Interferon-induced transcriptional and post-transcriptional modulation of factor H and C4 binding-protein synthesis in human monocytes

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Cultured human monocytes synthesize factor H (H) and C4 binding protein (C4-bp), as assessed by measuring their presence in culture fluids, and demonstrating the presence of their corresponding mRNAs in total monocyte RNA by Northern blot analysis and by nuclear run-on experiments. H mRNA (4.3 kb and 1.8 kb) could only be detected when cycloheximide (2.5 μ g/ml) was present in monocyte culture fluid. Recombinant interferon- γ (IFN- γ) and lymphoblastoid interferon- α (IFN- α) produce dose-related increases in the synthesis of H and C4-bp and in the abundance of C4-bp mRNA (2.5 kb). Increased abundance of H mRNA was also seen when cycloheximide (2.5 μ g/ml) was present in the cultures. Both cytokines increased the transcription rates of the H and C4-bp genes. These changes in transcription were rapid, with significant increases being observed within 30 min of exposure. Following the removal of the cytokines from the cultures the transcription rates of both genes returned to control levels within 4 h. The effects of combining IFN- α and IFN- γ on H and C4-bp transcription rates, mRNA abundances and protein secretion rates were not quantitatively additive. The half-life of H mRNA in monocytes was 15 min, whereas that of C4-bp mRNA was 2 h 45 min. Neither half-life was altered by IFN- γ .

INTRODUCTION

Activation of the complement system by either the classical or the alternative pathway results in the production of enzymes (convertases) which activate the third (C3) and fifth (C5) components, with the generation of inflammatory peptides, anaphylatoxins and the lytic membrane attack complex (Whaley, 1987). The expression of the C3 and C5 convertases of both pathways is regulated by the enzyme factor I and its two cofactors C4-binding protein (C4-bp) and factor H (H), which bind to C4b (Gigli *et al.*, 1979) and C3b (Whaley & Ruddy, 1976) respectively. In the presence of C4-bp, factor I enzymically degrades C4b so that it cannot bind C2 to form the classical pathway C3 and C5 convertases (C4b2a and C4b3b) (Gigli *et al.*, 1979). Similarly, in the presence of H, factor I converts C3 to C3bi so that the alternative pathway C3 and C5 convertases [C3bBbP and (C3b)_nBbP] cannot be assembled (Pangburn *et al.*, 1977). In addition to cofactor activity, both proteins are able to accelerate the decay of preformed convertases. Thus C4-bp displaces C2a from the classical pathway convertases (Gigli *et al.*, 1979), and H displaces Bb from the alternative pathway enzymes (Weiler *et al.*, 1976).

Studies of the primary structures of C4-bp and H have shown them to have a large degree of sequence identity, each consisting of numerous short consensus repeat sequences of some 60 amino acids (Holers *et al.*, 1985; Klickstein *et al.*, 1987). The genes encoding H and C4-bp have been localized to chromosome 1 (Reid *et al.*, 1986; Kristensen *et al.*, 1987) along with the genes encoding complement receptors types 1 and 2 (CR1 and CR2), decay-accelerating factor (DAF) and membrane cofactor protein

(MCP) (Rey Campos *et al.*, 1987; Hing *et al.*, 1988) which also regulate complement activation, as part of the RCA (regulation of complement activation) gene cluster (Rodrigues de Cordoba & Rubinstein, 1987).

Although H and C4-bp mRNA have been detected in Northern blots of RNA extracted from the liver (Chung *et al.*, 1985; Schwaebler *et al.*, 1987; Ripoché *et al.*, 1987) the primary site of synthesis of these two proteins has not been determined. However, H has been shown to be synthesized by mononuclear phagocytes (Whaley, 1980), monocyte-like U937 cells (Malhotra & Sim, 1985), human (Katz & Strunk, 1988) and murine fibroblasts (Munos-Canoves *et al.*, 1989) and endothelial cells (Ripoché *et al.*, 1988a; Brooimans *et al.*, 1989) in culture *in vitro*. C4-bp synthesis by these cells has not been reported. In the present study we have investigated the synthesis of H and C4-bp by human monocytes in the presence and absence of interferons (IFNs).

MATERIALS AND METHODS

Reagents

The following substances were purchased from the sources shown. RPMI 1640 and Linbro multiwell tissue culture dishes (Flow Laboratories, Rickmansworth, Herts., U.K.); foetal calf serum (FCS), antibiotic anti-mycotic solution, restriction enzymes, modifying enzymes (Gibco, Paisley, Scotland, U.K.); [α -³²P]dCTP (PB 10205), [α -³²P]UTP (PB 10203), [³H]uridine (TRK 410), Hybond-N nylon membranes (Amersham International, Amersham, Bucks., U.K.); alkaline phosphatase, random-

Abbreviations used: the nomenclature of complement is that published by the World Health Organisation (1968, 1981); H, factor H; C4-bp, C4 binding protein; DAF, decay-accelerating factor; C1-inh, C1 inhibitor; C2, second component of complement; B, factor B; C3, third component of complement; C5, fifth component of complement; IFN- α , lymphoblastoid interferon- α ; IFN- β , fibroblast interferon- β ; IFN- γ , recombinant interferon- γ ; IFNs, interferons; ABS, human AB serum, heat-inactivated for 2 h at 56 °C; RPMI, RPMI 1640 medium containing Hepes buffer; FCS, foetal calf serum, heat-inactivated for 2 h at 56 °C; NP-40, Nonidet P-40; PBS, potassium phosphate (100 mM)-buffered saline, pH 7.4; SSC, 150 mM-NaCl/15 mM-trisodium citrate, pH 7; SCR, short consensus repeat; CR1, complement receptor type 1; CR2, complement receptor type 2; MCP, membrane cofactor protein.

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primed DNA labelling kit (Boehringer Mannheim, Mannheim, Germany; the dye H33258, BSA, dithiothreitol, ATP, GTP, CTP, UMP, IDP and UTP (Sigma Chemical Co., Poole, Dorset, U.K.); lymphoblastoid interferon- α (IFN- α), fibroblast interferon- β (IFN- β) (Strattech Scientific, London, U.K.); RNazol (Biogenesis, Bournemouth, U.K.).

Recombinant interferon- γ (IFN- γ ; Immuneron B68201, lot no. 10M05, 14×10^6 international reference units/mg) was a gift from Biogen SA, Geneva, Switzerland. Human AB serum (ABS) was supplied by the Scottish Blood Transfusion Service (Law Hospital, Carlisle, Scotland, U.K.). The plasmid B38-1 (H cDNA) was a gift from Dr. R. B. Sim, Department of Biochemistry, University of Oxford, Oxford, U.K.) (Ripoche *et al.*, 1988b). The plasmid BP-8 (C4-bp cDNA) was a gift from Dr. K. B. M. Reid (Immunochemistry Unit, Department of Biochemistry, University of Oxford) (Chung *et al.*, 1985). The plasmid p749 (actin cDNA) was a gift from Dr. F. Frischauf (European Molecular Biology Laboratory, Heidelberg, Germany) (Katcoff *et al.*, 1980).

Preparation of DNA probes

The cDNA inserts were excised from their plasmid vectors as follows: actin cDNA (p749) with *Pst*I; H cDNA (B38-1) and C4-bp cDNA (BP8) with *Bam*HI and *Hind*III. The inserts were isolated by electrophoresis in agarose gels, purified by phenol/chloroform (1:1, v/v) extraction and 0.3 M-sodium acetate/ethanol (1:3, v/v) precipitation. For the run-on transcription assays and mRNA stability studies, each insert cDNA was prepared at a concentration of $2 \mu\text{g}/\mu\text{l}$ in $10 \times \text{SSC}$, heated to 65°C for 5 min, cooled on ice, dotted on to a Hybond-N filter and immobilized by u.v. irradiation (10 min). Linearized pAT 153 pvu II-8 vector was used as a control.

For probing Northern blots and double dilution dot-blots, the cDNA inserts were labelled with [α - ^{32}P]dCTP using the random-primed DNA labelling reaction (Feinberg & Vogelstein, 1983, 1984).

Monocyte cultures

Human monocyte monolayers were prepared from the buffy coats of blood donations in 24-well Linbro tissue culture plates (Lappin *et al.*, 1984) and the cells were cultured in RPMI 1640 containing 10% ABS at 37°C in a humidified air/ CO_2 (19:1) atmosphere. After 3 days, the medium was changed to RPMI 1640 containing 20% FCS and the cells were incubated under the same conditions for 24 h before any experiments were started. At this time IFNs were added and the cultures were incubated for defined periods. Replicate cultures were used for the preparation of nuclei for run-on transcription assays and RNA for Northern blot and dot-blot analyses. A further set of cultures was incubated for 24 h for the measurement of H and C4-bp concentrations in the culture supernatants. The monolayers were lysed in 0.05% (v/v) SDS and the DNA content was determined by fluorimetry (Cesarone *et al.*, 1979) to determine the number of cells in each monolayer ($1 \mu\text{g}$ of DNA = 10^5 monocytes).

Run-on transcription assay

Nuclei were prepared using a modification (Lappin *et al.*, 1990b) of the method of Greenberg & Ziff (1984).

Nuclei (2×10^7 in $100 \mu\text{l}$) in 10 mM-Tris (pH 8.3) containing 10 mM-NaCl, 3 mM-MgCl₂ and 0.5% (v/v) Nonidet P-40 (NP-40) were mixed with $100 \mu\text{l}$ of 10 mM-Tris/HCl containing 5 mM-MgCl₂, 300 mM-KCl, 0.5 mM each of ATP, GTP and CTP, 2 mM-dithiothreitol and $100 \mu\text{Ci}$ of [α - ^{32}P]UTP, and incubated for 30 min at 30°C , after which time RNA was prepared from the nuclei using a modification (Lappin *et al.*, 1990b) of the method

of Chomczynski & Sacchi (1987). [^{32}P]RNA was dissolved in hybridization buffer (10^6 c.p.m./ml) (Lappin *et al.*, 1990b) and hybridization was carried out at 42°C for 36 h (Wahl *et al.*, 1979) to cDNA immobilized on Hybond-N membranes. The membranes were then washed sequentially with $2 \times \text{SSC}$ containing 0.1% SDS for 30 min at room temperature, $2 \times \text{SSC}$ containing 0.1% SDS for 30 min at 65°C , $0.5 \times \text{SSC}$ containing 0.1% SDS for 30 min at 65°C , and $0.1 \times \text{SSC}$ containing 0.1% SDS for 30 min at 65°C , before they were air-dried and subjected to autoradiography (Fulton *et al.*, 1985). Radiolabelled RNA was also treated with RNAase A ($1 \mu\text{g}/\text{ml}$) for 10 min at 37°C prior to hybridization, to serve as a control.

In a preliminary experiment, RNA was isolated from nuclei of untreated monocytes which had been subjected to a run-on transcription assay. This RNA was analysed by electrophoresis on a denaturing agarose gel and transferred on to a Hybond-N membrane that had been soaked in H insert cDNA ($20 \mu\text{g}/\text{ml}$ in $10 \times \text{SSC}$) and baked for 2 h at 80°C . The membrane was washed to the same stringency as above, and subjected to autoradiography.

Total cellular RNA

Total cellular RNA was prepared using RNazol, and Northern blot and double-dilution dot-blot analyses were carried out as described previously (Lappin *et al.*, 1990a).

Scanning of autoradiographs

Autoradiographs were scanned using a Helena Laboratories Autoscanner (Helena Laboratories, Gateshead, Tyne and Wear, U.K.). An arbitrary value of 1.00 was assigned to the control level of expression. Actin gene expression was assumed to remain constant, so any variations in actin densitometry readings were regarded as indicating variations in RNA loading and were therefore used to correct the results of H and C4-bp readings.

Complement component synthesis rates

Concentrations of H and C4-bp in the 24 h culture supernatants were measured by e.l.i.s.a. (Lappin *et al.*, 1986). As the medium was changed at the beginning of the 24 h culture period, and as the rate of accumulation of both components over a 48 h period was linear (D. F. Lappin & K. Whaley, unpublished work), we have calculated the average secretion rates of H and C4-bp over the 24 h period and expressed them as molecules/min per cell.

Measurement of half-lives of H mRNA and C4-bp mRNA

The half-lives of H and C4-bp mRNAs were measured using a modification (Lappin *et al.*, 1990b) of the method described by Brinckerhoff *et al.* (1986). [^3H]Uridine ($40 \mu\text{Ci}/\text{ml}$) was added and the cells were incubated for 18 h with or without IFN- γ . After three rapid washes with Hanks balanced salt solution, the monocytes were incubated in RPMI/FCS containing 5 mM-uridine. RNA was prepared at time 0 and after chase periods of 1, 2, 3, 4, 5, 6, 8, 12, 16, 20 and 24 h. The amounts of mRNA were assessed by hybridization to Hybond-N-immobilized cDNA inserts (Lappin *et al.*, 1990b). The size of the [^3H]UTP pool was measured using h.p.l.c. (Gilson 302; Anachem, Luton, Beds., U.K.). A replicate set of monocyte cultures were lysed in 0.5 ml of ice-cold HClO_4 . The lysate was centrifuged to remove debris, neutralized with 5 M-KOH, left for 30 min on ice and filtered through $0.2 \mu\text{m}$ Acrodisc filters (Gelman, Brackley, Northants., U.K.). A 0.25 ml portion of each sample was applied to an anion-exchange column (TSK DEAE-25W; Scotlab, Bellshill, Scotland, U.K.). The nucleotides were eluted with a 10–850 mM-KH₂PO₄/K₂HPO₄ (pH 7.0) gradient. The absorbance of the eluate was measured at 254 nm and the positions of UMP, UDP and UTP

pools were determined by comparison with standard nucleotides (each at 0.5 μ M).

The time required during the chase period to deplete the [3 H]UTP pool to less than 10% of its original value was 4 h. The half-life of each mRNA was determined from this time point onwards.

RESULTS

Effects of interferon concentration on H and C4-bp synthesis and mRNA abundance

IFNs α , β and γ all produced similar dose-dependent increases in the levels of H and C4-bp in monocyte culture supernatants (Fig. 1; results shown only for IFN- α and IFN- γ). Increases in the abundance of C4-bp mRNA (2.5 kb) were also observed (Figs. 2a and 2b). However, H mRNA (4.3 and 1.8 kb bands) could only be detected when monocytes had been cultured in the presence of cycloheximide (2.5 μ g/ml) (Fig. 2b). Under these conditions all three IFNs produced dose-dependent increases in the abundances of both species of H mRNAs (Fig. 2; results shown only for IFN- γ). The abundance of actin mRNA was not affected by IFNs (Fig. 2).

Effect of IFNs on transcription

In preliminary experiments IFNs α , β and γ (all at 10 ng/ml) produced a 2–3-fold increase in the rates of transcription of the genes encoding H and C4-bp, whereas the transcription of the actin gene was not affected. As H mRNA was not detected in monocytes in the absence of cycloheximide, we decided to analyse the RNA from the run-on transcription assay by Northern blotting, in order to confirm the specificity of the hybridization reaction. Two bands of radioactivity of 4.3 kb and 1.8 kb were

Fig. 1. Effects of IFNs on the production of H and C4-bp by human monocytes

The effects of IFN- α (0–100 ng/ml) and IFN- γ (0–100 ng/ml) on the synthesis rate (mol/min per cell) of (a) H (IFN- α , Δ ; IFN- γ , $\circ$), and (b) C4-bp (IFN- α , $\blacktriangle$; IFN- γ , $\bullet$) are shown. Results are the means of three experiments performed in duplicate.

Fig. 2. Effect of IFN- γ and cycloheximide on H, C4-bp and actin mRNA abundances

(a) Results of scanning densitometry on autoradiographs of Northern blots of 10 μ g of RNA isolated from control and IFN- γ (0.01–100 ng/ml)-treated (for 2 h) monocytes. The Northern blots were probed sequentially for H ($\circ$), C4-bp ($\bullet$) and actin and subjected to autoradiography. The results are expressed relative to control levels of expression, which had a value of 1.00. Insets show the autoradiographs from this experiment. Lanes 1–6 indicate IFN concentrations of 0, 0.01, 0.1, 1, 10 and 100 ng/ml respectively. (b) Results of scans made on autoradiographs of Northern blot analysis of 10 μ g of RNA isolated from cycloheximide (2.5 μ g/ml)-treated control monocytes and monocytes pretreated for 2 h with IFN- γ (0.01–100 ng/ml). The Northern blots were probed sequentially for H ($\square$), C4-bp ($\blacksquare$) and actin. Insets show the autoradiographs from this experiment; IFN concentrations in lanes 1–6 are as in (a).

detected (Fig. 3c). Since all three IFNs produced similar effects on the synthesis of H and C4-bp and on the transcription rates of their genes, we decided to limit further studies to IFN- α and IFN- γ .

The effects of IFN- α and IFN- γ on H and C4-bp transcription rates were dose-dependent over concentration ranges of 1–100 ng/ml (Figs. 3a and 3b). IFN- α and IFN- γ (both at 10 ng/ml) produced detectable increases in the levels of transcription of the genes encoding H and C4-bp within 30 min of exposure. After 2 h of incubation, stimulation of transcription was maximal for both genes (Fig. 4a).

Within 4 h of the removal of the IFNs from monocyte culture fluid, the rates of transcription of the H and C4-bp genes returned to their control values (Fig. 4b).

The effects of the combination of IFN- α (1 ng/ml) and IFN- γ (1 ng/ml) on the synthetic rates of H and C4-bp (Table 1), on the relative abundances of H mRNA and C4-bp mRNA and on the transcription rates of the H and C4-bp genes were not quantitatively additive (Table 1).

Fig. 3. Effects of IFNs on H and C4-bp gene transcription

The Figure shows the effects of IFN- α and IFN- γ (both at 0–100 ng/ml) on the transcription rates of the genes encoding (a) H (IFN- α , Δ ; IFN- γ , $\circ$), and (b) C4-bp (IFN- α , $\blacktriangle$; IFN- γ , $\bullet$) relative to control levels of transcription of these genes, which had a value of 1. Autoradiographs from these experiments including the actin signal are shown alongside the densitometry measurements. Plasmid pAT153 (pAT) was included as a control for non-specific hybridization in this experiment. Pre-treatment of RNA isolated from nuclei following a run-on transcription assay with RNAase A (10 μ g/ml) abrogated the hybridization signals obtained. (c) RNA prepared from 10^7 monocyte nuclei following a run-on transcription assay was sized by electrophoresis on a denaturing agarose gel and transferred to a membrane impregnated with H cDNA.

Half-lives of mRNAs

The half-life of H mRNA, which was extremely short, was estimated to be about 15 min or less, whereas the half-life of C4-bp mRNA was 2 h 45 min. These half-lives were not altered significantly by IFN- γ treatment (Fig. 5). The half-life of total RNA was the same in both control and IFN- γ -treated monocytes (16 h 20 min).

The specific activity of total RNA remained unchanged by IFN- γ treatment of monocytes (1.52×10^5 c.p.m./ μ g of RNA in control; 1.54×10^5 c.p.m./ μ g of RNA in IFN- γ -treated monocytes).

DISCUSSION

IFNs have been known to stimulate the synthesis of C2, B (Strunk *et al.*, 1985) and C1-inh (Hamilton *et al.*, 1987) by human monocytes, whereas IFN- γ inhibits C3 (Hamilton *et al.*, 1987) and properdin (Lappin & Whaley, 1987) synthesis. Recently we have demonstrated that changes in the secretion rates of C2, B, C1-inh and C3 are paralleled by changes in the abundances of their corresponding mRNAs (Lappin *et al.*, 1990a). In the present study, we have shown that the synthesis of C4-bp is increased by IFNs, and that the increase is associated with increased abundance of C4-bp mRNA. Although we were also

Fig. 4. Kinetics of IFN- γ effect

(a) Time required for IFN- γ (10 ng/ml) to elicit a maximum effect on the transcription of the genes encoding H ($\circ$) and C4-bp ($\bullet$). Measurements of the level of transcription of these genes were made at 30 min, 2 h and 8 h following the addition of this cytokine. The results are shown relative to control levels of transcription (at time 0; which had a value of 1.00) of these genes, as assessed by scanning densitometry on autoradiographs (inset, including actin signal) obtained from run-on transcription assays. (b) Duration of the IFN effect on transcription of the genes encoding H and C4-bp. Monocytes were treated for 2 h with IFN- γ (10 ng/ml). This cytokine was then removed and nuclei were prepared at time intervals of 0, 1, 2, 4 and 24 h (following the removal of this cytokine). The levels of transcription of these genes are shown relative to control levels of transcription assessed by scanning densitometry on autoradiographs (inset) obtained from run-on transcription assays. Plasmid pAT153 (pAT) was used as a control for non-specific hybridization in these experiments. C indicates results in the absence of IFN- γ .

Table 1. Effect of IFNs on the expression of H and C4-bp genes

Differences in the actin signal were taken to signify variations in loading of RNA and the results obtained were adjusted accordingly. Secretion rate values are means $\pm$ S.E.M. Unless otherwise stated, all IFNs were used at a concentration of 1 ng/ml. In the experiment in which IFN- β was used at a concentration of 1 ng/ml, C4-bp mRNA abundance was not determined. However, at 10 ng/ml, this cytokine increased C4-bp abundance 2.4-fold. N.d., none detected; N.D., not determined.

Protein	Treatment	Relative rate of transcription	Relative mRNA abundance	Secretion rate (mol/min per cell)
H	Control	1.00*	N.d.	10.2 $\pm$ 1.4
	IFN- α	2.95	N.d.	22.5 $\pm$ 1.4
	IFN- β	2.51	N.D.	24.7 $\pm$ 2.0
	IFN- γ	3.00	N.d.	25.0 $\pm$ 2.1
	IFN- α + IFN- γ	3.63	N.d.	31.5 $\pm$ 2.5
C4-bp	Control	1.00*	1.00*	51.5 $\pm$ 1.2
	IFN- α	1.95	1.99	102.6 $\pm$ 2.9
	IFN- β	2.62	N.D.	100.5 $\pm$ 4.2
	IFN- γ	2.15	2.05	107.7 $\pm$ 5.3
	IFN- α + IFN- γ	2.90	2.87	128.0 $\pm$ 9.8

* Means of two experiments.

Fig. 5. Half-lives of (a) H mRNA and (b) C4-bp mRNA assessed by pulse-chase with [³H]uridine

The abundance of each ³H-labelled mRNA in control (○) and IFN-γ-treated (●, 10 ng/ml) monocytes was measured at time intervals (0, 1, 2, 4, 8, 12 and 16 h) during the chase period, following depletion of the [³H]UTP pool, by hybridization to immobilized H cDNA and C4-bp cDNA inserts. The results are expressed as c.p.m./μg of RNA and represent the means of two determinations. The specific activity of total RNA in control cells and in IFN-γ-treated monocytes was 1.52×10^5 c.p.m./μg of RNA and 1.54×10^5 c.p.m./μg of RNA respectively at time 0, and 0.767×10^5 c.p.m./μg of RNA and 0.781×10^5 c.p.m./μg of RNA at 16 h, with half-lives of 16 h 20 min and 16 h 30 min respectively.

able to show that IFNs increased the synthesis rate of H, initially we were unable to demonstrate increases in H mRNA by Northern blotting. However, the addition of cycloheximide (2.5 μg/ml) to the culture medium permitted the demonstration of two H mRNA species of 4.3 kb and 1.8 kb. This is in contrast with liver RNA, in which three species of H mRNA (4.3 kb, 1.8 kb and 1.4 kb) can be detected (Schwaebler *et al.*, 1987; Ripoché *et al.*, 1988b). The 1.4 kb mRNA does not appear to be expressed in monocytes. When monocytes were treated with IFNs and cycloheximide together, we were able to show that the IFNs produced a dose-related increase in the abundances of the 4.3 kb and 1.8 kb species of H mRNA. Thus these results confirm the previous report from our laboratory that H is synthesized by human monocytes (Whaley, 1980). The failure of Katz & Strunk (1988) to demonstrate H mRNA on Northern blots of monocyte RNA was obviously due to the inherent instability of H mRNA. However, we cannot explain their failure to demonstrate H synthesis in monocytes by biosynthetic labelling.

The synthesis of H by fibroblasts (Katz & Strunk, 1988; Munos-Canoves *et al.*, 1989) and endothelial cells (Ripoché *et al.*, 1988a) has previously been shown to be modulated by IFNs. Our results show that IFNs α, β and γ regulate both H and C4-bp synthesis at the transcriptional level and that the effect on transcription occurs rapidly (within 30 min of exposure) and depends upon the continued presence of IFNs to be sustained. Our results also show that when IFN-α and IFN-γ were present simultaneously, their combined effects on H and C4-bp synthesis were not quantitatively additive, as assessed by measurement of synthesis rates, mRNA abundances and transcription rates. These results are similar to those found in a study of the effects of IFNs on monocyte C2 synthesis, but differ from those of IFNs on C1-inh and B synthesis (Hamilton *et al.*, 1987; Lappin *et al.*, 1990a); in these studies IFN-α and IFN-β acted synergistically with IFN-γ to stimulate synthesis. The effects of the IFNs on the transcription of the C1-inh and B genes have been shown not to

be quantitatively additive (Lappin *et al.*, 1990b), but IFN-γ was also found to increase the stability of both C1-inh mRNA and B mRNA (Lappin *et al.*, 1990b). It was therefore concluded that a combination of increased transcription (caused by IFN-α and IFN-γ treatment) and increased mRNA stability (produced by IFN-γ treatment) was required for the synergistic effect. Thus the failure to demonstrate synergism in the present experiments can be explained on the basis that IFN-γ did not affect the stabilities of either H mRNA or C4-bp mRNA. The extremely short half-life of H mRNA (< 15 min) probably accounts for our inability to detect H mRNA unless cycloheximide was added to monocyte culture fluid. Synthesis of H and C4-bp by monocytes may influence complement activation at sites of inflammation.

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