Modulation by interferons of the expression of monocyte complement genes

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Interferons- α , - β and - γ (IFNs - α , - β and - γ) stimulated the synthesis of the second complement component (C2), Factor B (B) and C1 inhibitor (C1-inh) by human monocytes *in vitro*. The degree of increase of the secretion rates of C2, B and C1-inh was dose-dependent and proportional to increases in the abundances of their respective mRNAs. IFN- γ was the most effective at stimulating monocyte C1-inh synthesis, whereas IFN- α and IFN- β were marginally more effective at stimulating monocyte C2 and B synthesis. Kinetic studies showed that the effect of the IFNs was rapid, with maximum stimulation occurring within 1–2 h for all three proteins. After the removal of IFNs from cultures the C1-inh mRNA abundance remained elevated for over 24 h in IFN- γ -treated monocytes but returned to control levels within 8 h in IFN- α -treated and IFN- β -treated monocytes. The abundances of C2 mRNA and B mRNA also returned to basal values within 8 h after removal of any of the three cytokines from the cultures. Both IFN- α and IFN- β acted synergistically with IFN- γ to stimulate synthesis of C1-inh and B. This synergistic effect only occurred when the cytokines were present in the cultures simultaneously. The effects of IFN- γ plus IFN- α or IFN- β on C2 synthesis appeared to be additive rather than synergistic. IFN- γ inhibited synthesis of C3 by monocytes, but IFN- α and IFN- β had no effect on the synthesis of this protein. Furthermore, none of the three cytokines had any effect on the expression of actin mRNA in monocytes.

INTRODUCTION

Human monocytes synthesize a number of complement components, including the second component (C2), the third component (C3), Factor B (B) and C1 inhibitor (C1-inh) (Einstein *et al.*, 1976; Whaley, 1980; Strunk *et al.*, 1983; Yeung-Laiweh *et al.*, 1985). Cells of this type (mononuclear phagocytes) appear to provide an extrahepatic source of complement *in vivo*, a function that may be important at the local tissue level, where in the interstitial space concentrations of complement proteins are likely to be low (Ezekowitz *et al.*, 1984). Activation of complement at such areas will lead to consumption and depletion of these proteins. The mobile nature of mononuclear phagocytes enables these cells to infiltrate sites of inflammation, where they can secrete complement components and perhaps replenish the extravascular fluids with these proteins. Macrophages isolated from inflamed tissues produce greater amounts of complement components than do resident macrophages of monocytes (De Ceulaer *et al.*, 1980; Lappin *et al.*, 1986). The factors that modulate complement biosynthesis by mononuclear phagocytes *in vivo* are still being defined. Many of these factors are likely to be derived from other cell types present at inflammatory sites. Supernatants of antigen-stimulated and mitogen-stimulated lymphocytes and of lymphocytes isolated from inflamed synovial membrane stimulate the synthesis of C2, B and C1-inh but inhibit the synthesis of C3 and properdin (Lappin & Whaley, 1989). The factor responsible for this effect has been identified as interferon- γ (IFN- γ) (Littman & Ruddy, 1977, 1979; Lappin & Whaley, 1989), which is a product of activated T-cells (Trinchieri & Perussia, 1985). IFN- γ has been shown to produce marked stimulation of C1-inh synthesis by human monocytes, with secretion rates increasing up to 150-fold (Hamilton *et al.*, 1987).

Interferon- α (IFN- α) and interferon- β (IFN- β), which are secretory products of myeloid cells and fibroblasts respectively (Revel, 1983), also stimulate synthesis of complement components by monocytes (Strunk *et al.*, 1985; Hamilton *et al.*, 1987). It has also been shown that IFN- γ and IFN- α or IFN- β act synergistically to stimulate synthesis of C1-inh and B.

The present work was undertaken to study the action of IFNs on the expression of complement genes in human monocytes, especially those encoding C1-inh, C2, C3 and B, by comparing the effects of these cytokines on the relative abundances of the mRNAs with the amounts of the corresponding protein secreted by the cells.

MATERIALS AND METHODS

Reagents

The following reagents were purchased from the sources shown: RPMI 1640 and Linbro multi-well tissue-culture dishes from Flow Laboratories (Rickmansworth, Herts., U.K.); foetal-calf serum (FCS; heat-inactivated for 2 h at 56 °C), antibiotic/antimycotic solution (containing penicillin, streptomycin and fungizone), *Eco*RI, *Pst*I and *Bam*HI from GIBCO-BRL (Paisley, Renfrewshire, U.K.); Bio-Gel A-1.5m (100–200 mesh) from Bio-Rad Laboratories (Richmond, CA, U.S.A.); [α -³²P]dCTP (PB 10205) and Hybond-N nylon membranes from Amersham International (Amersham, Bucks., U.K.); *Hind*III and random-primed DNA-labelling kit from Boehringer Mannheim (Mannheim, Germany); the dye H33258 and cycloheximide from Sigma Chemical Co. (Poole, Dorset, U.K.); lymphoblastoid interferon (IFN- α ; lot no. 85054, 1.0×10^5 IRU/mg) and fibroblast interferon (IFN- β ; lot no.

Abbreviations used: the nomenclature of complement components is that recommended by the World Health Organisation (1968, 1981); C1-inh, C1 inhibitor; C2, second component of complement; C3, third component of complement; B, Factor B; IFN- α , lymphoblastoid interferon- α ; IFN- β , fibroblast interferon- β ; IFN- γ , recombinant interferon- γ ; ABS, human AB serum heat-inactivated for 2 h at 56 °C; RPMI, RPMI 1640/Hepes buffer; FCS, foetal-calf serum heat-inactivated for 2 h at 56 °C; RPMI/ABS, RPMI 1640 containing 10% (v/v) heat-inactivated human AB serum; RPMI/FCS, RPMI 1640 containing 20% (v/v) heat-inactivated foetal-calf serum.

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84032, 1.5×10^5 IRU/mg) and fibroblast interferon (IFN- β ; lot no. 84032, 1.5×10^5 IRU/mg) from Stratech Scientific (London, U.K.); RNAzol from Biogenesis (Bournemouth, U.K.). Recombinant interferon- γ (IFN- γ ; Immuneron, B68201, lot no. 10M05, 14×10^6 IRU/mg) was a gift from Biogen SA (Geneva, Switzerland). The main details of these preparations have been described previously (Hamilton *et al.*, 1987). Human AB serum was supplied by the Scottish Blood Transfusion Service (Law Hospital, Carlisle, Lanarkshire, U.K.) and heat-inactivated at 56 °C for 2 h before use (ABS).

The plasmids pAT153PVUII-8 PC201 (C2 cDNA) and pAT153PVUII-8 P2FB (B cDNA) were gifts from Dr. David Bentley and Dr. Duncan Campbell (M.R.C. Immunochimistry Unit, Department of Biochemistry, University of Oxford, Oxford, U.K.) (Bentley & Porter, 1984; Morley & Campbell, 1984). The plasmid pC351 (C3 cDNA) was a gift from Dr. George Fey (Scripps Research Clinic, La Jolla, CA, U.S.A.) (De Bruijn & Fey, 1985). The C1-inh cDNA (PC1) was a gift from Dr. Philip Carter (Department of Biochemistry, University of Aberdeen, Aberdeen, U.K.) (Carter *et al.*, 1988). The rat actin cDNA (p749) was a gift from Dr. F. Frischauf (European Molecular Biology Laboratory, Heidelberg, Germany) (Katcoff *et al.*, 1980).

Monocyte cultures

Monocyte monolayers were prepared in 24-well Linbro tissue-culture plates and the cells were cultured in RPMI 1640 containing 10% (v/v) ABS (RPMI/ABS) at 37 °C in a humidified air/CO₂ (19:1) atmosphere (Lappin *et al.*, 1984). After 3 days the culture medium was removed and replaced with fresh medium consisting of RPMI 1640 containing 20% (v/v) FCS (RPMI/FCS) and incubated under the same conditions for a further day before any experiments were started. This day was termed day 0, and at this time IFNs were added to the cultures, which were then incubated for defined periods. At the end of the incubation period the supernatants were collected and stored at -70 °C for measurement of concentrations of complement components, and RNA was extracted from the monocytes. A replicate series of cultures was set up to determine the numbers of monocytes by measuring the DNA concentration of the monolayers (Lappin *et al.*, 1984).

Isolation of total cellular RNA

RNA was prepared by lysing each monolayer in 100 μ l of RNAzol, which was then transferred to a Microcap centrifuge tube. Each well was then flushed with a further 100 μ l of RNAzol, which was combined with the original lysate (Chomczynski & Sacchi, 1987).

Northern-blot and doubling-dilution dot-blot analyses

RNA (2–5 μ g) was electrophoresed on a denaturing agarose gel (1% agarose) and blotted on to Hybond-N membranes by the use of a capillary transfer technique (Thomas, 1980). RNA was also diluted in 2-fold dilutions, and 4 μ l of each dilution was dotted on to Hybond-N membranes (with the highest amount being 2 μ g) (Fulton *et al.*, 1985).

Preparation of probes

The cDNA inserts were excised from their plasmid vectors by digestion with *Bam*HI and *Hind*III (C1-inh, C2 and B cDNAs) or with *Eco*RI (C3 cDNA) or with *Pst*I (actin cDNA), isolated by agarose-gel electrophoresis and labelled with [α -³²P]dCTP by using the random-primed DNA-labelling reaction (Feinberg & Vogelstein, 1983, 1984). Nucleic acids were fixed to the membranes by u.v. irradiation. Hybridization (Wahl *et al.*, 1979)

and autoradiography (Fulton *et al.*, 1985) were performed as described previously.

Stripping of membranes for re-probing

Membranes were stripped for reprobing by boiling in 0.1% (w/v) SDS for 20 min.

Scanning of autoradiographs

Autoradiographs of Northern blots and double-dilution dot-blots were scanned with a Helena Laboratories Autoscanner. An arbitrary value of 1.00 was assigned nominally to the control degree of expression, except when stated otherwise. The actin-probed blots were used to correct for variability in the loading of the agarose gels with RNA and the efficiency of transfer.

Effects of concentration of IFNs on mRNA abundances

At time 0 IFNs were added to the culture medium. After 24 h the supernatants were removed (for e.l.i.s.a.) and RNA was extracted from the monocytes. Replicate cultures were used for DNA determination.

Time required for effects of IFNs on mRNA abundances

In two separate experiments (1 and 2) IFNs (1 ng/ml) were added to replicate cultures at time 0, and RNA was extracted after incubation periods of 0, 2, 4, 8 and 24 h (Expt. 1) and of 0, 15, 30 min and 1, 2, and 24 h (Expt. 2).

Duration of effects of IFNs on mRNA abundances

Monocyte cultures were treated with IFNs (1 ng/ml) for 2 h. After this time they were removed and the monocytes washed five times with RPMI 1640. Fresh medium not containing IFNs was added and RNA was extracted after incubation periods of 0, 1, 2, 4, 8 and 24 h.

Synergism between IFNs

The synergistic effects between IFNs on mRNA abundances were studied at IFN concentrations of 1 ng/ml for each cytokine. After 24 h the culture supernatants were removed for measurement of concentrations of complement components, and RNA was extracted from the monocytes.

Duration of the synergistic effect of IFNs

Monocytes were treated for 2 h with one of the IFNs (1 ng/ml) before it was removed and a second IFN was added at time intervals of 0, 1, 2, 4, 8 and 24 h. RNA was prepared 24 h after the addition of the second cytokine and the supernatants were assayed for complement proteins by e.l.i.s.a.

Measurement of secretion rates of complement components

Concentrations of C2, C3, B and C1-inh were measured by e.l.i.s.a. (Lappin *et al.*, 1986) in the supernatants of cultures that had been exposed to IFNs for 24 h. The average secretion rates were calculated and expressed as molecules/cell per min over the 24 h period (1 μ g of DNA $\equiv 10^5$ cells).

RESULTS

Effects of concentration of IFNs on mRNA abundances

IFN- α , IFN- β and IFN- γ produced dose-related increases in the abundances of C1-inh, C2 and B mRNAs (Fig. 1) in association with dose-dependent increases in the secretion rates of these three proteins (Figs. 1 and 2). IFN- γ was the most effective cytokine for stimulating C1-inh synthesis. A 28-fold increase in the C1-inh secretion rate and a 20-fold increase in C1-inh mRNA abundance occurred when IFN- γ was used at a concentration of 100 ng/ml. IFN- α and IFN- β appeared to be

Fig. 1. Effects of concentration of IFNs on mRNA abundance in monocytes

A 5 μ g portion of total cellular RNA was run on each lane of a denaturing agarose gel, transferred by Northern blotting, probed sequentially for C1-inh mRNA, C2 mRNA, C3 mRNA, B mRNA and actin mRNA and subjected to autoradiography. The appearance of total cellular RNA on agarose gel stained with ethidium bromide (E) was used to assess the loading in each lane. The dose-related effects (0, 0.1, 1, 10 and 100 ng/ml) of IFN- α (lanes 1–5), IFN- β (lanes 6–10) and IFN- γ (lanes 11–15) are shown. The autoradiogram was exposed for 7 days.

more effective at stimulating the synthesis of C2 and B (Figs. 1 and 2). In addition, IFN- γ inhibited the synthesis of C3, whereas IFN- α and IFN- β had no detectable effect on the synthesis of this protein (Fig. 2). None of these cytokines altered the abundance of actin mRNA (Fig. 1).

Time required for effects of IFNs on mRNA abundances

In Expt. 1 it was shown that the abundances of C2 mRNA, B mRNA and C1-inh mRNA were maximal 2 h after the addition of each cytokine, and no further increase was seen in the 4 h and 8 h cultures (results not shown). In Expt. 2 all three IFNs produced detectable increases in C1-inh mRNA, C2 mRNA and B mRNA abundances within 30 min of exposure. The maximum effects were observed after 1–2 h of treatment (Fig. 3). These increases in C1-inh mRNA, C2 mRNA and B mRNA abundances did not require protein synthesis, since they occurred in the presence of cycloheximide (2.5 μ g/ml), which effectively inhibits synthesis of C1-inh, C2 and B proteins. In the presence of cycloheximide and the cytokines the abundances of C1-inh mRNA, C2 mRNA and B mRNA were increased above the level achieved with each IFN alone. Abundances of these mRNAs were also increased in control cultures treated with cycloheximide in the absence of IFNs. In the presence of cycloheximide (2.5 μ g/ml) alone the abundances of C1-inh mRNA, C2 mRNA and B mRNA were 2.9, 2.0 and 3.0 times greater than their respective control levels. In the presence of IFN- γ (1.0 ng/ml) alone these relative mRNA abundances were increased to 7.5-, 2.5- and 4.1-fold, and in the presence of both cycloheximide and IFN- γ they were increased to 10.2, 4.1 and 5.9 times the levels in the control cultures (Fig. 4). The abundance of C3 mRNA was unaffected by cycloheximide treatment when the results were adjusted for the expression of actin mRNA (Fig. 4).

Duration of effects of IFNs on mRNA abundances

After the removal of the IFNs (α , β or γ) from the cultures the abundances of C2 mRNA and B mRNA declined over the next 8 h towards basal levels of expression. This also occurred for C1-

Fig. 2. Effects of concentration of IFNs on the rates of synthesis of C1-inh, C2, B and C3 by human monocytes (left) and on the abundances of C1-inh mRNA, C2 mRNA, B mRNA and C3 mRNA (right) obtained by scanning the autoradiographs of several dot-blots of the same RNA prepared from the same cultures used in the Northern blots in Fig. 1

○, IFN- α ; △, IFN- β ; ●, IFN- γ . Each dilution of RNA was tested in duplicate and the mean relative abundance of specific mRNA is given for each concentration of IFN used in the cultures. An arbitrary value of 1.00 was assigned to the mean relative abundance of mRNA in unstimulated monocytes.

inh mRNA in monocytes that had been treated with IFN- α and IFN- β . In contrast, in IFN- γ -treated cells the C1-inh mRNA abundance remained elevated over the 24 h period (Fig. 5).

Synergistic effects between IFNs

The effects of IFN- α (1 ng/ml) and IFN- β (1 ng/ml) on the secretion rates of complement components and mRNA abundances were additive (Fig. 6). In contrast, IFN- α (1 ng/ml) and IFN- β (1 ng/ml) each acted synergistically with IFN- γ (1 ng/ml) on the stimulation of secretion rates of C1-inh and B and the relative abundances of C1-inh mRNA and B mRNA (Fig. 6). Measurements of protein synthesis rates and mRNA abundances revealed no evidence of a synergistic effect on the synthesis of C2 (Fig. 6) or of C3 (results not shown). C2 mRNA and B mRNA were not detected on the untreated monocytes in this experiment (Fig. 6).

Duration of the synergistic effect of IFNs

For the maximum synergistic effect on C1-inh and B synthesis both cytokines had to be present simultaneously in the monocyte

Fig. 3. Time required for the effects of IFNs on mRNA abundance in monocytes

A 2 μ g portion of total cellular RNA was run on each lane, and the blots were probed sequentially for C1-inh mRNA, C2 mRNA, B mRNA and subjected to autoradiography. The relative abundances of C1-inh mRNA, C2 mRNA and B mRNA were obtained by scanning the autoradiographs and plotted against the time of exposure to each IFN: $\circ$, IFN- α ; $\triangle$, IFN- β ; $\bullet$, IFN- γ . [In this experiment B mRNA was not detectable at time 0 so the abundance measured at 30 min (0–5 h) was ascribed an arbitrary value of 1.0.]

cultures (Fig. 7). The synergistic effect was not observed when the second IFN was added to monocyte cultures 1 h after the removal of the first IFN.

DISCUSSION

IFNs α and β (type I IFNs) and IFN- γ (type II IFN) act through distinct receptors (Auger & Morgensen, 1983), to modulate a number of monocyte/macrophage functions, in-

Fig. 5. Duration of effects of IFNs on mRNA abundance in monocytes

A 5 μ g portion of total cellular RNA, obtained from monocytes at timed intervals after the removal of IFNs from the culture medium, was run on each lane of a denaturing agarose gel, subjected to Northern blotting, probed sequentially for C1-inh mRNA, C2 mRNA and B mRNA and subjected to autoradiography. The relative abundances of C1-inh mRNA, B mRNA and C2 mRNA (obtained by scanning the autoradiographs) are plotted against the time interval (0, 1, 2, 4, 8 and 24 h) after the removal of the IFNs: $\circ$, IFN- α ; $\triangle$, IFN- β ; $\bullet$, IFN- γ .

Fig. 4. Effects of cycloheximide on the abundances of C1-inh mRNA, C2 mRNA, B mRNA, C3 mRNA and actin mRNA in IFN- γ (1 ng/ml)-treated monocytes and control (untreated) monocytes

A 5 μ g portion of total cellular RNA was run on each lane of denaturing agarose gel, transferred by Northern blotting, probed for each of the above mRNAs sequentially and subjected to autoradiography. Lanes a and b, IFN- γ -treated monocytes; lanes c and d, untreated monocytes; and lanes a and c cultures to which cycloheximide (2.5 μ g/ml) was added. Numerical values for the degree of expression of each of the genes were obtained by scanning the autoradiograph. As some variability of RNA loading was observed in this experiment, the relative mRNA abundances of these genes were adjusted for the degree of actin expression, which was assumed to be unchanged.

Fig. 6. Synergism between IFNs

A 2 μ g portion of total cellular RNA was run on each lane of an agarose gel, subjected to Northern blotting, probed sequentially for C1-inh mRNA, C2 mRNA and B mRNA and subject to autoradiography. The effects of combinations of IFNs on the rates of secretion of C1-inh, C2 and B (left) and the relative abundances (obtained by scanning the autoradiographs) of C1-inh mRNA, C2 mRNA and B mRNA (right) are shown. Combinations of IFNs (each at 1 ng/ml) were added as follows: column a, IFN- α ; column b, IFN- α plus IFN- β ; column c, IFN- α plus IFN- γ ; column d, IFN- α plus IFN- β plus IFN- γ ; column e, IFN- β ; column f, IFN- β plus IFN- γ ; column g, IFN- γ ; column h shows control (untreated) monocytes. [For C2 mRNA and B mRNA an arbitrary value of 1.0 was ascribed to the degree of expression measured in the IFN- γ -treated monocytes (column g).]

cluding stimulation of macrophage tumoricidal activity (Meltzer *et al.*, 1982) and expression of HLA-DR protein (Basham & Merigen, 1983), interleukin-2 receptor (Holler *et al.*, 1987), transferrin receptor (Taetle & Honeysett, 1988), Fc γ -receptor and Ia antigen (Warren & Vogel, 1985). The IFNs also stimulate Fc- γ -receptor-mediated phagocytosis (Warren & Vogel, 1985) and antibody-dependent cytotoxicity (Firestein & Zvaifler, 1987), and increase the synthesis of a number of mononuclear phagocyte secretory products, including interleukin-1 β (Vermuelen *et al.*, 1987), plasminogen activator (Mokoena & Gordon, 1985), C2, B (Strunk *et al.*, 1985) and C1-inh (Hamilton *et al.*, 1987). They also inhibit some monocyte functions, such as the expression of differentiation antigens (Firestein & Zvaifler, 1987), synthesis of 28 S rRNA (Varesio *et al.*, 1987), C3 (Strunk *et al.*, 1985; Hamilton *et al.*, 1987) and properdin (Lappin & Whaley, 1987). This present study examines the effects of the IFNs on the synthesis of C2, B, C1-inh and C3 by human monocytes, by comparing the effects of these cytokines on the relative abundance of each mRNA and the concentrations of each of these proteins in culture fluid. All three IFNs increased the synthesis and secretion of C2, B and C1-inh, in a dose-dependent manner, but, whereas IFN- γ inhibited the synthesis of C3, neither IFN- α nor IFN- β had any effect on the production of this protein. These

Fig. 7. Duration of the synergistic effects of IFNs

A replicate series of monocyte cultures were incubated with IFN- α (1 ng/ml) or IFN- γ (1 ng/ml) for 2 h at 37 °C. After being washed the cultures were continued, and at 0, 1, 2, 4, 8, and 24 h IFN- γ (1 ng/ml) was added to those pretreated with IFN- α (●) and IFN- α (1 ng/ml) to those pretreated with IFN- γ (○). For the time 0 point, the second of the cytokines was added without removal of the first cytokine. The cells were lysed and the RNA was extracted 24 h after the addition of the second cytokine. A 2 μ g portion of total cellular RNA was electrophoresed on each lane of an agarose gel, subjected to Northern blotting and probed sequentially for C1-inh mRNA and B mRNA. This Figure shows the relative abundance (obtained by scanning densitometry) of C1-inh mRNA. The control cultures were incubated in the absence of IFN (□) and the presence of IFN- α (1 ng/ml; ■) or IFN- γ (1 ng/ml; ■) for 24 h.

findings are generally in agreement with those obtained by Strunk *et al.* (1985) and Hamilton *et al.* (1987). IFN- α and IFN- β appear to be more effective than IFN- γ at stimulating the synthesis of C2 and B, but IFN- γ was far more effective than either IFN- α or IFN- β at stimulating the synthesis of C1-inh. These results do differ, however, from those obtained by Hamilton *et al.* (1987), who failed to show changes in C2 synthesis with either IFN- α or IFN- β , and also showed that higher concentrations of IFN- γ were required to stimulate synthesis of B than that of C2. The results of the present study, like those of Strunk and colleagues, showed that the C2 and B genes respond to IFN- α and IFN- β , although the results reported in the earlier publication (Strunk *et al.*, 1985) suggested that both C2 and B genes were less responsive to IFN- α than to IFN- β , whereas our present results demonstrate their equipotency. These relatively minor discrepancies could be due to variability in the activities of the different IFN preparations used in the different studies, or to methodological differences.

All three IFNs exerted their maximum effect on the synthesis of C2, B and C1-inh within 1–2 h of exposure. These findings are similar to those obtained by Hamilton *et al.* (1987), who showed that the maximum effect occurred after 60 min incubation with IFN- γ , but are in disagreement with those obtained by Strunk *et al.* (1985) with regard to synthesis of C2. Those workers reported that at least 8 h exposure to IFN- γ was required for stimulation of C2 synthesis, although the stimulation of B was observed after 2 h exposure. In these biosynthetic labelling experiments (Strunk *et al.*, 1985) the high background in the C2 immunoprecipitate might have influenced the results.

The changes observed in the secretion rates of each of these proteins parallel changes in the abundances of the corresponding mRNAs, indicating that the effect of the IFNs is not at the level of translation of the mRNAs. The rapidity of the onset of the

stimulatory effects of these cytokines on the synthesis of C2, B and C1-inh suggests an effect on transcription.

After removal of the cytokines from the monocyte cultures the abundances of C2 mRNA and B mRNA began to decrease after 2 h, and had almost reached basal levels of expression by 24 h. These findings are in agreement with previously published work (Strunk *et al.*, 1985), which showed that C2 mRNA and B mRNA abundances returned to control levels within 24 h. The rate of decline of the C1-inh mRNA abundance was similar to the rates of C2 mRNA and B mRNA when IFN- α and IFN- β were removed from the culture supernatants. However, in IFN- γ -treated cultures the C1-inh mRNA abundance remained elevated throughout the subsequent 24 h period. This finding suggests that, in addition to increasing transcription, IFN- γ may stabilize C1-inh mRNA. The observation that cycloheximide increased the abundances of C2 mRNA, B mRNA and C1-inh mRNA indicates that these mRNAs are normally degraded rapidly, suggesting the action of mRNA-destabilization factors (Shapiro *et al.*, 1987). A labile destabilizing factor of *c-myc* mRNA has been identified that modifies the rate of degradation of this mRNA (Brewer & Ross, 1989). This mRNA is stabilized when protein synthesis is inhibited (Dani *et al.*, 1984), presumably because of the inhibition of the synthesis of this labile destabilization factor. The mechanism by which IFN- γ modifies the stability of C1-inh mRNA is unknown, but could be related to the presence or absence of a specific destabilization factor.

Hamilton *et al.* (1987) have previously shown that IFN- α and IFN- β act synergistically with IFN- γ in the stimulation of monocyte B and C1-inh synthesis. The results reported in the present paper support and extend these findings. Parallel increases in C1-inh mRNA abundance and C1-inh secretion rates and in B mRNA abundance and B secretion rates were observed. For the maximum synergistic effect on the synthesis of C1-inh and B to occur it is necessary that the IFNs be added simultaneously to the cells. The mechanisms involved in the synergistic action of the IFNs on the synthesis of B and C1-inh are unclear. It is possible that their synergistic effects are due in part to an increase in the transcription of these genes combined with an increase in mRNA stability.

Further studies are required to investigate the effects of the IFNs on the transcription of the complement genes and on the stability of complement component mRNAs.

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