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Effect of interferon-γ on complement gene expression in different cell types

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We have studied the expression of the complement components C2, C3, factor B, C1 inhibitor (C1-inh), C4-binding protein (C4-bp) and factor H in human peripheral blood monocytes, skin fibroblasts, umbilical vein endothelial cells (HUVEC) and the human hepatoma cell line G2 (Hep G2) in the absence and the presence of interferon-γ (IFN-γ). E.l.i.s.a. performed on culture fluids, run-on transcription assays, Northern blot and double-dilution dot-blot techniques confirmed that monocytes expressed all six components, whereas fibroblasts, HUVEC and HepG2 each expressed five of the six components. Fibroblasts and HUVEC did not synthesize C4-bp, and Hep G2 did not produce factor H. In addition to these differences, the synthesis rates of C3, C1-inh and factor H were not the same in all cell types. However, the synthesis rates of C2 and factor B were similar in all four cell types. The half-lives of the mRNAs were shorter in monocytes than in other cell types. Monocyte factor H mRNA had a half-life of 12 min in monocytes, compared with over 3 h in fibroblasts and HUVEC. The instability of factor H mRNA in monocytes may contribute to their low factor H secretion rate. IFN-γ produced dose-dependent stimulation of C2, factor B, C1-inh, C4-bp and factor H synthesis by all cell types expressing these proteins, but decreased C3 synthesis in all four cell types. Cell-specific differences in the response to IFN-γ were observed. The increased rates of transcription of the C1-inh and factor H genes in HUVEC were greater than in other cell types, while the increased rate of transcription of the C2, factor B and C1-inh genes in Hep G2 cells was less than in other cell types. IFN-γ did not affect the stability of C3, factor H or C4-bp mRNAs, but increased the stability of factor B and C1-inh mRNAs and decreased the stability of C2 mRNA. Although these changes occurred in all four cell types studied, the half-life of C1-inh mRNA in monocytes was increased almost 4-fold, whereas the increases in the other cell types were less than 30%. These data show that the constitutive synthesis rates of complement components may vary in the different cell types. They also show that the degree of change in synthesis rates in response to IFN-γ in each of the cell types often varies due to differences in transcriptional response, sometimes in association with changes in mRNA stability.

INTRODUCTION

The plasma levels of most complement proteins are maintained by hepatic synthesis (Alper *et al.*, 1968; Colten, 1976), and cytokines such as interleukin-1, tumour necrosis factor α, interleukin-6 and interferon-α (IFN-γ) act on hepatocytes to regulate the synthesis of complement components (Miura *et al.*, 1987; Andus *et al.*, 1987; Ramadori *et al.*, 1988; Anthony *et al.*, 1989). However, synthesis of complement also occurs at a number of extrahepatic sites, including sites of inflammation (Ruddy & Colten, 1974; Moffat *et al.*, 1989). The potential sources of extrahepatic synthesis *in vivo* include mononuclear phagocytes, fibroblasts, endothelial cells and epithelial cells, all of which have been shown to synthesize complement proteins *in vitro* (Colten, 1976). The ability of these cells to synthesize complement components locally at the tissue level, particularly at sites of inflammation, may be important for determining the extent of local complement activation. The second component of complement (C2) and factor B are the rate-limiting components in the classical and alternative pathways of complement activation respectively, and along with the third complement component (C3) are constituents of the classical pathway C5 convertase and the alternative pathway C3 and C5 convertases respectively. Activation of these pathways leads to formation of inflammatory mediators, anaphylatoxins and the membrane attack complex (for reviews see Whaley, 1987; Muller-Eberhard, 1988). C1-inh, a plasma protease inhibitor, regulates C1 activation and the activities of enzymes of the coagulation, fibrinolytic and kinin-generating systems (Carrell & Boswell, 1988). C4-binding protein (C4-bp) and factor H act as cofactors for the enzyme factor I to regulate the formation of the classical and alternative pathway C3 and C5 convertases (Whaley & Ruddy, 1976; Pangburn *et al.* 1977; Gigli *et al.*, 1979).

The secretion of cytokines by one cell type may modulate the synthesis of complement components by another cell type. The modulation of monocyte complement synthesis by interferons-α and -β (synthesized by fibroblasts) and -γ (synthesized by T-lymphocytes) are examples of such paracrine effects (Strunk *et al.*, 1985; Hamilton *et al.*, 1987; Lappin & Whaley, 1989, 1990; Lappin *et al.*, 1990*a*,*b*). Cytokine-mediated modulation of complement component synthesis by fibroblasts and human umbilical vein endothelial cells (HUVEC) are other examples (Katz & Strunk, 1988; Brooimans *et al.*, 1989; Dauchel *et al.*, 1990; Schwaeble *et al.*, 1991).

In the present study we have compared the synthesis rates of C2, factor B, C3, C1-inh, C4-bp and factor H by cultures *in vitro* of human peripheral blood monocytes, human skin fibroblasts, HUVEC and the hepatoma cell line (Hep G2) in the presence or absence of IFN-γ.

Abbreviations used: The nomenclature of complement is that used by the World Health Organisation (1968, 1981): C1-inh, C1-inhibitor; C2, second component of complement; C3, third component of complement; C4-bp, C4-binding protein. HUVEC, human umbilical vein endothelial cells; ABS, human AB serum (heat-inactivated for 2 h at 56 °C); RPMI, RPMI 1640 containing Hepes buffer; DMEM, Dulbecco's modified Eagle's medium; ECGF, endothelial cell growth factor; FCS, fetal calf serum (heat-inactivated for 2 h at 56 °C); IFN-γ, recombinant interferon-γ; PBS, potassium phosphate (10 mM)-buffered saline, pH 7.4; SSC, 150 mM-NaCl/15 mM-trisodium citrate, pH 7.

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MATERIALS AND METHODS

Reagents

The following reagents were purchased from the sources shown: Linbro multiwell tissue culture dishes, Linbro 75 cm^2 tissue culture flasks, Dulbecco's modified Eagle's medium (DMEM) and RPMI 1640 from Flow Laboratories (Rickmansworth, Herts., U.K.); fetal calf serum (FCS), antibiotic/antimycotic solution, restriction enzymes and trypsin/EDTA solution (trypsin 0.5 g/l, EDTA 0.2 g/l in Puck's saline) from Gibco BRL (Paisley, Renfrewshire, Scotland, U.K.); [α-^{32}P]dCTP (PB 10205, 3000 Ci/mmol), [α-^{32}P]UTP (PB 10203, 3000 Ci/mmol) and Hybond-N nylon membranes from Amersham International (Amersham Bucks., U.K.); Random-primed DNA labelling kit from Boehringer Mannheim (Mannheim, Germany); dye H33258, BSA, dithiothreitol, endothelial cell growth factor (ECGF), ATP, GTP, CTP and UTP from Sigma Chemical Co. (Poole, Dorset, UK); and RNAzol from Biogenesis (Bournemouth, Dorset, U.K.). Recombinant IFN-γ (Immuneron B68201, lot no. 10 MOS, 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, Carluke, Scotland, U.K.). Plasmids pC201 (C2 cDNA) and p2FB (factor B cDNA) were gifts from Dr. David Bentley and Dr. Duncan Campbell respectively (both at Medical Research Council Immunochemistry Unit, Department of Biochemistry, University of Oxford, Oxford, U.K.) (Bentley & Porter, 1984; Morley & Campbell, 1984). Plasmid B38-1 (factor H cDNA) was a gift from Dr. R. B. Sim (Department of Biochemistry, University of Oxford) (Ripoche *et al.*, 1988*a*). 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). Plasmid pC351 (C3 cDNA) was a gift from Dr. George Fey (Scripps Research Clinic, La Jolla, CA U.S.A.) (De Bruijn & Fey, 1985). C1-inh cDNA (pC1) was a gift from Dr. Philip Carter (Department of Biochemistry, University of Aberdeen) (Carter *et al.*, 1988). Actin cDNA (p749) was a gift from Dr. A. M. Frischauf (ICRF, London, U.K.).

Preparation of DNA probes

The cDNA inserts were excised from their plasmid vectors as follows: actin with *Pst*1; C1-inh, C2, factor B, factor H and C4-bp with *Bam*H1 and *Hin*dIII; and C3 with *Eco*R1. The inserts were isolated by electrophoresis on low-melting-point agarose gels and purified by phenol/chloroform (1:1, v/v) extraction and 0.3 M-sodium acetate/ethanol (1:3, v/v) precipitation (Sambrook *et al.*, 1989). For the run-on transcription assays, each insert cDNA was diluted to 2 μg/μl in 10 × SSC (1 × SSC = 0.15 M-NaCl/0.015 M-sodium citrate, pH 7), denatured, dotted on to a Hybond-N filter and fixed by u.v. irradiation (Lappin *et al.*, 1990*b*). The cDNA inserts were also labelled with [α-^{32}P]dCTP using the random-primed DNA labelling reaction (Feinberg & Vogelstein, 1983, 1984) and used for probing Northern blots and dot-blots.

Cell cultures

Human monocytes, skin fibroblasts, HUVEC and Hep G2 cells were all cultured in a CO_2/air (1:19) atmosphere. Cultures of fibroblasts, HUVEC and Hep G2 cells were passaged at confluence by incubation with trypsin/EDTA solution (10 ml/75 cm^2 flask; 5 ml/10 cm^2 flask) at 37 °C for 5 min until the cells were detached. Cells from 25 cm^2 flasks were transferred to 75 cm^2 flasks and those from the larger flasks were divided into three portions, each of which was transferred to a 75 cm^2 flask. Experiments on Hep G2 cells, fibroblasts and HUVEC were performed at confluence during the third passage.

Human skin fibroblasts were isolated from a skin biopsy (Mielke *et al.*, 1990) and cultured in DMEM supplemented with 10% FCS, initially in 25 cm^2 and then in 75 cm^2 tissue culture flasks.

HUVEC were isolated by cannulation of the umbilical vein and incubation with collagenase (0.2%, w/v) in cord buffer (140 mM-NaCl, 4 mM-KCl, 11 mM-glucose, 10 mM-phosphate, pH 7.4) for 15 min at 37 °C (Jaffe *et al.*, 1973). After three washes in RPMI, the endothelial cells were cultured in RPMI 1640 supplemented with 10% FCS and ECGF (15 ng/ml) in 25 cm^2 tissure culture flasks (10^6 cells/flask) which had been precoated with a 1% (w/v) gelatin solution (1 h, 37 °C), and then transferred to 75 cm^2 flasks.

Hep G2 cells were cultured in DMEM containing 10% FCS in 75 cm^2 flasks (Aden *et al.*, 1979).

Monocyte monolayers were prepared from the buffy coats of donated blood samples in 24-well Linbro tissue culture plates (Lappin *et al.*, 1984, and the cells were cultured in RPMI 1640 containing 10% ABS. After 3 days the medium was changed to RPMI 1640 containing 20% FCS.

Replicate cultures were used for the preparation of nuclei (for the run-on transcription assay) and RNA (for Northern and dot-blot analyses).

Measurements of proteins in culture fluids

A set of each type of cell culture was incubated for 72 h. At 24 and 48 h, samples of culture fluid were removed and stored at −70 °C, with the sample volume being replaced. At 72 h the culture supernatant was harvested. The concentrations of complement proteins in the culture fluid were determined by e.l.i.s.a. (Lappin *et al.*, 1986).

DNA content of monolayers

The monolayers were lysed in 0.05% (v/v) SDS and the DNA content was determined by fluorimetry (Cesarone *et al.*, 1979) in order to estimate the number of cells in each monolayer (1 μg of DNA is equivalent to 3.5×10^4 Hep G2 cells, 6×10^4 skin fibroblasts, 7×10^4 HUVEC and 1×10^5 monocytes).

Total cellular RNA

Total cellular RNA was prepared using RNAzol, and Northern blot and double dilution dot-blot techniques were carried out as described previously (Lappin *et al.*, 1990*b*). Blots were hybridized (incubation at 42 °C overnight) to the α-^{32}P-labelled cDNAs, washed to high stringency [0.1 × SSC containing 0.1% (w/v) SDS] at 65 °C (Lappin *et al.*, 1990*a*) and subjected to autoradiography (Fulton *et al.*, 1985).

Run-on transcription assay

Nuclei were prepared as previously described (Greenberg & Ziff, 1984) and suspended to 2×10^8/ml in 50 mM-Tris (pH 8.3)/40% (v/v) glycerol/5 mM-$MgCl_2$/0.1 mM-EDTA. A 100 μl portion of nuclei was mixed with an equal volume of 10 mM-Tris (pH 8.3)/5 mM-$MgCl_2$/300 mM-KCl/0.5 mM each of ATP, GTP, CTP/2 mM-dithiothreitol/100 μCi of [α-^{32}P]UTP, and incubated for 30 min at 30 °C. RNA, prepared from the nuclei using RNAzol (Lappin *et al.*, 1990*b*), was dissolved in hybridization buffer (10^6 c.p.m./ml) and hybridization was carried out at 42 °C to cDNAs immoblized on Hybond-N membranes. After high-stringency washes (Lappin *et al.*, 1990*b*) filters were subjected to autoradiography (Fulton *et al.*, 1985). Radiolabelled RNA was also treated with RNAase A (1 μg/ml) for 10 min at 37 °C prior to hybridization, to serve as a control.

mRNA half-life

The half-life of mRNA in control, untreated cells and IFN-γ-treated cells was measured following the inhibition of transcription with actinomycin D (5 μg/ml). The cells were incubated with and without IFN-γ for 2 h before the addition of actinomycin D, and total cellular RNA was prepared (as described above) at timed intervals (0, 1, 2, 4, 6, 8 and 24 h) after the addition of actinomycin D. The abundance of each mRNA was assessed by dot-blot analysis (Fulton *et al.*, 1985) of the total cellular RNA prepared at each time point. The relative abundance and the half-life of each mRNA were determined from scanning densitometric measurements of the autoradiographs.

Scanning of autoradiographs

Autoradiographs were scanned using a Joyce–Loebl Chromoscan-3 (Joyce–Loebl, Gateshead, Tyne and Wear, U.K.). An arbitrary value of 1.00 was assigned to the control level of expression.

RESULTS

Expression of C2, factor B, C3, C1-inh, C4-bp and factor H by four different cell types

The secretion rate of each of the complement proteins was determined (mol/min per cell) as their rate of accumulation in culture fluids (as measured by e.l.i.s.a.) and was linear over the culture period.

Peripheral blood monocytes synthesized and secreted all six components, whereas fibroblasts, HUVEC and Hep G2 cells expressed only five of the six components. Fibroblasts and HUVEC did not express C4-bp, whereas Hep G2 cells did not synthesize factor H (Table 1). These data on cell specificity were confirmed by the detection of the respective mRNAs (C2 mRNA, 2.9 kb; factor B mRNA, 2.6 kb; C3 mRNA, 5.2 kb; C1-inh mRNA, 2.1 kb; C4-bp mRNA; 2.5 kb; factor H mRNAs, 1.8 kb and 4.3 kb) by Northern blot and double dilution dot-blot analyses and run-on transcription assays in the cells expressing these genes (results not shown). In monocytes, factor H mRNA could not be detected unless cycloheximide (2.5 μg/ml) was present in the cultures. Synthesis rates of C2 and factor B were similar in all four cell types. In contrast, rates of C3 and C1-inh synthesis were far lower in HUVEC than in Hep G2 cells, monocytes and fibroblasts. The C4-bp synthesis rate was similar in Hep G2 cells and monocytes, and factor H synthesis rate was extremely low in monocytes compared with fibroblasts and HUVEC.

Effect of IFN-γ on expression of complement genes

IFN-γ produced dose-dependent increases in the transcription rates of the C2, factor B, C1-inh, factor H and C4-bp genes, and a dose-dependent decrease in the transcription of the C3 gene in each of the cell types in which they were expressed (Figs. 1 and 2). In general, the changes in transcription rates were accompanied by changes of similar magnitude and direction in mRNA abundances and protein secretion rates (Figs 1 and 2; Table 2). The major exception was C1-inh gene expression in monocytes, for which the changes in mRNA abundance and protein secretion rate was grossly in excess of changes in transcription.

IFN-γ did not induce the expression of those complement genes which were not expressed in control untreated cultures.

Relative mRNA stabilities in the four cell types

The half-life of actin mRNA was similar in Hep G2 cells, fibroblasts and HUVEC, and slightly shorter in monocytes. The half-lives of each mRNA were different in each of the cell types, generally being shortest in monocytes and longest in Hep G2 cells (Table 3). In addition, when each mRNA in a single cell type was ranked according to half-life, the ranking order was different in all cell types (Table 3).

Effect of IFN-γ on mRNA stability

IFN-γ did not alter the half-lives of the mRNAs for actin, C3,

Table 1. Expression of C2, factor B, C3, C1-inh, C4-bp and factor H by four different cell types

The secretion rate of each of the complement proteins was determined as their rate of accumulation in culture fluids, as measured by e.l.i.s.a, and was linear over the culture period. Each value represents the mean ± S.E.M. of four experiments.

Complement component	Secretion rate (mol/min per cell)			
	Hep G2	Monocytes	Fibroblasts	HUVEC
C2	44±2[2]	42±4	45±5	60±2
Factor B	80±5	75±8	97±26	53±2
C3	749±59	980±104	1005±37	81±7
C1-inh	82±19	187±31	207±37	32±15
C4-bp	21±4	37±6	0	0
Factor H	0	10±1	108±19	84±8

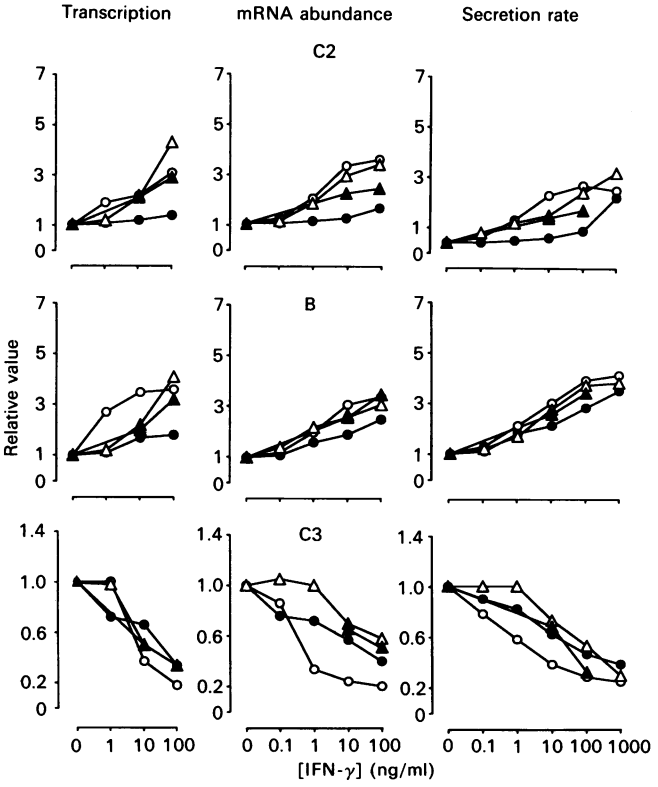

Fig. 1. Effect of IFN-γ on the expression of C2, factor B (B) and C3 by Hep G2 cells (●), human peripheral blood monocytes (○), human skin fibroblasts (△) and HUVEC (▲)

Dose-related effects of IFN-γ (0–100 ng/ml) are shown on transcription rate (left) and mRNA abundance (middle), and dose-related effects of IFN-γ (0–1000 ng/ml) are shown on the secretion rate of each protein (right). The results are expressed relative to control levels (cells not exposed to IFN-γ) which are assigned the arbitrary value of 1.00. Each point represents the mean of two experiments, except for the secretion rate, values which are the means of four experiments.

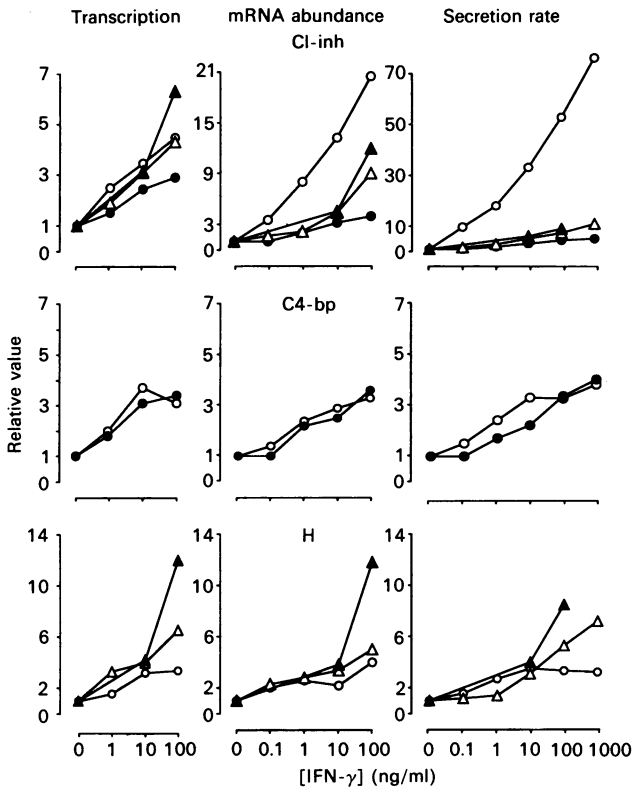

Fig. 2. Effect of IFN-γ on the expression of C1-inh, factor H (H) and C4-bp by Hep G2 cells (●), human peripheral blood monocytes (○), human skin fibroblasts (△) and HUVEC (▲)

Dose-related effects of IFN-γ (0–100 ng/ml) are shown on transcription rate (left) and mRNA abundance (middle), and dose-related effects of IFN-γ (0–1000 ng/ml) are shown on the secretion rate of each protein (right). The results are expressed relative to control levels (cells not exposed to IFN-γ), which are assigned the arbitrary value of 1.00. Each point represents the mean of two experiments, except for the secretion rate values, which are the means of four experiments.

C4-bp or factor H in any of the cells in which their genes were expressed (Table 2). The half-lives of C1-inh mRNA and factor B mRNA were prolonged by IFN-γ, particularly the former in monocytes. The half-life of C2 mRNA was shortened by IFN-γ in all four cell types (Table 2).

DISCUSSION

Expression of mRNA in eukaryotic cells is a complex process which requires both transcriptional and post-transcriptional mechanisms. Modulation of the abundance of an mRNA may occur as a result of changes in gene transcription or in the processing, transport and degradation of the mRNA (Darnell, 1982; Nevins, 1983).

We have used a combination of immunochemical and molecular biological techniques to study the effects of IFN-γ on the biosynthesis of six complement components (C2, factor B, C3, C1-inh, C4-bp and factor H) by Hep G2 cells, monocytes, skin fibroblasts and HUVEC *in vitro*. With the exception of showing, for the first time, that C4-bp is synthesized by Hep G2 cells, our data confirm previous observations on the ability of Hep G2 cells (Morris *et al.*, 1982; Strunk *et al.*, 1985; Prandini *et al.*, 1986), monocytes (Einstein *et al.*, 1976; Whaley, 1980; Strunk *et al.*, 1983; Yeung-Laiwah *et al.*, 1985; Lappin & Whaley, 1990), fibroblasts (Katz & Strunk, 1988, 1989) and HUVEC (Uekl *et al.*, 1987; Ripoche *et al.*, 1988*b*; Brooimans *et al.*, 1989; Donaldson *et al.*, 1989; Dauchel *et al.*, 1990) to synthesize individual components of complement.

Cell-specific differences between unstimulated cells

Apart from differences in the number of complement components synthesized by each cell type, differences in the synthesis rates of some components were also observed (Table 1). The greatest differences were noted for the rates of synthesis of C3 and C1-inh, which were low in HUVEC compared with the other cell types, and factor H, which was extremely low in monocytes compared with fibroblasts and HUVEC. Major differences in the synthesis rates of C2, factor B and C4-bp were not found.

In addition to cell-specific differences in protein secretion rates, cell-specific differences in mRNA stability were observed (Table 3). Thus, in contrast to actin mRNA which had a similar half-life in all four cell types, the half-life of each complement mRNA, with the exception of factor H mRNA in HUVEC, decreased progressively in Hep G2 cells, fibroblasts, HUVEC and monocytes. However, as the rank order of each mRNA half-life was not the same in all cell types, the intrinsic properties of each mRNA species must also influence its stability. The cell-specific differences in mRNA stability could be due to cell-specific expression of a labile destabilizing factor, such as that described for c-*myc* mRNA (Brewer & Ross, 1989). The observation that the half-lives of the mRNAs for all six complement components were lower in monocytes than in the other cell types

Table 2. Effect of IFN-γ (100 ng/ml) on transcription rates, mRNA abundances and protein secretion rates in different cell types

Values for transcription rates (TR), mRNA abundances (mRNA) and protein secretion rates (SR) are expressed relative to the value in control cultures which were not treated with IFN-γ. Results for transcription rates and mRNA abundances are the means of two experiments. Changes in protein secretion rates are the means of four experiments.

	Hep G2			Fibroblasts			HUVEC			Monocytes		
Component	TR	mRNA	SR	TR	mRNA	SR	TR	mRNA	SR	TR	mRNA	SR
C2	1.4	1.6	1.5	4.3	3.4	3.0	2.9	2.4	2.3	3.1	3.5	3.3
Factor B	1.8	2.5	2.8	4.1	3.4	3.7	3.2	3.5	3.4	3.6	3.5	3.9
C3	0.4	0.4	0.5	0.4	0.4	0.5	0.4	0.6	0.3	0.2	0.2	0.3
C1-inh	4.8	4.0	4.5	7.6	9.0	7.5	11.6	12.0	9.0	7.9	20.5	53.0
C4-bp	3.4	3.6	3.3	–	–	–*	–	–	–	3.1	3.3	3.3
Factor H	–	–	–	6.5	5.0	5.3	12.0	11.8	8.5	3.4	5.0	3.3

* Synthesis not detected.

Table 3. Effects of IFN-γ on mRNA half-lives

Each point represents the mean of two determinations.

		Half-life (h) of mRNA encoding													
		C2		B		C3		C1-inh		C4-bp		H		Actin	
Cells	INF-γ...	−	+	−	+	−	+	−	+	−	+	−	+	−	+
Hep G2		9.5	7.0	6.5	8.5	4.0	4.1	6.9	8.6	6.3	6.5	–	–	7.0	6.8
Fibroblasts		8.6	5.0	5.2	7.1	3.7	3.7	6.0	7.6	–	–	3.3	3.2	6.9	6.8
HUVEC		5.7	4.1	4.7	8.4	2.7	2.8	3.3	3.8	–	–	3.7	3.7	6.7	6.6
Monocytes		3.7	2.4	2.1	4.2	1.2	1.2	1.5	5.8	3.7	3.7	0.2	0.2	6.4	6.3

(Table 3), although the secretion rates of all components except factor H were not significantly lower in monocytes (Table 1), suggests that the half-life of mRNA is usually not a major determinant of protein synthesis rate unless the mRNA is extremely unstable, as is the case for factor H in monocytes. It also appears that, in monocytes, the transcription rates of the complement genes or the translational efficiency of their mRNAs are greater than in the other cell types studied.

Cell-specific differences in IFN-γ-treated cells

With the exception of C1-inh, the levels of IFN-γ-induced changes in gene transcription were associated with changes of similar magnitude and direction in the relative abundances of the respective mRNAs and in the secretion rates. Many studies have shown that IFNs stimulate the transcription of a large number of genes in different cell types. The regulatory DNA elements involved in expression of IFN activity (IFN-stimulated response elements; ISREs) are generally found in regions upstream from the coding sequences of responsive genes. Although an ISRE has been identified at position −154 to −127 of the factor B gene (Wu *et al.*, 1987), there are no published data regarding their presence in relation to the C2, C3, C1-inh, C4-bp or factor H genes. The changes in transcription of each of the complement genes in response to IFN-γ varied in the different cell types. The C2, factor B and C1-inh genes were least responsive in Hep G2 cells, while the factor H and C1-inh genes were most responsive in HUVEC (Figs. 1 and 2; Table 2). The finding that IFN-γ inhibited C3 expression in all four cell types by decreasing transcription confirms and extends the results of earlier reports on C3 synthesis by monocytes (Strunk *et al.*, 1985; Hamilton *et al.*, 1987; Lappin *et al.*, 1990*a*,*b*). However, this differs from the findings of Katz & Strunk (1988), who showed that IFN-γ increased C3 expression in fibroblasts. We are unable to explain this discrepancy.

The disproportionate increase in monocyte C1-inh synthesis in response to IFN-γ was due to a combination of increased transcription and a 5-fold increase in C1-inh mRNA stability, as has been documented previously (Lappin *et al.*, 1990*b*). IFN-γ produced only a modest increase in C1-inh mRNA stability in fibroblasts, HUVEC and Hep G2 cells. However, it should be noted that the half-lives of C1-inh mRNA in these latter three cell types were far longer (6.0 h, 3.3 h and 6.9 h respectively) than its half-life in monocytes (1.5 h). IFN-γ did not alter the half-lives of C3 mRNA, C4-bp mRNA or factor H mRNA. However, the half-life of factor B mRNA was prolonged, and the half-life of C2 mRNA was decreased. The mechanism by which INF-γ stabilizes C1-inh and factor B mRNAs is unknown, but could be due to changes in the expression of specific stabilizing or destabilizing factors (Shapiro *et al.*, 1987).

The results of the present study show that the levels of complement gene expression vary in different cell types. The mechanisms responsible for these differences have not been defined, although the low synthesis rate of factor H by monocytes is probably related to the extreme lability of factor H mRNA in these cells. In contrast, the differences in the expression of complement genes in response to IFN-γ were shown to be mainly due to variations in the transcriptional responses of the individual genes, and, in some cases, to changes in the stability of the individual mRNAs. The factors which regulate transcription of the complement genes and the stability of their respective mRNAs merit further investigation.

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REFERENCES

Aden, D. P., Fosel, A., Plotkin, S., Dowjanov, I. & Knowles, B. B. (1979) Nature (London) **282**, 615–616
Alper, C. A., Johnson, A. M., Birch, A. G. & Moore, F. D. (1968) Science **163**, 286–288
Andus, T., Heinrich, P. C., Bauer, J., Tran-Thi, T. A., Dekker, K., Männel, D. & Northoff, H. (1987) Eur. J. Immunol. **17**, 1193–1197
Anthony, R., El-Omar, R., Lappin, D. F., MacSween, R. N. M. & Whaley, K. (1989) Eur. J. Immunol. **19**, 1405–1412
Bentley, D. R. & Porter, R. R. (1984) Proc. Natl. Acad. Sci. U.S.A. **81**, 1212–1215
Brewer, G. & Ross, J. (1989) Mol. Cell Biol. **9**, 1996–2006
Brooimans, R. A., Hiemstra, P. S., Van Der Ark, A. A. J., Sim, R. B., Van Es, L. A. & Daha, M. R. (1989) J. Immunol. **142**, 2024–2030
Carrell, R. W. & Boswell, D. R. (1988) in Proteinase Inhibitors (Barrett, A. & Salvesen, G., eds.), pp. 403–420, Elsevier, Amsterdam
Carter, P. E., Dunbar, B. & Fothergill, J. E. (1988) Eur. J. Biochem. **173**, 163–169
Cesarone, D. F., Bolognesi, C. & Santi, C. (1979) Anal. Biochem. **100**, 188–197
Chung, L. P., Bentley, D. R. & Reid, K. B. M. (1985) Biochem. J. **230**, 133–141
Colten, H. R. (1976) Adv. Immunol. **22**, 67–118
Darnell, J. E. (1982) Nature (London) **297**, 265–271
Dauchel, H., Julen, N., Lemercier, C., Daveau, M., Ozanne, D., Fontaine, M. & Ripoche, J. (1990) Eur. J. Immunol. **20**, 1669–1675
De Bruijn, M. C. & Fey, G. H. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 708–712
Donaldson, V. H., Olivi, L. B., Radzicjewska, E., Bock, S. C. & Wagner, C. J. (1989) Behring Inst. Mitt. **84**, 194 (Abstract)
Einstein, L. P., Schneeberger, E. E. & Colten, H. R. (1976) J. Exp. Med. **143**, 114–126
Feinberg, A. P. & Vogelstein, A. (1983) Anal. Biochem. **132**, 6–13
Feinberg, A. P. & Vogelstein, A. (1984) Anal. Biochem. **137**, 266–267
Friedman, R. L. & Stark, G. R .(1985) Nature (London) **314**, 637–639
Fulton, R., Birnie, G. D. & Knowler, J. (1985) Nucleic Acids Res. **13**, 6467–6482
Gigli, I., Fujita, T. & Nussenzweig, V. (1979) Proc. Natl. Acad. Sci. U.S.A. **76**, 6596–6600

Greenberg, M. E. & Ziff, E. B. (1984) Nature (London) **311**, 433–435
Hamilton, A. O., Jones, L., Morrison, L. & Whaley, K. (1987) Biochem. J. **242**, 809–815
Jaffe, E. A., Nachmann, R. L., Becker, C. G. & Minick, C. R. (1973) J. Clin. Invest. **52**, 2743–2756
Katz, Y. & Strunk, R. C. (1988) Arthritis Rheum. **31**, 1365–1370
Katz, Y. & Strunk, R. C. (1989) J. Immunol. **142**, 2041–2045
Lappin, D. & Whaley, K. (1989) Clin. Exp. Immunol. **76**, 86–91
Lappin, D. F. & Whaley, K. (1990) Biochem. J. **271**, 767–772
Lappin, D. F., Damereau, B., Riches, D. W. H. & Whaley, K. (1984) Biochem. J. **222**, 477–486
Lappin, D., Hamilton, A. O., Morrison, L., Aref, M. & Whaley, K. (1986) J. Clin. Lab. Immunol. **20**, 101–106
Lappin, D. F., Birnie, G. D. & Whaley, K. (1990*a*) Biochem. J. **268**, 387–392
Lappin, D. F., Birnie, G. D. & Whaley, K. (1990*b*) Eur. J. Biochem. **194**, 177–148
Mielke, V., Bauman, J. G. G., Sticherling, M., Timke, I. B. S., Zomershoe, A. G., Seligmann, K., Hennicke, H. H., Schöder, J.-M., Sterry, W. & Christophers, E. (1990) J. Immunol. **144**, 153–161
Miura, N., Prentice, H. L., Scheider, P. M. & Perlmutter, D. H. (1987) J. Biol. Chem. **262**, 7298–7305
Moffat, G. J., Lappin, D., Birnie, G. D. & Whaley, K. (1989) Clin. Exp. Immunol. **78**, 54–60
Morley, B. J. & Campbell, R. D. (1984) EMBO J. **3**, 153–157
Morris, K. M., Aden, D. P., Knowles, B. B. & Colten, H. R. (1982) J. Clin. Invest. **70**, 906–913
Muller-Eberhard, H. J. (1988) Annu. Rev. Biochem. **57**, 321–347
Nevins, J. R. (1983) Annu. Rev. Biochem. **52**, 441–446
Pangburn, M. K., Schreiber, R. D. & Muller-Eberhard, H. J. (1977) J. Exp. Med. **146**, 257–270
Prandini, M. H., Reboul, A. & Colomb, M. G. (1986) Biochem. J. **237**, 93–98
Ramadori, G., Van Damme, J., Rieder, H. & Meyer Zum Buschenfeble, K. H. (1988) Eur. J. Immunol. **18**, 1259–1264
Ripoche, J., Day, A. J., Harris, T. J. R. & Sim, R. B. (1988*a*) Biochem. J. **249**, 593–602
Ripoche, J., Mitchell, A., Erdei, A., Madin, C., Moffatt, B., Mokoena, T., Gordon, S. & Sims, R. B. (1988*b*) J. Exp. Med. **168**, 1917–1922
Ruddy, S. & Colten, H. R. (1974) N. Engl. J. Med. **290**, 1284–1288
Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd edn., pp. 6.1–6.33, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
Schwaeble, W., Schwanger, H., Brooimans, R. A., Barbeiri, A., Most, J., Tiefenthaler, M., Hirsch-Kauffmann, M., Lappin, D. F., Daha, M. R., Whaley, K. & Dierich, M. P. (1991) Eur. J. Biochem. **198**, 399–404
Shapiro, D. J., Blume, J. E. & Nielsen, D. A. (1987) BioEssays **6**, 221–226
Strunk, R. C., Kunke, K. S. & Giclas, P. C. (1983) Immunology **49**, 169–174
Strunk, R. C., Cole, F. S., Perlmutter, D. H. & Colten, H. R. (1985) J. Biol. Chem. **260**, 15280–15285
Uekl, A., Sai, T., Oka, H., Tabata, M., Hosokawa, K. & Mochizuki, Y. (1987) Immunology **61**, 11–14
Whaley, K. (1980) J. Exp. Med. **151**, 501–506
Whaley, K. (1987) in Complement in Health and Disease (Whaley, K., ed.), pp. 1–17, MTP Press, Lancaster
Whaley, K. & Ruddy, S. (1976) J. Exp. Med. **144**, 1147–1163
World Health Organisation (1968) Bull. WHO **39**, 935–936
World Health Organisation (1981) Eur. J. Immunol. **11**, 668–669
Wu, L. C., Morley, B. J. & Campbell, R. D. (1987) Cell **48**, 331–342
Yeung-Laiwah, A. C., Jones, L., Hamilton, A. O. & Whaley, K. (1985) Biochem. J. **226**, 199–205

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