SYNERGISM BETWEEN γ INTERFERON AND LIPOPOLYSACCHARIDE FOR SYNTHESIS OF FACTOR B, BUT NOT C2, IN HUMAN FIBROBLASTS

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The complement system, a set of 20 plasma proteins, is an effector of several functions associated with inflammation, immunologic regulation, and cytotoxicity (1). Two complement proteins, C2 of the classical pathway and factor B of the alternative pathway, have many structural and functional similarities, including amino acid composition, molecular size, electrophoretic mobility, and genetic linkage to the MHC on the short arm of human chromosome 6 (2). The primary site of synthesis of C2 and factor B is the liver, although synthesis of C2 and factor B has also been detected in cells of the monocyte/macrophage lineage (3) and in mouse L cells (C2 only) (4).

The synthesis of C2 and factor B in monocytes is regulated characteristically by LPS and IFN-γ. LPS, a constituent of cell walls of Gram-negative bacteria and a potent activator of humoral and cellular immunity (5), stimulated 15-fold more factor B than C2 synthesis, while IFN-γ, an immunoregulator lymphokine, stimulated approximately equal increases (6). Distinct effects of each substance have been noted on other monocyte functions. Synergistic responses of combinations of LPS and IFN-γ have also been observed. One of the most widely studied responses to these stimuli is tumor cell killing by macrophages, where IFN-γ increases the sensitivity of the macrophages to LPS, either priming otherwise LPS-unresponsive cells (7), allowing a response to subthreshold amounts of LPS (8), or preventing the decay of LPS-stimulated cytotoxicity (9). Synergism between LPS and IFN-γ has also been observed for the synthesis of IL-1 in human monocytes (10).

Fibroblasts are widely distributed in the human body. Besides being a source of IFN-β, they synthesize C1 (11) and C3 (12), two proteins of the complement system. Thus, fibroblasts may participate in local regulation of the inflammatory response. When stimulated by IFN-γ, fibroblasts express MHC class II antigens (13) and present antigen to lymphocytes (14). IFN-γ also increases proliferation of lung fibroblasts (15).

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In this report we provide data that human fibroblasts synthesized C2 and factor B constitutively and that this synthesis was modulated by LPS and IFN-γ. Synergism was present between LPS and IFN-γ for factor B, but not for C2. This synergism was significant only when the two stimuli were present simultaneously. LPS and IFN-γ each increased rates of protein synthesis and levels of mRNA for each protein. However, LPS either alone or in combination with IFN-γ induced a greater increase in factor B protein synthesis than in mRNA levels. These data suggest that LPS acts to increase factor B synthesis at both pretranslational and translational sites, while IFN-γ acts at a pretranslational step only. The synergism on factor B synthesis was the result of both synergism on the production of mRNA and an increased efficiency of translation of the newly produced mRNA.

Materials and Methods

Cells. Human fibroblast lines were obtained from American Type Culture Collection (Rockville, MD) (CRL 1471 from the abdomen of a healthy 20-yr-old female and 1507 from the abdomen of a healthy 21-yr-old male), Human Genetic Mutant Cells (NIGMS) (Camden, NJ) (GM 3440 from the leg of a healthy 20-yr-old male), and Dr. P. M. Henson (National Jewish Center for Immunology and Respiratory Medicine, Denver, CO) (primary foreskin). Cells from passages 4 through 8 were used.

Reagents. Low endotoxin DME and FCS (endotoxin <0.1 ng/ml) were purchased from Hazelton Systems, Inc. (Lenexa, KS). DME without methionine was purchased from Gibco Laboratories (Chagrin Falls, OH). Streptomycin and L-glutamine were from Flow Laboratories, Inc. (McLean, VA); and albumin bovine crystallized (purity: 98% minimum) was from Nutritional Biochemicals (Cleveland, OH). This BSA product had the lowest LPS contamination (0.1 ng/mg) (limulus amebocyte lysate quantitative assay, QCL-1000; M. A. Bioproducts, Walkersville, MD) of several commercially available preparations tested; synthesis of complement proteins in cells cultured in medium containing this BSA in concentrations up to 1 mg/ml was not different than synthesis in cells cultured in medium alone. L-[^{35}S]Methionine (specific radioactivity, ~1,000 Ci/mmol), ^{32}P-labeled nucleotides (dCTP, dATP, dGTP, dTTP; specific radioactivity, ~667 Ci/mmol), and EN^{3}HANCE were purchased from New England Nuclear (Boston, MA). Antisera were purchased as follows: sheep anti–human C2 from Seward Labs (London, England) and Miles Laboratories Inc. (Elkhart, IN) and the IgG fraction of goat anti–human factor B from Atlantic Antibodies (Scarborough, ME). Formalin-fixed *Staphylococcus aureus* (Cowan strain 2, IgGsorb) was purchased from The Enzyme Center (Malden, MA). Recombinant human IFN-γ was kindly provided by Dr. Peter Sorter, Hoffman-La Roche, Inc. (Nutley, NJ) in 0.12 M NaCl, 0.025 M ammonium acetate, pH 5.0, in a concentration of 10^8 U/ml. The specific activity of the IFN-γ was 1.9×10^7 IU of antiviral activity per milligram of protein. IFN-γ was diluted in DME containing 1 mg/ml BSA to a concentration of 10^6 U/ml for storage at −70°C. LPS, the Westphal extract of *Escherichia coli* 0111:B4, PMSF, cycloheximide, and polymyxin B were purchased from Sigma Chemical Co. (St. Louis, MO). Leupeptin was purchased from Calbiochem-Behring Corp. (La Jolla, CA).

Cell Cultures, Biosynthetic Labeling, and Immunoprecipitation. Monolayers of human fibroblasts were grown to confluency in tissue culture multiwell plates in medium containing 10% FCS (each 2-cm^2 well contained 1.5×10^5 cells; three wells were used per condition). The medium was discarded, the cells were washed twice and cultured for various time intervals in DME with 1 mg/ml BSA alone (control) or in media containing various concentrations of LPS and/or IFN-γ. The presence of FCS during the stimulation period reduced the IFN-γ-induced increase in factor B synthesis from approximately six- to eightfold to two- to threefold. FCS was eliminated during the stimulation period so that the results would not be affected by the unknown factors present in this serum. Cell number, determined by counting nuclei (16), was not affected by the concentrations of

LPS or IFN-γ that were used. Preliminary experiments demonstrated that constitutive C2 and factor B synthesis and the response to various stimuli were not dependent on the degree of confluency. Biosynthetic labeling experiments were performed by incubating the cells for 2 h in methionine-free DME containing [^{35}S]methionine, 500 μCi/ml (17). At the end of the pulse period, monolayers were rinsed and either lysed by freeze thawing (17), or, in pulse-chase experiments, transferred to medium containing 1,000-fold excess of cold methionine and incubated for varying time periods (chase periods). At the end of the various periods, extracellular media were harvested and monolayers were lysed. Total protein synthesis was estimated by incorporation of [^{35}S]methionine into TCA-insoluble protein (18). Cell lysates and extracellular media were prepared for immunoprecipitation, preabsorbed with formalin-fixed *S. aureus*, and immunoprecipitated sequentially for the relevant proteins (17). Immunoprecipitates were subjected to SDS-PAGE, and the gels were fixed, impregnated with EN^{3}HANCE, dried, and exposed at −70°C to Kodak XAR-5 film. Incorporation of [^{35}S]methionine into individual immunoprecipitated proteins was determined in gel slices after digestion with 15% hydrogen peroxide for 16 h at 60°C and addition of ScintiVerse II (Fisher Scientific Co., Fair Lawn, NJ).

RNA Extraction, Oligo(dT) Chromatography, and Northern Blot Analysis. Total cellular RNA was extracted from monolayers by lysis with guanidium thiocyanate and isolated by cesium chloride density gradient ultracentrifugation (19). Recovery of RNA from LPS and/or IFN-γ-treated cells and control monolayers was similar. Poly(A)$^+$ RNA was purified from 100 μg total cellular RNA using oligo(dT)-cellulose (type 7; P-L Biochemicals, Milwaukee, WI), and subjected to Northern blot analysis (6) using double-stranded cDNA probes radiolabeled with ^{32}P by nick translation. The isolation and characterization of the cDNA probes for C2 (pC2-2a) (20) and factor B (pBfA28) (21) have been described previously. The concentration of cycloheximide used (5 μg/ml) reversibly inhibited protein synthesis > 90% within 1 h and did not affect the yield of total mRNA after 24 h of incubation.

Results

C2 and Factor B Synthesis by Human Fibroblasts. Newly synthesized intracellular and extracellular C2 and factor B proteins were detected in fibroblast cultures (Fig. 1). Pulse-chase experiments demonstrated precursor–product relationships and kinetics of secretion/catabolism exactly similar to those observed previously for human monocytes (6), including the two cell-associated forms for C2 (6, 22). The two extra bands in the area of 200 kD are unidentified proteins synthesized by fibroblasts but not by monocytes. These proteins adhered nonspecifically to *S. aureus* (Fig. 1, lanes *A* and *B*): these bands were present when *S. aureus* was added without specific antibody, the intensities correlated with the amount of the *S. aureus* added and decreased gradually with multiple preclearings without affecting amounts of C2 or factor B proteins precipitated subsequently by addition of monospecific antibodies. C2 represented 0.00012% of the TCA-precipitable protein labeled with [^{35}S]methionine in the cell lysates and 0.00068% in the media. The corresponding values for factor B were 0.00037% and 0.0014%, respectively.

Effects of LPS and IFN-γ on C2 and Factor B Synthesis in Human Fibroblasts. Confluent cells were incubated for 24 h in the presence of different concentrations of LPS or IFN-γ before being analyzed for synthesis of C2 and factor B. While LPS increased synthesis of both factor B and C2, the effects on factor B (12.1-fold for 50 ng/ml) were much greater than for C2 (2.1-fold) (Table I). These effects were observed for LPS concentrations as low as 1 ng/ml, with a maximal response being observed between 50 and 100 ng/ml. The effects

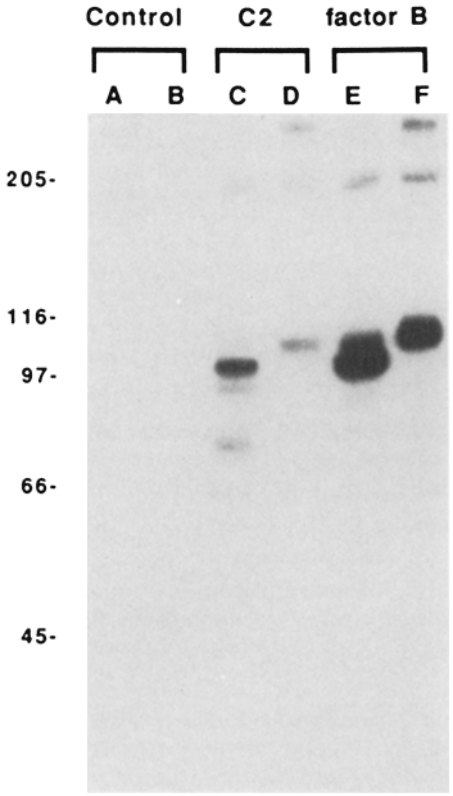

FIGURE 1. Synthesis of C2 and factor B by human fibroblasts. An autoradiograph of SDS-PAGE analysis under reducing conditions of C2 and factor B is shown. Human skin fibroblasts were incubated in medium containing 10% FCS until confluent and 4.5×10^5 cells were pulse labeled for 2 h in 1 ml of DME without methionine containing 500 μCi [^{35}S]methionine. Intracellular lysates and extracellular media were precipitated initially with *S. aureus* without antibody (*A* and *B*). Subsequently the same samples were immunoprecipitated sequentially with anti-C2 and then anti–factor B and the products were analyzed by SDS-PAGE and fluorography. (*A* and *B*) Two proteins are demonstrated in the area of 200 kD that adhered nonspecifically with *S. aureus* alone. (*C* and *D*) Immunoprecipitates with monospecific antisera to C2 showed three radiolabeled bands at ~93, 87, and 67 kD in the intracellular lysates, and a single radiolabeled band at ~103 kD in the extracellular media. (*E* and *F*) Immunoprecipitates with monospecific antisera to factor B showed single radiolabeled bands in the intracellular lysates at ~95 kD and in the media at ~105 kD.

TABLE I

Effect of LPS and IFN-γ on Synthesis of C2 and Factor B

LPS	C2	Factor B	IFN-γ	C2	Factor B
0	1.1 ± 0.2	3.7 ± 1.0	0	1.1 ± 0.2	3.7 ± 1.0
1	2.2 ± 0.7	18.3 ± 7.3	1	3.1 ± 2.6	11.0 ± 3.3
10	3.4 ± 1.2	46.0 ± 17.3	10	5.2 ± 1.6	18.0 ± 9.0
50	2.3 ± 0.6	45.0 ± 11.8	100	7.0 ± 1.0	26.4 ± 8.0
100	0.7 ± 0.9	32.0 ± 11.0	1,000	11.6 ± 2.4	57.0 ± 13.0
500	2.0 ± 0.4	41.0 ± 15.3			

Confluent cultures of human fibroblasts were washed and incubated for 24 h in the presence of medium containing BSA (1 mg/ml), alone, or with various concentrations of LPS (in ng/ml) or IFN-γ (in U/ml) and pulse labeled as described in legend to Fig. 1. Areas of SDS–polyacrylamide gels containing specific C2 and factor B proteins were cut out, digested, and counted after addition of scintillation fluid. Specific protein counts were corrected for nonspecific background contained in the appropriate lane of the gel. Data points represent 4–8 different experiments and are expressed as the ratio of specific protein to total TCA-precipitable protein in the same condition $\times 10^6 \pm 1$ SEM.

of LPS on the synthesis of C2 and factor B were not accompanied by changes in the size or subunit composition of the proteins or their rates of secretion. LPS concentrations as high as 50 μg/ml did not affect the number of cells adherent to the tissue culture plastic or the total synthesis of proteins as measured by incorporation of [^{35}S]methionine into TCA-precipitable protein (data not shown).

IFN-γ stimulated comparable increases in C2 (6.3-fold at 100 U/ml) and factor B (7.1-fold) (Table I). These effects were noted for concentrations as low as 1 U/ml and increased in a concentration-dependent fashion up to 1,000 U/ml. IFN-γ did not affect the number of cells adherent, total protein synthesis, or the size or subunit composition of C2 and factor B and their rates of secretion.

Because of the possibility that the response to IFN-γ was influenced by trace amounts of LPS contaminating the medium, FCS and BSA, cells were incubated with polymyxin B for 96 h before and during the stimulation with IFN-γ. Preliminary experiments indicated that 5 μg/ml polymyxin abrogated the response of these cells to LPS; i.e., cells incubated with LPS + polymyxin B synthesized the same amount of factor B as cells incubated in polymyxin B. Cells in medium containing 5 μg/ml polymyxin B synthesized factor B constitutively and this synthesis was increased approximately fourfold by 100 U/ml IFN-γ. Thus, the trace amounts of LPS in the medium did not appreciably affect the function of the cells.

Effect of Simultaneous Incubation with LPS and IFN-γ on C2 and Factor B Synthesis. Since both LPS and IFN-γ increased C2 and factor B synthesis when added to the cells separately, the effects of the combination of the two stimuli were compared with the additive effects of the individual stimuli. A synergistic effect was present when the cells that were incubated in both stimuli together synthesized more protein than would have been predicted from the combined effects of the individual stimuli. A significant synergistic effect was demonstrated for factor B synthesis (2.6-times greater than the combined effects of LPS and IFN-γ when added separately), but not for C2 synthesis (Fig. 2). The rate of factor B synthesis in the presence of LPS and IFN-γ together could not be achieved by increasing concentrations of, or the times of incubation with, either stimulus separately.

To determine if incubation with one stimulus increased the sensitivity of the cells to the other stimulus, cells were incubated in different combinations of LPS and IFN-γ and synthesis of factor B was quantitated. Synergism was observed between 100 U/ml IFN-γ and concentrations of LPS as low as 1 ng/ml (Fig. 3*A*). The maximum response to LPS was reached between 10 and 50 ng/ml whether the cells were incubated in LPS alone or with LPS in combination with IFN-γ (Fig. 3*A*). Similar results were obtained when the concentrations of IFN-γ were varied while the LPS concentration was constant (Fig. 3*B*). Synergistic effects were observed between 50 ng/ml LPS and concentrations of IFN-γ as low as 1 U/ml. The response to IFN-γ was concentration dependent up to 1,000 U/ml whether the cells were incubated in IFN-γ alone or in combination with LPS. These results indicated that neither IFN-γ nor LPS altered the sensitivity of the cells to the other stimulus.

The time of incubation with the cells required to observe a response was different with each stimulus. Cells incubated with IFN-γ for periods as short as 4 h had an increased capacity to synthesize factor B (2.6-fold) (Fig. 4*C*). Cells had to be incubated with LPS at least 12 h for the synthesis of factor B to increase. Syngergism on factor B synthesis was not noted until the effect of each stimulus was observed independently: the increase in the factor B synthesis in cells incubated in both stimuli for 4 h was comparable only to the increase with

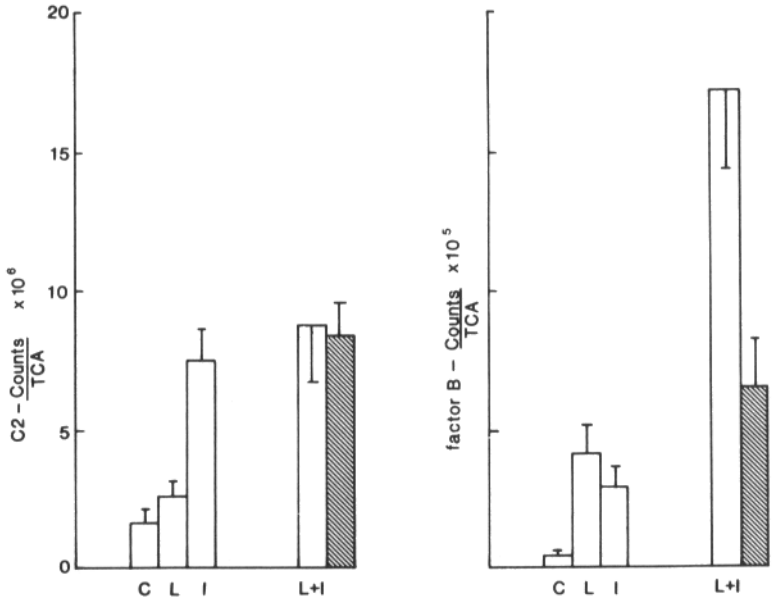

FIGURE 2. Effect of simultaneous incubation with LPS and IFN-γ on C2 and factor B synthesis. Confluent monolayers were incubated for 24 h in medium containing 1 mg/ml BSA alone (control or *C*) or with 50 ng/ml LPS (*L*), 100 U/ml IFN-γ (*I*), or LPS + IFN-γ (*L* + *I*). Amounts of biosynthetically labeled C2 and factor B in the intracellular lysates for each condition were determined as described in the legend for Table I. Data points (mean ± 1 SEM) represent eight different experiments and are expressed as specific counts divided by total TCA-precipitable protein in the appropriate experiment. The expected additive effects (calculated from counts/TCA from L alone + I alone − 1 control, *dashed bars*) were compared with the actual effects (*open bars*).

IFN-γ alone (2.5-fold) (Fig. 4, *C* and *D*), and a significant synergistic effect was demonstrated only after 12 h (16-fold increase or 2.5-fold greater than the sum of the individual effects) (Fig. 4, *F–H*).

To determine if the synergistic response to the two stimuli could be reproduced by preincubation with one stimulus followed by incubation with the other stimulus, factor B synthesis was quantitated after two consecutive 18-h periods of incubation with the stimuli in various sequences. Significant synergism between the two stimuli was demonstrated only when both were present together (Fig. 5). Preincubation with one stimulus did not have a significant effect on the subsequent response to the other stimulus. For example, preincubation with IFN-γ and subsequent incubation with LPS (Fig. 5*I*) increased factor B synthesis 8.3-fold over control. This response can be compared with the increase for IFN-γ followed by control (6.3-fold; Fig. 5*J*) plus the increase for control followed by LPS (2.1-fold; Fig. 5*B*), or a total of 8.4. The 8.3-fold increase in factor B synthesis with preincubation with IFN-γ followed by LPS (Fig. 5*I*) is contrasted to 45.2-fold increase in the synthesis of factor B when the cells were incubated in both stimuli together (Fig. 5*K*). Also apparent in Fig. 5 is the persistence of the effects of LPS, IFN-γ, and their combination on factor B synthesis after their removal (Fig. 5, compare *G*, *J*, and *L*, with *A*).

Northern Blot Analysis of RNA in Control and Stimulated Fibroblasts. Comparable

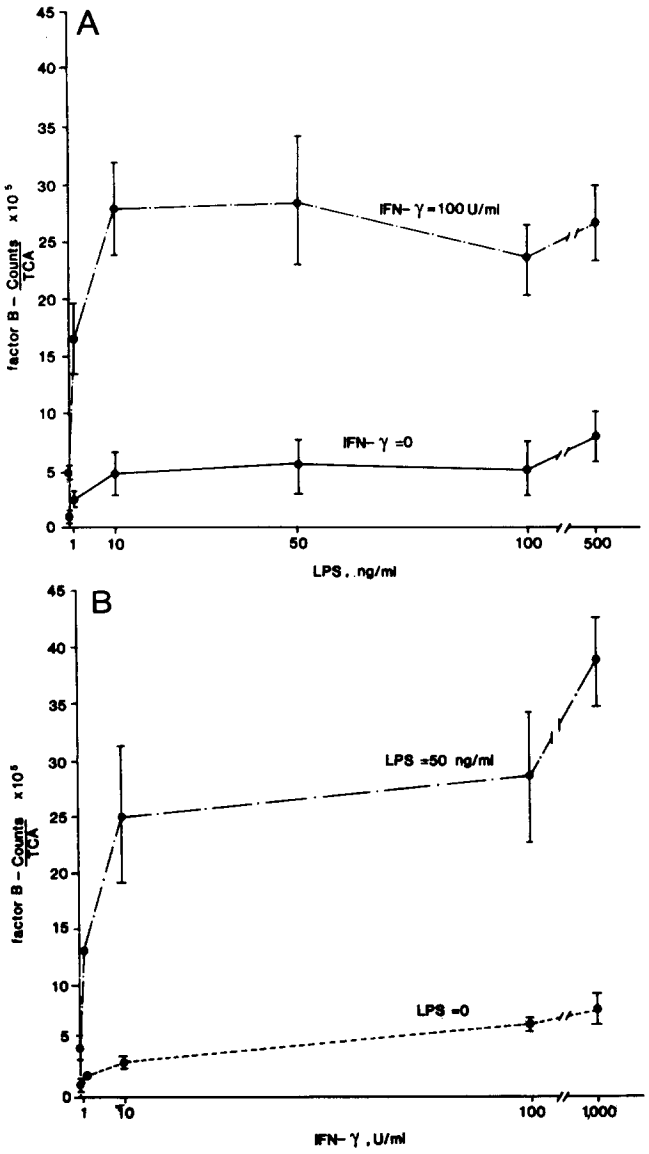

FIGURE 3. Factor B synthesis: effect of one stimulus on the response to the other stimulus. (*A*) Confluent monolayers were incubated for 24 h in medium containing LPS alone in concentrations ranging from 1 to 500 ng/ml (IFN-γ = 0) or with IFN-γ (100 U/ml). (*B*) Monolayers were incubated in medium containing concentrations of IFN-γ alone in concentrations ranging from 1 to 1,000 U/ml (LPS = 0) or with LPS (50 ng/ml). The synthesis of factor B in the intracellular lysates was determined as described in the legend to Table I. The data are represented as the mean of three experiments (±1 SEM) and are expressed as the specific counts divided by total TCA-precipitable protein in the appropriate experiment.

amounts of poly(A)$^+$ RNA isolated from fibroblasts incubated in the medium alone or in the different stimuli were subjected to Northern blot analysis using human cDNA probes for C2 and factor B. The effects of the different stimuli on amounts of C2 and factor B mRNA were analyzed with soft laser densitometry. Both LPS and IFN-γ increased levels of factor B mRNA (Table II). These increases were not prevented by incubation with cycloheximide in concentrations sufficient to reversibly inhibit protein synthesis by >90% (data not shown), indicating that new protein synthesis was not required for the effects of these stimuli on the mRNA levels. LPS induced a greater increase in factor B protein synthesis than in factor B mRNA, whereas IFN-γ stimulated comparable increases in mRNA and protein (Table II). These data suggest that LPS acts to

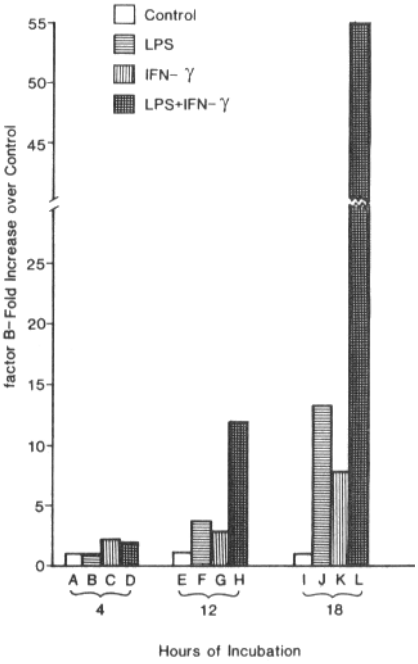

FIGURE 4. Time of incubation with LPS, IFN-γ, and LPS + IFN-γ required to increase the synthesis of factor B. Confluent fibroblasts were biosynthetically labeled after incubation with LPS alone, IFN-γ alone, or their combination for periods ranging from 4 to 18 h. Because of the possibility that differences of total time in culture might affect the capacity of the cells to synthesize factor B, all cells were pulse labeled at the same time by varying the times of addition of the stimuli. The synthesis of factor B was analyzed as described in legend for Table I.

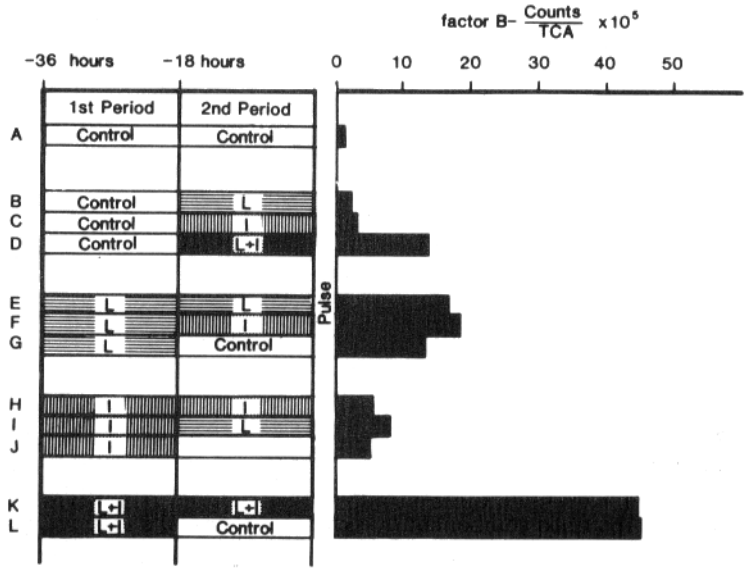

FIGURE 5. Effect of preincubation with one stimulus on the response of the cells to the other stimulus. Confluent fibroblasts were incubated for two consecutive 18 h periods in varying combinations of control medium (*C*), 50 ng/ml LPS (*L*), 100 U/ml IFN-γ (*I*), or LPS + IFN-γ (*L* + *I*); in each case media were discarded and the monolayers were rinsed between the two periods. After the second incubation period the monolayers were biosynthetically labeled and factor B synthesis in the cell lysates was quantitated as described in the legend to Table I. The data are the means of two separate experiments and are expressed as the specific counts divided by total TCA-precipitable protein in the appropriate experiment.

TABLE II
Effects of LPS, IFN-γ, and LPS + IFN-γ on Factor B mRNA

Treatment	Fold increase over control					
	Factor B mRNA levels				Mean	Mean for protein
	Exp. 1	Exp. 2	Exp. 3	Exp. 4		
LPS	4.0	1.2	3.7	1.9	2.7	12.1
IFN	1.9	6.1	4.0	15.5	6.9	7.1
LPS + IFN	24	8.1	13.8	101	36.7	54.5

Fibroblasts were incubated for 24 h in medium alone or medium containing LPS (50 ng/ml), IFN-γ (100 U/ml), or LPS + IFN-γ, and total cellular RNA was extracted using guanidine and cesium chloride density ultracentrifugation. 100 μg of total cellular RNA for each condition were extracted with oligo(dT) and subjected to Northern blot analysis using cDNA probes for factor B. The intensity and area of density of the relevant band on the autoradiograms were determined using soft laser scanning densitometry, and expressed as fold increase over control.

increase factor B synthesis at both pretranslational and translational sites, while IFN-γ acts primarily at a pretranslational step. LPS and IFN-γ together induced an ~36.7-fold increase in factor B mRNA over control, which was 3.5-fold greater than predicted from the increase when the stimuli were added separately, comparable to the synergistic effect (2.8-fold) on protein synthesis (Table II).

LPS increased C2 mRNA 2.5-fold (data not shown), similar to the increase in factor B mRNA, but the increase in C2 protein synthesis was only 2.0-fold. For IFN-γ, increases in C2 mRNA (6.4-fold) and protein synthesis (6.4-fold) were comparable, similar to the results for factor B. As with the protein data, there was no synergism between LPS and IFN-γ for C2 mRNA. Cycloheximide in concentrations that reversibly inhibited protein synthesis did not prevent the increases in levels of factor B or C2 mRNA in response to LPS, IFN-γ, or their combination.

Discussion

We report that human fibroblasts synthesize C2 and factor B and that this synthesis is regulated by LPS and IFN-γ. The size and subunit structure of C2 and factor B produced in human fibroblasts were similar to that observed in both Hep-G2 cells (22, 23) and in human monocytes (6). Unstimulated fibroblasts and monocytes synthesized approximately comparable amounts of factor B per cell; however, monocytes synthesized ~100-fold more C2 per cell than fibroblasts (24).

IFN-γ increased synthesis of both proteins, but LPS had a significant effect only on synthesis of factor B. This pattern and the levels of stimulation of C2 and factor B were comparable to those observed with these stimuli in monocytes (6, 24). The molecular mechanisms underlying these effects, as reflected in the relationships between rates of protein synthesis and levels of mRNA, were different for the two stimuli. For IFN-γ, increases in rates of protein synthesis and mRNA levels were comparable for both C2 and factor B, suggesting that IFN-γ affects both C2 and factor B synthesis at a pretranslational level. For LPS,

the increase in the rate of factor B protein synthesis was much greater than for factor B mRNA; the small increase in the rate of C2 protein synthesis was accompanied by a comparable increase in C2 mRNA. These results suggest that the major effect of LPS (on factor B synthesis) occurs at the translational level. The absence of a significant effect of LPS on C2 synthesis may be due to the absence of translational regulation for the C2 mRNA.

Translational regulatory events also occur for the mechanism of action of LPS on the synthesis of cachectin, or TNF (25). Mouse macrophages contained mRNA for TNF, but did not synthesize TNF protein until stimulated with LPS. The events that govern these effects are not known, but it has been speculated that they may be related to an octameric sequence (UUAUUUAU) present in the 3′ untranslated region of the mRNA (26). This sequence is also present in the 3′ untranslated regions of other inflammatory mediators that respond to LPS (27). The factor B gene does not contain this octameric sequence through 100 nucleotides 3′ to the stop codon region (28), but the sequence is found between 100 and 200 nucleotides 3′ to the untranslated region of the other genes.

The combination of LPS and IFN-γ had a significant synergistic effect on the synthesis of factor B. Synergism may be dependent, at least in part, on LPS-induced translational regulation of factor B mRNA. Synergism was observed only after the cells had been incubated for periods long enough to produce an effect of LPS alone and did not occur after periods of incubation when only IFN-γ increased factor B synthesis. And there was no synergism for C2, for which only the transcriptional effect of LPS was observed. The wide scatter of the data points for mRNA levels prevents a definitive assignment of the level of the synergistic effect. Synergism was also evident for factor B mRNA levels, suggesting that pretranslational steps may also be affected synergistically. There was no synergism for C2 mRNA levels.

Synergism between LPS and IFN-γ has been studied in several cell types with different cell functions as the marker of the effect. Most studies of the combined effects of LPS and IFN-γ have examined complex cell functions. In the only study demonstrating synergism for a single protein product of a cell (IL-1), the mechanism of synergism was not investigated (10). Synergism has been studied most for tumor cell killing in macrophages, where IFN-γ either increased the sensitivity of the cells to LPS (29), allowed an otherwise unresponsive cell to respond to the LPS (30), or prevented the decay of LPS-induced cytotoxicity that occurs in some cells even in the continued presence of LPS (9, 31). The effect of IFN-γ on LPS action was observed whether the IFN-γ increased killing when added by itself (8) or not (29). IFN-γ prevented the decay of LPS-induced cytotoxicity either by preventing a negative feedback of PGE_2 (9, 31) or by maintaining production of a soluble cytotoxic factor that otherwise decreased with time in culture (32). Another possible mechanism of the effect of IFN-γ on LPS action is increasing the binding of LPS to the cell surface (30). It is unlikely that the mechanisms proposed for synergism in these other systems account for synergism on factor B synthesis: preventing the negative feedback of PGE_2 is unlikely since the effect of LPS on fibroblasts did not decay with continued incubation with LPS through 48 h and addition of PGE_2 to the cultures did not abolish the LPS-induced stimulation (Katz, Y., and R. C. Strunk, unpublished

observations). Increased binding of LPS to the cell surface is unlikely because the effects of the combination of even low concentrations of the two stimuli could not be reproduced by simply increasing the concentrations of LPS (Fig. 3, *A* and *B*).

The role of IFN-γ in regulation of TNF synthesis was different from its role in regulation of factor B synthesis. IFN-γ by itself did not stimulate TNF production. In addition, IFN-γ did not increase the LPS-induced stimulation of TNF production in normal cells. However, in cells resistant to the effects of LPS (C3H/HeJ), IFN-γ allowed translation of otherwise nonfunctioning TNF mRNA, amounts of which had been stimulated by LPS (33). The mechanism of interaction between LPS and IFN-γ in normal fibroblasts observed in our study is probably distinct from the mechanism in these otherwise LPS-unresponsive cells.

Synergism between LPS and IFN-γ for synthesis of factor B required close interaction between the two stimuli, as evidenced by the absence of an effect of preincubation with one stimulus on the subsequent response to the other stimulus. This was true even though the effect of the first stimulus persisted during the stimulation with the second compound. These observations are in contrast to the other systems where the enhancing effects of IFN-γ were reproduced by preincubation of the cells with IFN-γ alone, followed by incubation of LPS in the absence of IFN-γ (15, 30, 34). IFN-γ priming of the cells for the increased response to LPS in these other systems was seen as early as 4 h after addition of the IFN-γ (15, 29).

The major protein products of fibroblasts are collagen, elastin, proteoglycans, and glycoproteins (35). Synthesis of proteins of the complement pathway C1 (11) and C3 (12) in these cells has also been documented. Synthesis of C2 and factor B in human fibroblasts may play a role in host defense. Fibroblasts are abundant in many tissues and synthesis by fibroblasts would provide complement proteins for participation in inflammatory reactions before increases in vascular permeability and recruitment of other complement producing inflammatory cells. Emphasis on synthesis of factor B over C2, both constitutively and in response to endotoxin, is consistent with the concept that the alternative pathway has a significant role in the initial response to inflammation at local sites before the availability of antibody.

Summary

Four different human fibroblast cell lines synthesized C2 and factor B. Factor B synthesis was increased 12.1-fold by 50 ng/ml LPS and 7.1-fold by 100 U/ml IFN-γ. C2 synthesis was increased only 2.1-fold by LPS, but 6.4-fold by IFN-γ. Both LPS and IFN-γ increased levels of factor B mRNA. LPS induced a 4.7-fold greater increase in factor B protein than in factor B mRNA, whereas IFN-γ stimulated comparable increases in protein and mRNA. These data suggest that LPS acts to increase factor B synthesis at both pretranslational and translational sites, while IFN-γ acts primarily at a pretranslational level. In contrast to factor B, increases in C2 protein and C2 mRNA were comparable for both stimuli. A synergistic effect between the two stimuli was observed for factor B only: protein synthesis was increased 54.5-fold or 2.8-fold greater than the additive effects of the stimuli separately. The rate of synthesis in the presence of LPS and IFN-γ

together could not be achieved by increasing concentrations of, or the times of incubation with, either stimulus separately. The synergism was not the result of an increased sensitivity of the cells to either stimulus and was not reproduced by preincubation with one stimulus before incubation with the other stimulus. Several lines of evidence suggest that the synergism, like the stimulation of factor B synthesis by LPS, was dependent on both translational and pretranslational regulation of factor B mRNA. C2 and factor B synthesized in human fibroblasts may play a role in host defense in inflammatory reactions before increases in vascular permeability and recruitment of other complement producing cells.

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