti-dinitrophenol antibody did not alter the effect of γ -interferon on the synthesis of these complement components. In a second experiment, different concentrations (1000, 100, 10, 1 and 0.1 ng/ml) of LPS were added to monocyte cultures and the SRs of all four complement components were measured. SRs in the LPS-treated cultures did not differ from those in control cultures (results not shown).

Pulse-chase studies demonstrated greatly increased incorporation of [³⁵S]methionine into C1 inhibitor, C2 and factor B (Fig. 3). Incorporation into C3, assessed by densitometry and by the radioactivity (c.p.m.) measured in the bands cut from the gels of the intracellular pro-form of C3, was reduced by 30%. The rates of disappearance of 50% of the intracellular precursors and the appearance of 50% of the secreted forms of C1 inhibitor and C3 were similar in control and γ -interferon-treated cultures (1.25–1.5 h). The rates of disappearance of the intracellular precursors of C2 and factor B and the rates of the appearance of their secreted forms could not be measured accurately in the control cultures. We believe that our failure to demonstrate immunoprecipitable factor B and only low levels of immunoprecipitable C2 is due to loss of these antigens during the previous steps of the sequential absorption procedure, although it could be related to differences in the properties of the various antisera used.

Incubation of monocytes with a fixed dose of γ -interferon (10 ng/ml) for different time periods demonstrated that exposure for as little as 5 min caused enhanced C2, factor-B and C1-inhibitor production (Fig. 4). C3 synthesis was unaffected, even after 8 h exposure (results not shown).

Effects of α - and β -interferons on monocyte complement synthesis

Both α - and β -interferons selectively stimulated synthesis of factor B and C1 inhibitor and reduced synthesis of C2. Neither compound produced detectable changes in C2 synthesis (Figs. 5 and 6). Lysozyme levels were decreased by β -interferon from 87 ng $\pm$ 14 ng/ml to 55 ng $\pm$ 3 ng/ml at doses of 100 and 1000 units. No effect was seen with α -interferon.

Interactions between γ -interferon and α - or β -interferon were studied and γ -interferon was found to act synergistically with both α - and β -interferons in the stimulation of synthesis of factor B and C1 inhibitor, but synergistic effects on C3 or C2 synthesis were not seen (Figs. 5 and 6).

Fig. 5. Effect of adding α -interferon on its own (●), or in combination with 1300 units (100 ng) of γ -interferon/ml (○) on the production of factor B, C1 inhibitor, C3 and C2 by monocytes over 24 h

Each point represents the mean $\pm$ S.E.M. for three cultures.

Fig. 6. Effect of adding β -interferon on its own (●) or in combination with 1300 units (100 ng) of γ -interferon/ml (○) on the production of factor B, C1 inhibitor, C3 and C2 by monocytes over 24 h

Each point represents the mean $\pm$ S.E.M. for three cultures.

DISCUSSION

The interferons represent a series of proteins which have antiviral, antiproliferative and immunoregulatory activities (reviewed by Clemens & McNurlan, 1985). Three distinct classes of interferons (α -interferon), fibroblast (β -interferon) and immune (γ -interferon) have been identified and cloned. γ -Interferon is a T-lymphocyte product which regulates a broad spectrum of macrophage activities including increasing the membrane expression of class II MHC antigens (Basham & Merigan, 1983), receptors for the Fc portion of immunoglobulins (Unkeless *et al.*, 1981), lymphocyte function associated antigen LFA-1 (Straussmann *et al.*, 1985), reducing the expression of the transferrin and mannose receptors and the F4/80 antigen (Hamilton *et al.*, 1985; Ezekowitz & Gordon, 1982), primary-tumour cytotoxicity (Marino & Adams, 1982; Pace *et al.*, 1983), modulation of the NADPH oxidase complex necessary for the execution of the respiratory burst (Nathan *et al.*, 1983) and enhancing the secretion of such products as plasminogen activator (Mokoena & Gordon, 1985), C2 and factor B (Strunk *et al.*, 1985).

In the present study, serial measurements of the concentrations of complement components in monocyte culture supernatants have shown that γ -interferon has two major effects on the synthesis of complement proteins. Firstly, the 2–3-day lag period before the onset of synthesis of C2 and C1 inhibitor in control monocyte cultures was not observed in cultures treated with γ -interferon. The delay in onset of synthesis in control monocytes is thought to represent a period of maturation during which the monocytes acquire certain macrophage characteristics (Einstein *et al.*, 1976). Thus γ -interferon appears to accelerate this maturation process. Secondly, γ -interferon altered the rate of synthesis of complement components. Synthesis of C2, factor B and C1 inhibitor was increased, whereas that of C3 was reduced. Although γ -interferon could be removed from monocyte cultures after only 5 min, increased synthesis of C2, factor B and C1 inhibitor occurred. However Strunk *et al.* (1985) found that it took several hours before the effect of C2 and factor B became apparent, suggesting that the initial

rapid signal is translated into enhancement of C2 and B synthesis by a slower, secondary, process. Strunk *et al.* (1985) also demonstrated that this process was not dependent on protein synthesis, since it was not blocked by cycloheximide. In contrast, for reduction of C3 synthesis to occur, it appears that γ -interferon had to be present for more than 8 h. This observation suggests that the signal involved for reduction in C3 synthesis differs from that involved in the stimulation of synthesis of C2, factor B and C1 inhibitor. Unlike us, Strunk & his colleagues (Strunk *et al.*, 1985) also noted that when γ -interferon was removed from monocyte cultures, synthesis of C2 and B returned towards normal levels within 24 h. The results of our experiment, in which levels of complement components were measured 3 days after the administration of γ -interferon to, and its removal from, the cultures, are not inconsistent with these findings.

The results of the pulse-label studies showed that the effect of γ -interferon on the incorporation of [35 S]methionine into the complement components was similar to the effect detected by the radioimmunoassay and enzyme-linked immunoadsorbent assays. In addition there was no obvious change in the way in which C1 inhibitor and C3 were secreted, suggesting that the effect of γ -interferon is not post-translational. Strunk *et al.* (1985) showed that the levels of mRNAs for C2 and factor B were increased in γ -interferon-treated monocytes. These data demonstrated that γ -interferon increased synthesis of C2 and factor B at the pretranslational level.

The results from the dose-response curves show that γ -interferon exerts quantitatively different effects on each of the C1-inhibitor, C2 and factor-B genes. C1-inhibitor synthesis was most responsive, the lowest dose used (0.01 ng/ml) producing a significant level of stimulation. Furthermore, despite increasing the concentration of γ -interferon to 10000 ng/ml, there was no suggestion that C1-inhibitor synthesis had reached its maximum value. In contrast, maximal stimulation of synthesis of C2 and factor B was achieved when the concentration of γ -interferon was 100 ng/ml. The sensitivity of the C2 gene to γ -interferon was greater than that of the factor-B gene, as significant stimulation of C2 synthesis was achieved by

a concentration of 0.01 ng/ml in some cultures, whereas synthesis of factor B was achieved only at concentrations of 1 ng/ml and above. However, factor-B synthesis was enhanced to a greater extent, as has been shown previously by Strunk *et al.* (1985). Like us, these authors were able to detect stimulation of C2 with concentration between 0.01 and 0.1 ng/ml. However, they found that similar concentrations of γ -interferon stimulated factor-B synthesis, whereas we were unable to produce stimulation until concentrations of between 1.0 ng/ml and 10 ng/ml were achieved. We are unable to explain this further discrepancy, but as the potencies of the two preparations was similar (19 units/ng, Strunk *et al.*, 1985; 14 units/ng, the present Biogen preparation), the difference cannot be dismissed on this basis.

The effects of α - and β -interferons also increased the expression of the factor-B and C1-inhibitor genes, but had no effect on the expression of the C2 gene and inhibited expression of C3. It could be noted that the degree of stimulation was less than that produced by γ -interferon. Previously Strunk *et al.* (1985) showed that α - and β -interferons stimulated factor-B synthesis, but did not affect C2 synthesis. Lysozyme is generally considered to be a constitutive secretory product of mononuclear phagocytes, as its rate of synthesis is not affected by the state of activation (Gordon *et al.*, 1974). Reduction in lysozyme synthesis is generally considered to be a sign of cell injury. In the present study β -interferon reduced lysozyme synthesis, but at the same time synthesis of factor B and C1 inhibitor was increased, suggesting that β -interferon-treated monocytes were healthy. The major effects of α - and β -interferons on complement synthesis was their ability to act synergistically with γ -interferon in the stimulation of factor-B and C1-inhibitor synthesis. The mechanism of this synergism is not yet understood. γ -Interferon has been shown to increase cell-membrane receptors for tumour necrosis factor (Tsuijimoto *et al.*, 1986). The synergism between γ - and the α - and β -interferons could be due, perhaps, to the enhanced expression of α - and β -interferon receptors, though this would not explain the selective nature of the effect.

In summary, our studies show that α -, β - and γ -interferons modulate the synthesis of complement components by human monocytes. Not only do they have their own individual effects, but there is synergism between them. The mode of action of the interferons on the expression of complement genes in monocytes requires study in order to further our understanding of the regulation of synthesis of complement components at sites of inflammation.

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REFERENCES

- Basham, T. Y. & Merigan, T. C. (1983) *J. Immunol.* **130**, 1492–1494
- Clemens, M. J. & McNurlan, M. A. (1985) *Biochem. J.* **226**, 345–360
- De Ceulaer, C., Papazoglou, S. & Whaley, K. (1980) *Immunology* **41**, 37–43
- Einstein, L. P., Schneberger, E. E. & Colton, H. R. (1976) *J. Exp. Med.* **143**, 114–126
- Ezekowitz, R. A. B. & Gordon, S. (1982) *J. Exp. Med.* **155**, 1623–1637
- Gordon, S., Todd, J. & Cohn, Z. A. (1974) *J. Exp. Med.* **139**, 1228–1248
- Hamilton, T. A., Becton, D. L., Somers, S. D., Gray, P. W. & Adams, D. O. (1985) *J. Biol. Chem.* **260**, 1378–1381
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–686
- Lappin, D. F., Riches, D. W. H., Damerau, B. & Whaley, K. (1984) *Biochem. J.* **222**, 477–486
- Lappin, D. F., Hamilton, A. O., Morrison, L., Aref, M. & Whaley, K. (1986) *J. Clin. Lab. Immunol.* **20**, 101–105
- Laskey, R. A. & Mills, A. D. (1975) *Eur. J. Biochem.* **56**, 335–341
- Littman, B. H. & Ruddy, S. (1977) *J. Exp. Med.* **145**, 1344–1345
- Marino, P. A. & Adams, D. O. (1982) *J. Immunol.* **128**, 2816–2823
- Mokoena, T. & Gordon, S. (1985) *J. Clin. Invest.* **75**, 624–631
- Müller, W., Hanauske-Abel & Loos, M. (1978) *J. Immunol.* **121**, 1578–1615
- Naama, J. K., Hamilton, A. O., Yeung-Laiweh, A. C. & Whaley, K. (1985) *Clin. Exp. Immunol.* **58**, 486–492
- Nathan, C. F., Murray, H. W., Wiebe, M. E. & Rubin, B. Y. (1983) *J. Exp. Med.* **158**, 670–689
- Pace, J. L., Russell, S. W., Torres, B. A., Johnson, H. M. & Gray, P. W. (1983) *J. Immunol.* **130**, 2011–2013
- Schreiber, R. D., Pace, J. L., Russell, S. W., Altman, A. & Katz, D. H. (1983) *J. Immunol.* **131**, 826–832
- Strassmann, G., Somers, S. D., Springer, T. A., Adams, D. O. & Hamilton, T. A. (1986) *Cell. Immunol.* **97**, 110–120
- Strunk, R. C., Kunke, K. S. & Musson, R. A. (1980) *J. Reticuloendothel. Soc.* **28**, 483–488
- Strunk, R. C., Kunke, K. S. & Giclas, P. C. (1983) *Immunology* **49**, 169–174
- Strunk, R. C., Cole, F. S., Perlmuter, D. H., Colten, H. R. (1985) *J. Biol. Chem.* **260**, 15280–15285
- Tsuijimoto, M., Yip, K. & Vilcek, J. (1986) *J. Immunol.* **136**, 2441–2444
- Unkless, J. C., Fleit, H. & Mellman, I. (1981) *Adv. Immunol.* **31**, 1–136
- Whaley, K. (1980) *J. Exp. Med.* **151**, 501–516
- Yeung-Laiwah, A. C., Jones, L., Hamilton, A. O. & Whaley, K. (1985) *Biochem. J.* **226**, 149–205

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