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Modulation of complement gene expression by glucocorticoids

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The addition of dexamethasone, prednisolone or cortisol (in order of efficacy) to human monocytes in culture produced dose-related increases in the synthesis rates of the complement components C1 inhibitor (C1-inh), factor B (B) and C2. In contrast, concentrations of C3 and lysozyme in the culture supernatants were decreased. Indomethacin stimulated synthesis of C1-inh, C2 and B, but had little effect on synthesis of C3 or lysozyme. The simultaneous addition of cycloheximide (2.5 μg/ml) abrogated the effects of dexamethasone on synthesis of C2, B and C1-inh, but the effect of indomethacin on the synthesis of these components was unchanged. These data suggest that protein synthesis is required for the effects of glucocorticoids on the synthesis of C2, B and C1-inh to occur. Dexamethasone and indomethacin increased the abundances of C1-inh mRNA, B mRNA and C2 mRNA in parallel with changes in the synthesis rates of these proteins. The changes in mRNA abundance were not transcriptional, but were shown to be due to increased mRNA stability. In contrast, dexamethasone decreased the expression of C3 and lysozyme by decreasing the rate of transcription of these genes. Indomethacin had no effect on transcription of the C3 and lysozyme genes. The half-lives of C3 mRNA, lysozyme mRNA and actin mRNA were not altered by dexamethasone or indomethacin. It is concluded that the effects of glucocorticoids on monocyte synthesis of C2, B and C1-inh are due to increased mRNA stability and may be related to inhibition of prostaglandin synthesis, as these effects are similar to those produced by indomethacin. The effects of dexamethasone on the synthesis of C3 and lysozyme differ from those on C2, B and C1-inh as they depend upon a decrease in gene transcription, which is not affected by indomethacin.

INTRODUCTION

Glucocorticoids are used therapeutically to inhibit the immune and inflammatory responses (Rohhut & Russo-Marie, 1984; Stevenson & Fauci, 1984; Di Rosa, 1985; Laue *et al.*, 1988). In many respects they have opposing effects to those of interferons on inflammation and the immune system. For instance, glucocorticoids inhibit a number of macrophage functions which are stimulated by interferons, such as the expression of Fc receptors, phagocytosis, antibody-dependent cytotoxicity (Warren & Vogel, 1985), anti-microbial activity (Schaffner, 1985) and interleukin-1 production (Snyder & Unanue, 1982; Fertsch-Ruggio *et al.*, 1988). In contrast, they have been shown to have effects similar to those of interferons on other macrophage functions, such as increased HLA-DR expression (Gerrard *et al.*, 1984).

At present, little is known of the effects of glucocorticoids on the biosynthesis of complement components. In earlier studies it was observed that glucocorticoids stimulated synthesis of the second component of complement (C2), factor B (B) and C1 inhibitor (C1-inh) (McPhaden *et al.*, 1982; Lappin & Whaley, 1989), and their effects on human umbilical vein endothelial cells were to increase factor H synthesis and decrease C3 and B synthesis (Dauchel *et al.*, 1990). However, the mode of action of these agents was not investigated. The present investigation was undertaken to study the effects of glucocorticoids on the synthesis of C2, B, C1-inh and C3 by monocytes and to determine whether these effects are exerted at the transcriptional or post-transcriptional level. Synthesis of lysozyme was also investigated.

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, RPMI 1640 and Dulbecco's modified Eagle's medium: Flow Laboratories, Rickmansworth, Herts., U.K. Foetal calf serum (FCS), antibiotic/antimycotic solution and restriction enzymes: Gibco BRL, Paisley, Renfrewshire, Scotland, U.K. [α-^{32}P]dCTP (PB 10205), [α-^{32}P]UTP (PB 10203) and Hybond-N nylon membranes: Amersham International, Amersham, Bucks., U.K. Random-primed DNA labelling kit: Boehringer–Mannheim, Mannheim, Germany. The dye H33258, BSA, dithiothreitol, ATP, GTP, CTP, UTP, cycloheximide, dexamethasone, cortisol and prednisolone: Sigma Chemical Co., Poole, Dorset, U.K. RNAzol was from Biogenesis, Bournemouth, U.K. Human AB serum (ABS) was supplied by the Scottish Blood Transfusion Service (Law Hospital, Carluke, Scotland, U.K.). The plasmids pC201 (C2 cDNA) and p2FB (B cDNA) were gifts from Dr. David Bentley and Dr. Duncan Campbell respectively (Medical Research Council Immunochemistry 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). C1-inh cDNA (pC1) was a gift from Dr. Philip Carter (Department of Biochemistry, University of Aberdeen, Aberdeen, Scotland, U.K.) (Carter *et al.*, 1988). Actin cDNA (p749) was a gift from Dr. A. M. Frischauf (European Molecular Biology Laboratory, Heidelberg, Germany) (Katcoff *et al.*, 1980). Chick lysozyme cDNA (pls 1023) was a gift from Dr. Gunther Shultz (Institute for Cell and Tumour Biology, Heidelberg, Germany) (Land *et al.*, 1981). Plasmid pGemini-1 (pGem) was purchased from Promega (Southampton, U.K.).

Preparation of DNA probes

The cDNA inserts were excised from their plasmid vectors as follows: actin cDNA with *Pst*1, chick lysozyme cDNA (pls 1023)

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; B, factor B; ABS, human AB serum, heat-inactivated for 2 h at 56 °C; RPMI, RPMI 1640 containing Hepes buffer; FCS, foetal calf serum, heat-inactivated for 2 h at 56 °C; NP-40, Nonidet P-40; PBS, potassium phosphate (10 mM)-buffered saline, pH 7.4; SSC, 150 mM-NaCl/15 mM-trisodium citrate, pH 7); HSP, heat-shock protein.

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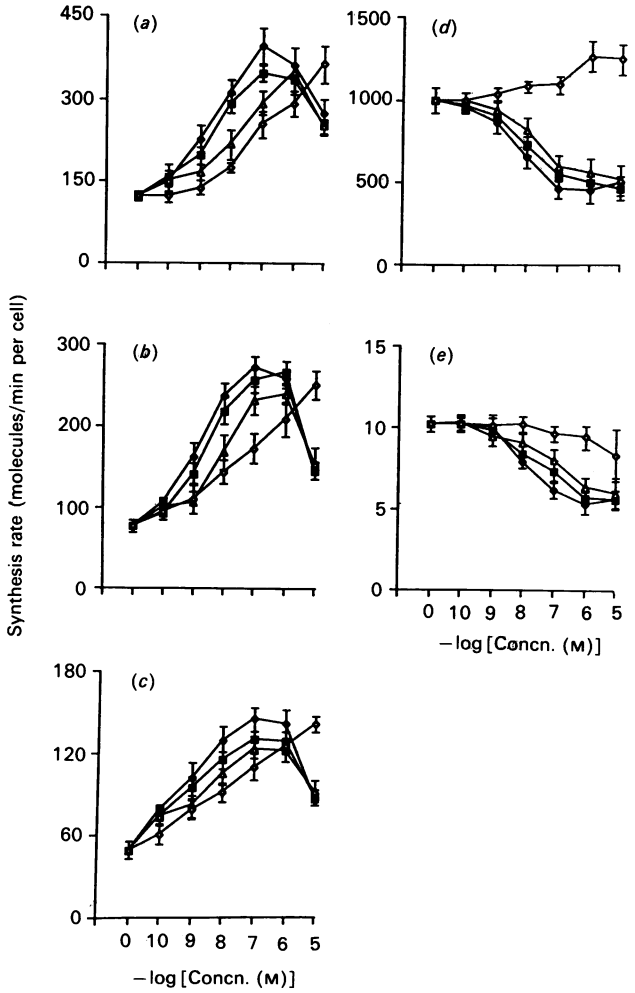

Fig. 1. Effects of glucocorticoids and indomethacin on complement protein and lysozyme synthesis

Dose-related effects of dexamethasone (●), cortisol (△), prednisolone (■) and indomethacin (◇) are shown on the synthesis rates (molecules/min per cell) of C1-inh (*a*), B (*b*), C2 (*c*), C3 (*d*) and lysozyme (*e*) from human peripheral blood monocytes. Each point represents the mean ± S.E.M. of four experiments, each performed in duplicate.

with *Rsa*1, C1-inh, C2 and B cDNA with *Bam*HI and *Hin*dIII, and C3 cDNA with *Eco*R1. The inserts were isolated by electrophoresis on agarose gels and purified by phenol/chloroform (1:1, v/v) extraction and 0.3 M-sodium acetate/ethanol precipitation. For the run-on transcription assays, each insert cDNA was diluted to 2 μg/μl in 10 × SSC, denatured, dotted on to a Hybond-N filter and fixed by u.v. irradiation. The cDNA inserts were also labelled with [α-^{32}P]dCTP using the random-primed DNA labelling reaction (Feinberg & Vogelstein, 1983, 1984) and were used for probing Northern blots and dot-blots.

Monocyte cultures

Human monocyte monolayers were prepared from the buffy coats of donated blood in 24-well Linbro tissue culture plates (Lappin *et al.*, 1984) and the cells were cultured in RPMI 1640 containing 10% (v/v) ABS at 37 °C in a humidified air/CO_2 (19:1) atmosphere. After 3 days the cells were washed extensively and the medium was changed to RPMI 1640 containing 20% (v/v) FCS, and the cells were incubated under these conditions for 24 h before any experiments were performed. At this time the culture medium was changed, the glucocorticoids or indomethacin were added and the monolayers were incubated for defined periods.

In some experiments cycloheximide (2.5 μg/ml) was administered simultaneously with the glucocorticoids and indomethacin to ascertain whether protein synthesis was required for these agents to exert their effects. After 2 h at 37 °C the supernatants were removed, the monolayers were washed twice in RPMI, and the culture was continued at 37 °C in the presence of fresh RPMI/FCS.

Measurement of proteins in the culture fluid

A set of monocyte cultures was incubated for a further 72 h. At 24 and 48 h, samples (250 μl) of culture fluid were removed (and stored at −70 °C), the sample volume was replaced and at 72 h the culture supernatant was harvested. The concentrations of complement proteins in the culture fluids were determined by e.l.i.s.a. (Lappin *et al.*, 1986). The secretion rate of each of the complement proteins was determined (mol/min per cell), as their rate of accumulation in culture fluids was linear. Lysozyme concentrations in the supernatant were assessed by an assay measuring the ability to lyse *Micrococcus luteus* (Strunk *et al.*, 1980).

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), to determine the number of cells in each monolayer (1 μg of DNA is equivalent to 10^5 monocytes).

mRNA abundance

Monocytes were cultured in the absence or the presence of dexamethasone (1 nM–1 μM) or indomethacin (0.1–10 μM) for 24 h. Total cellular RNA was then prepared using RNAzol, and Northern blotting and double-dilution dot-blotting techniques were carried out as described previously (Lappin *et al.*, 1990*b*). With the exception of blots probed with chick lysozyme cDNA, the Northern and double-dilution dot-blots were then hybridized 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*). Blots probed with chick lysozyme cDNA were washed to a stringency of 0.5 × SSC containing 0.1% (w/v) SDS at 65 °C. The blots were then subjected to autoradiography (Fulton *et al.*, 1985).

Run-on transcription assay

Monocytes were cultured in the absence or the presence of dexamethasone (0.1 μM), cortisol (0.1 μM) or indomethacin (10 μM) for 24 h. Nuclei were prepared as previously described (Greenberg & Ziff, 1984) and were 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. Portions of nuclei (100 μl) were 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 and CTP/2 mM-dithiothreitol/100 μCi of [α-^{32}P]UTP and incubated for 30 min at 30 °C. RNA was prepared from the nuclei using RNAzol (Lappin *et al.*, 1990*a*). The [^{32}P]RNA was dissolved in hybridization buffer (10^6 c.p.m./ml) and hybridization was carried out at 42 °C to cDNA immobilized on Hybond-N membranes. After high-stringency washes (Lappin *et al.*, 1990*a*), filters were subjected to autoradiography (Fulton *et al.*, 1985). When the [^{32}P]RNA was hybridized to filters containing chick lysozyme cDNA, washes were carried out with 0.5 × SSC containing 0.1% (w/v) SDS at 65 °C. Radiolabelled RNA was also treated with RNAase A

Table 1. Effect of cycloheximide on glucocorticoid- and indomethacin-mediated alterations in complement and lysozyme synthesis

Dexamethasone was added at 1 μM and indomethacin at 10 μM. Cycloheximide (Chx) was used at a concentration of 2.5 μg/ml. See the Materials and methods section for details. Results are means ± S.E.M. of four experiments.

	Relative secretion rate					
	Control		Dexamethasone		Indomethacin	
Protein	−Chx	+Chx	−Chx	+Chx	−Chx	+Chx
C1-inh	1.00	0.98±0.05	2.92±0.30	1.04±0.07	2.45±0.22	2.41±0.39
B	1.00	0.97±0.07	2.95±0.28	1.21±0.17	2.67±0.42	2.55±0.25
C2	1.00	0.95±0.07	3.05±0.42	0.91±0.09	3.25±0.35	2.65±0.18
C3	1.00	1.02±0.03	0.43±0.10	0.52±0.11	1.20±0.04	1.26±0.12
Lysozyme	1.00	0.92±0.08	0.52±0.07	0.57±0.05	0.90±0.03	0.90±0.08

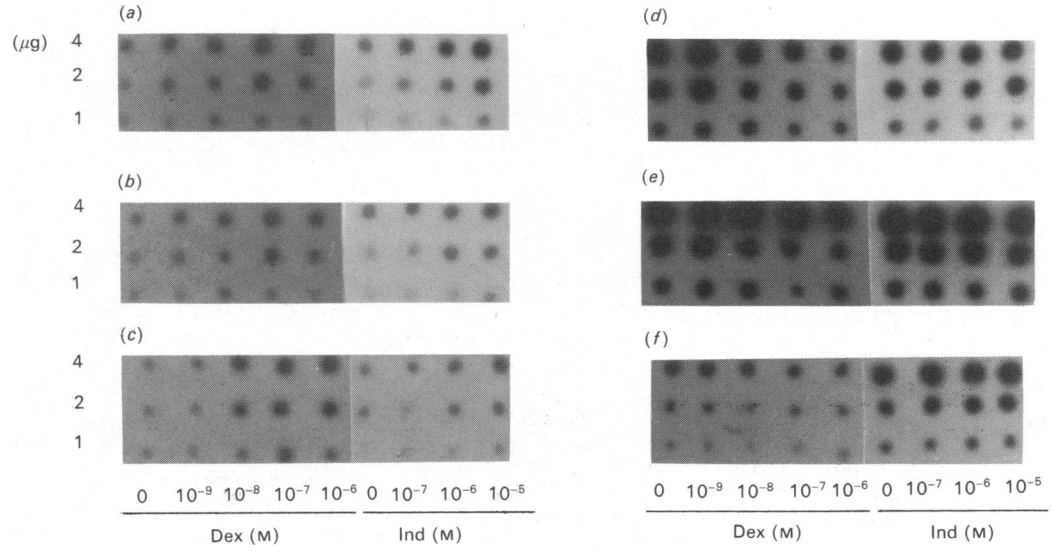

Fig. 2. Double-dilution dot-blot analysis of total cellular RNA

Dilutions of total cellular RNA (4, 2 and 1 μg, as indicated) were dotted and immobilized on to Hybond-N membranes. The membrane was then probed sequentially for C1-inh mRNA (*a*), C2 mRNA (*b*), B mRNA (*c*), C3 mRNA (*d*), lysozyme mRNA (*e*) and actin mRNA (*f*) and subjected to autoradiography. Dex, dexamethasone; Ind, indomethacin.

(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, 0.1 μM-dexamethasone-treated and 1 μM-indomethacin-treated monocytes was measured after the inhibition of transcription with actinomycin B (5 μg/ml). The cells were incubated with or without dexamethasone or indomethacin for 2 h prior to 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 abundances and the half-lives of each mRNA were determined from scanning densitometric measurements of the autoradiographs.

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.

RESULTS

Complement protein and lysozyme synthesis rates

The synthesis rates of C1-inh, B and C2 were increased when dexamethasone, prednisolone, cortisol or indomethacin (in order of efficacy) was present in monocyte culture supernatants (Fig. 1). This effect was dose-dependent and the most effective dose of each of the glucocorticoids was found to be between 0.1 and 1.0 μM (Fig. 1), whereas the most effective dose of indomethacin was 10 μM (results shown only for concentrations of 10 μM and lower in Fig. 1).

The concentrations of C3 and lysozyme were decreased in glucocorticoid-treated cultures. High concentrations of indomethacin stimulated synthesis of C3 slightly, but had no effect on lysozyme production (Fig. 1). The maximum effects of the glucocorticoids and indomethacin occurred following 2 h of treatment (results not shown).

As the effects of all glucocorticoids were essentially the same, most of the further studies were limited to dexamethasone.

Effect of cycloheximide

Dexamethasone (1 μM) or indomethacin (10 μM) was added to

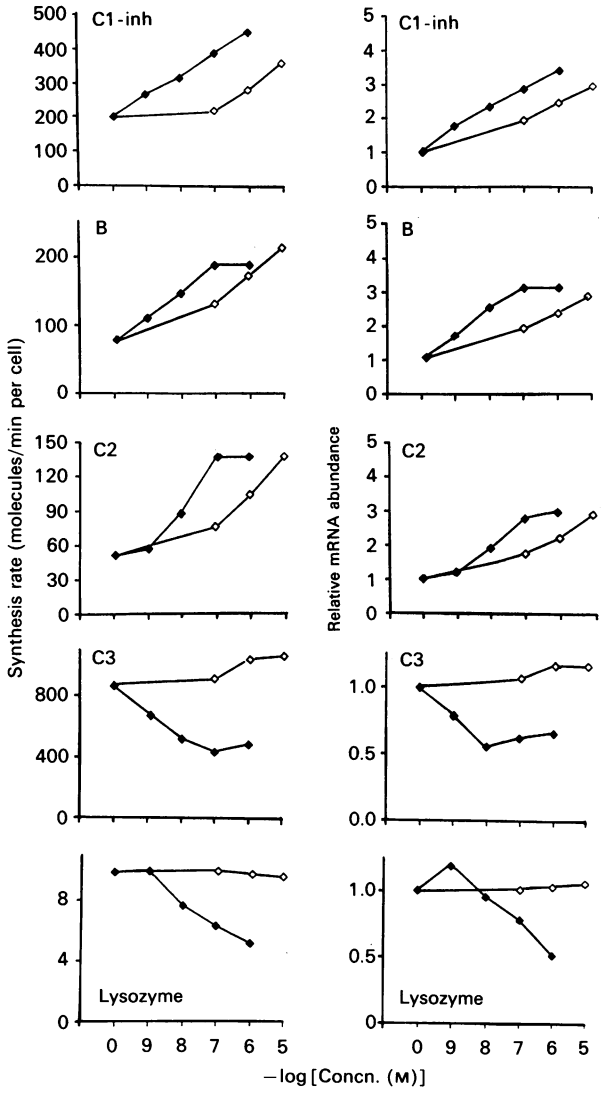

Fig. 3. Comparison of protein synthesis rates with mRNA abundances

C1-inh, C2, B, C3 and lysozyme protein synthesis rates (left) are compared with the relative abundance of each of their respective mRNAs (right) in control, dexamethasone- (◆) and indomethacin- (◇) treated monocytes. The relative abundance of each mRNA compared with the control value (1.00) was determined by scanning densitometry performed on the autoradiographs of double-dilution dot-blot analysis shown in Fig. 2.

Table 2. Effect of dexamethasone and indomethacin on mRNA abundance

Results are of scanning densitometry performed on the double-dilution dot-blot shown in Fig. 2. Values are adjusted for the actin hybridization signal, which was assumed to remain constant. A relative mRNA abundance of 1.00 is that in untreated cells.

	Relative abundance of mRNA						
	Dexamethasone (M)				Indomethacin (M)		
mRNA	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-7}	10^{-6}	10^{-5}
C1-inh	1.74	2.25	2.95	3.29	1.85	2.31	2.92
B	1.43	3.11	3.26	3.52	1.25	1.76	2.90
C2	1.79	2.03	3.02	2.98	1.25	1.30	1.75
C3	0.95	0.54	0.50	0.39	0.99	1.12	1.13
Lysozyme	1.05	1.02	0.78	0.62	0.97	1.08	0.97

monocyte cultures in the presence of cycloheximide (2.5 μg/ml) and for 2 h before exchanging the medium for fresh RPMI/FCS. After a further 24 h the culture supernatants were assayed for complement components and lysozyme. Cycloheximide inhibited the effect of 0.1 μM-dexamethasone on the production of C2, B and C1-inh. However, it did not alter the changes in the synthesis rates of these complement components produced by indomethacin (0.1 μM) or the effects of dexamethasone on C3 and lysozyme synthesis (Table 1).

mRNA abundance

Dexamethasone and indomethacin increased the abundance of C1-inh mRNA (2.1 kb band), B mRNA (2.6 kb band) and C2 mRNA (2.9 kb band). Dexamethasone decreased the abundance of C3 mRNA (5.2 kb band) and lysozyme mRNA (0.8 kb band), while indomethacin had no effect on the abundance of these mRNAs (results not shown). These results were confirmed by double-dilution dot-blot analyses (Fig. 2), which were subjected to scanning densitometry to determine the abundance of each mRNA. A slight decrease in the abundance of actin mRNA was observed in dexamethasone-treated monocytes (Fig. 2). Alterations in the abundances of each mRNA species were correlated with changes in the amounts of the respective proteins present in the culture fluids (Fig. 3, Table 2).

Gene transcription

The addition of dexamethasone or cortisol (0.1 μM) did not affect the transcription rate of the genes encoding C1-inh, B or C2, whereas the levels of transcription of the genes encoding C3 and lysozyme were decreased. Indomethacin had no effect on the transcription of any of these genes (Fig. 4, Table 3).

mRNA half-life

The half-lives of the mRNAs encoding C1-inh, C2 and B were increased approx. 2-fold by dexamethasone (0.1 μM) and indomethacin (1 μM). A slight increase in the half-life of C3 mRNA was also observed, while the half-lives of lysozyme mRNA and actin mRNA were unchanged (Fig. 5, Table 4).

DISCUSSION

Glucocorticoids stimulate mononuclear phagocytes to synthesize a phospholipase A_2 inhibitor, lipocortin (lipomodulin, macrocortin) (Carnuccio *et al.*, 1981; Blackwell, 1983; Flowers, 1986). Lipocortin prevents the conversion of membrane phospholipid to arachadonic acid and thus inhibits the formation of the inflammatory cyclo-oxygenase and lipoxygenase products. Many of the other effects of glucocorticoids are due to modulation of the expression of genes other than the lipocortin gene, and in many cases are due to changes in gene transcription (Yamamoto, 1985). Following their entry into the cytoplasm, glucocorticoids bind to a 9 S complex consisting of the glucocorticoid receptor and a heat-shock protein (HSP-70 or HSP-90) (Sanchez *et al.*, 1990). A 4 S glucocorticoid-ligand/receptor complex then dissociates from the HSP moiety (Rousseau, 1984; Harmon *et al.*, 1988) and migrates to the nucleus, where it binds to specific sites (i.e. enhancer sequences) to influence initiation of transcription from promoter sequences on responsive genes (Yamamoto, 1985; Godowski *et al.*, 1987; Munck & Holbrook, 1988). However, glucocorticoids have also been shown to exert some of their effects on gene expression post-transcriptionally, by altering the stability of some mRNAs (Friedman & Stark, 1985; Diamond & Goodman, 1985; Peck & Axel, 1987) or by altering the transport of mRNA from the nucleus to the cytoplasm (Fulton *et al.*, 1985).

In the present study it has been demonstrated that gluco-

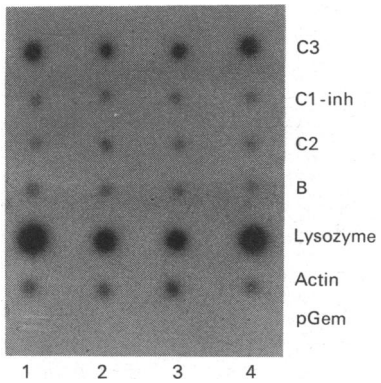

Fig. 4. Effects of glucocorticoids and indomethacin on transcription of complement genes

The transcription of the genes encoding C1-inh, C2, B, C3, lysozyme and actin in monocytes was measured. The presence of each mRNA was detected by immobilized cDNA inserts from RNA isolated from nuclei (2×10^7) following the run-on transcription assay. Lane 1, control; lane 2, dexamethasone (0.1 μM); lane 3, cortisol (0.1 μM); lane 4, indomethacin (10 μM). pGem DNA was immobilized on the filter as a negative control.

corticoids modulate the expression of a number of genes in human monocytes which encode complement components and lysozyme. Dexamethasone, prednisolone and cortisol, in decreasing order of potency, stimulated the synthesis of C1-inh, C2 and B in a dose-dependent manner, while inhibiting the synthesis of C3 and lysozyme.

Table 3. Effect of glucocorticoids and indomethacin on transcription rates

Results are of scanning densitometry on the gel in Fig. 4. The results for dexamethasone and cortisol are the means of three measurements of transcription rates (values adjusted for the actin signal, which was assumed to remain constant). The results for indomethacin are the means of two measurements adjusted for the actin signal.

	Relative transcription rate		
Gene	Dexamethasone (1 μM)	Cortisol (1 μM)	Indomethacin (10 μM)
C1-inh	0.95	0.94	1.03
B	1.03	0.89	1.04
C2	1.10	0.90	1.08
C3	0.63	0.54	1.09
Lysozyme	0.62	0.51	0.94

Alterations in the secretion rates of these proteins were parallelled by changes in the abundances of their respective mRNAs, which shows that these glucocorticoids exerted their effects at a pre-translational level. The glucocorticoid-mediated increases in the abundances of C1-inh, B and C2 mRNAs were not due to changes in transcription, but appeared to be entirely dependent upon increased mRNA stability. In contrast, the decreases observed in the abundance of C3 mRNA and lysozyme mRNA appeared to be transcriptionally mediated, since major

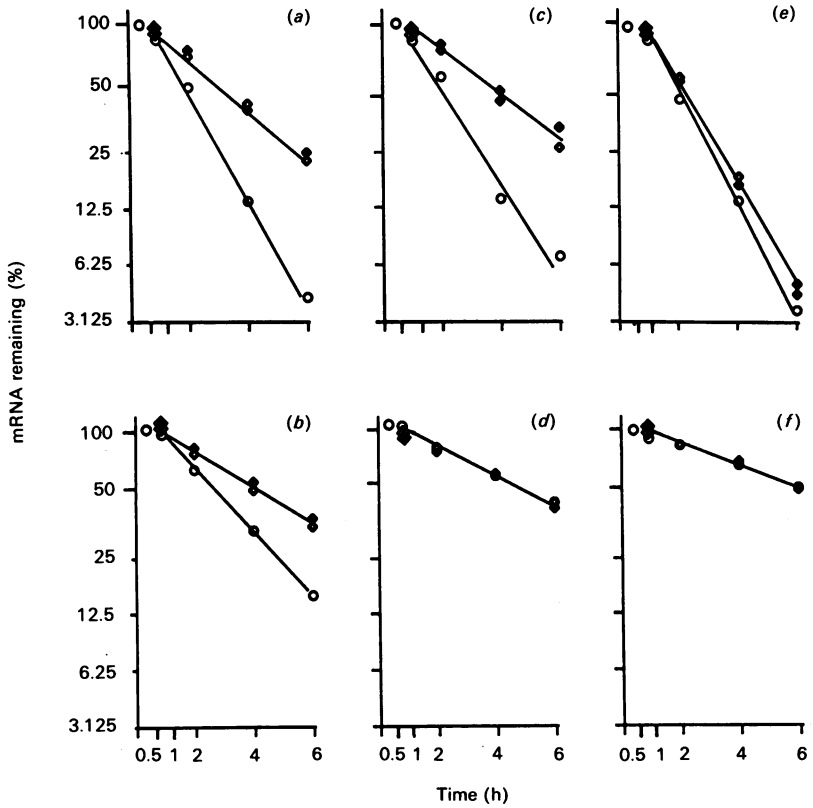

Fig. 5. Effects of dexamethasone and indomethacin on mRNA half-lives

Comparisons are shown of the residual amounts (% of time zero value) of C1-inh mRNA (*a*), C2 mRNA (*b*), B mRNA (*c*), lysozyme mRNA (*d*), C3 mRNA (*e*) and actin mRNA (*f*) in total cellular RNA in control (○), dexamethasone-treated (◆, 0.1 μM) and indomethacin-treated (◇; 10 μM) monocytes, at timed intervals (0, 1, 2, 4, 6, 8 and 24 h) after the addition of actinomycin D to halt transcription. The abundance of each mRNA was assessed by double-dilution dot-blot analysis and scanning densitometry. Each point represents the mean of two experiments performed in duplicate.

Table 4. Effect of dexamethasone and indomethacin on mRNA half-lives

Results are means of two determinations (data shown in Fig. 6).

	mRNA half-life (min)		
mRNA	Control	Dexamethasone (1 μM)	Indomethacin (10 μM)
C1-inh	70	155	160
B	80	175	180
C2	115	230	240
C3	65	70	75
Lysozyme	270	275	275
Actin	295	300	290

changes in the half-lives of these mRNAs were not observed and transcription rates were shown to be decreased.

The ability of cycloheximide to abolish the effects of the glucocorticoids on the expression of C1-inh, B and C2 suggests that glucocorticoids have an indirect action involving synthesis of a protein which then modulates the stability of the C1-inh, C2 and B mRNAs. Indomethacin, a cyclo-oxygenase inhibitor, also stabilized the mRNAs encoding C1-inh, B and C2, but in this case its effect was not cycloheximide-sensitive. Thus, if the effects of glucocorticoids and indomethacin on C2, B and C1-inh expression are mediated by inhibition of prostaglandin synthesis, the effect of glucocorticoids is likely to be mediated by lipocortin. This conclusion is supported by the results of our earlier studies in which it was shown that endogenous synthesis of prostaglandins modulated the synthesis of C2 in monocytes (Lappin & Whaley, 1983; Lappin *et al.*, 1984). Both glucocorticoids and indomethacin decrease monocyte cyclic AMP levels, whereas prostaglandin E_2 inhibits monocyte C2 synthesis and increases cyclic AMP levels (Lappin *et al.*, 1984). It is possible that a decrease in cyclic AMP is involved in the effects of glucocorticoids and indomethacin on the synthesis of C2, B and C1-inh.

In contrast, glucocorticoids decreased transcription of the C3 and lysozyme genes. Decreased transcription accounts for glucocorticoid-mediated down-regulation of major histocompatibility locus class II antigen expression in the mouse (Fertsch-Ruggio *et al.*, 1988). The failure of glucocorticoids and indomethacin to influence the stability of C3 and lysozyme mRNAs implies that the stabilities of these mRNA species are subject to different regulatory mechanisms than those encoding C1-inh, B and C2.

As 50% of the C3 present in inflamed joints is synthesized locally (Ruddy & Colten, 1974), glucocorticoid-mediated inhibition of C3 and lysozyme synthesis in mononuclear phagocytes may be of biological importance. It is well known that patients undergoing glucocorticoid therapy are predisposed to infections with micro-organisms (Schaffner, 1985). Both C3 and lysozyme have important antimicrobial functions: C3 is an opsonin, which promotes the phagocytosis and killing of micro-organisms by phagocytic cells (Kjilstra *et al.*, 1979; Leigh *et al.*, 1979), while lysozyme (mucopeptide-*N*-acetylmuramoyl-hydrolase) hydrolyses the mucopeptide–*N*-acetylmuramoyl bond causing direct lysis of bacteria (Ghuysen, 1968) or renders them more susceptible to complement-mediated lysis. Thus inhibition of mononuclear phagocyte synthesis of C3 and lysozyme may play a role in the predisposition of glucocorticoid-treated patients to bacterial infections.

On the other hand, decreased synthesis of C3 would lead to lowered C3a production and lowered C5 convertase formation, with secondary decreases in the production of C5a and the C5b-9 membrane attack complex. Thus the inhibition of mononuclear phagocyte C3 synthesis by glucocorticoids could be a significant anti-inflammatory effect.

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REFERENCES

Aden, D. P., Fogel, A., Plotkin, S., Dowjanou, I. & Knowles, B. B. (1979) Nature (London) **282**, 615–616
Bentley, D. R. & Porter, R. R. (1984) Proc. Natl. Acad. Sci. U.S.A. **81**, 1212–1215
Blackwell, C. J. (1983) Br. J. Pharmacol. **79**, 587–594
Carnuccio, R., Di Rosa, M., Flower, R. J. & Pinto, A. (1981) Br. J. Pharmacol. **74**, 322–324
Carter, P. E., Dunbar, B. & Fothergill, J. E. (1988) Eur. J. Biochem. **173**, 163–169
Cesarone, D. F., Bolognesi, C. & Santi, L. (1979) Anal. Biochem. **100**, 188–197
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. H. & Fey, G. H. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 708–712
Diamond, D. J. & Goodman, H. M. (1985) J. Mol. Biol. **181**, 41–62
Di Rosa, M. (1985) Prog. Biochem. Pharmacol. **20**, 55–62
Feinberg, A. P. & Vogelstein, A. (1983) Anal. Biochem. **132**, 6–13
Feinberg, A. P. & Vogelstein, A. (1984) Anal. Biochem. **137**, 266–267
Fertsch-Ruggio, D., Schoenberg, D. R. & Vogel, S. W. (1988) J. Immunol. **141**, 1582–1590
Flowers, R. J. (1986) Nature (London) **320**, 20–23
Friedman, R. L. & Stark, G. R. (1985) Nature (London) **314**, 637–639
Fulton, R., Birnie, G. D. & Knowler, J. T. (1985) Nucleic Acids Res. **13**, 6467–6482
Gerrard, T. L., Cupps, T. R., Jurgensen, C. H. & Fauci, A. S. (1984) Cell. Immunol. **85**, 330–339
Ghuysen, J. (1968) Bacteriol. Rev. **32**, 425–450
Godowski, R. J., Rusconi, S., Miesfeld, R. & Yamamoto, K. R. (1987) Nature (London) **325**, 365–368
Greenberg, M. E. & Ziff, E. B. (1984) Nature (London) **311**, 433–435
Harmon, J. M., Elasser, M. S., Urda, L. A. & Eisen, L. P. (1988) J. Steroid Biochem. **31**, 275–281
Katcoff, D., Nudel, U., Zevin-Soukin, D., Carmon, Y., Shani, M., Lehrach, H., Frischauf, A. M. & Yaffe, D. (1980) Proc. Natl. Acad. Sci. U.S.A. **77**, 960–964
Kjilstra, A., Van Es, L. A. & Daha, M. R. (1979) J. Immunol. **123**, 2488–2493
Land, H., Grez, M., Hanser, H., Lindenmaier, W. & Schutz, G. (1981) Nucleic Acids Res. **10**, 2251–2267
Lappin, D. & Whaley, K. (1983) Clin. Exp. Immunol. **49**, 623–630
Lappin, D. & Whaley, K. (1989) Behring Inst. Mitt. **84**, 180–203
Lappin, D., Riches, D. W. H., Damerau, B. & 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**, 101–105
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**, 177–184
Laue, L., Shinichi, K., Brandon, D. D., Brightwell, D., Barnes, K., Kuazek, R. A., Loriaux, D. L. & Chrousos, G. P. (1988) J. Steroid Biochem. **29**, 591–598
Leigh, P. J. C., Van den Barselaar, M. Th., Van Zivet, T. L., Daha, M. R. & Van Furth, R. (1979) J. Clin. Invest. **63**, 772–784
McPhaden, A. R., Lappin, D. & Whaley, K. (1982) J. Clin. Lab. Immunol. **8**, 1–7
Morley, B. J. & Campbell, R. D. (1984) EMBO J. **3**, 153–157
Munck, A. & Holbrook, N. J. (1988) J. Steroid Biochem. **31**, 599–606
Peck, I. & Axel, R. (1987) Mol. Cell Biol. **7**, 1496–1501
Rohhut, B. & Russo-Marie, F. (1984) Agents Actions (Suppl.) **14**, 171–180
Rousseau, R. (1984) Mol. Cell. Endocrinol. **38**, 1–11
Ruddy, S. & Colten, H. R. (1974) N. Engl. J. Med. **290**, 1284–1288
Sanchez, E. R., Faber, L. E., Henzel, W. & Pratt, W. B. (1990) Biochemistry **29**, 5145–5152
Schaffner, A. (1985) J. Clin. Invest. **76**, 1755–1764

Snyder, D. S. & Unanue, E. R. (1982) J. Immunol. **129**, 1803–1805
Stevenson, H. L. & Fauci, A. S. (1984) in Cellular Functions in Immunity and Inflammation (Oppenheim, J. J., Rosenstreich, D. L. & Potter, M., eds.), pp. 431–445, Elsevier, New York
Strunk, R. C., Kumker, K. S. & Musson, R. A. (1980) J. Reticuloendothel. Soc. **28**, 483–493
Warren, M. K. & Vogel, S. N. (1985) J. Immunol. **134**, 2462–2472
World Health Organisation (1968) Bull. W.H.O. **39**, 935–936
World Health Organisation (1981) Eur. J. Immunol. **11**, 668–669
Yamamoto, K. R. (1985) Annu. Rev. Genet. **19**, 209–252

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