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Research Article

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Counterregulatory Effects of Interferon-γ and Endotoxin on Expression of the Human C4 Genes

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Abstract

Susceptibility to autoimmune disease is associated with null alleles at one of the two genetic loci encoding complement protein C4. These two genetic loci, C4A and C4B, are highly homologous in primary structure but encode proteins with different functional activities. Expression of C4A and C4B genes is regulated by IFN-γ in human hepatoma cells and in murine fibroblasts transformed with the respective genes. In these cell lines, IFN-γ has a significantly greater and longer-lasting effect on expression of C4A than that of C4B. In this study we examined synthesis and regulation of C4A and C4B in peripheral blood monocytes from normal, C4A-null, and C4B-null individuals. Synthesis of C4 in human peripheral blood monocytes decreases during time in culture. IFN-γ mediates a concentration- and time-dependent increase in steady-state levels of C4 mRNA and a corresponding increase in synthesis of C4 in normal human monocytes. LPS decreases monocyte C4 expression and completely abrogates the effect of IFN-γ on the expression of this gene. In contrast, LPS and IFN-γ have a synergistic effect in upregulating expression of another class III MHC gene product, complement protein factor B. The effect of LPS on constitutive and IFN-γ–regulated C4 synthesis is probably not mediated via release of endogenous monokines IL-1β, TNF-α, or IL-6. Synthesis of C4, and regulation of its synthesis by IFN-γ and LPS, are similar in normal, C4A-, and C4B-null individuals. These results demonstrate the synthesis of C4 at extrahepatic sites and tissue-specific regulation of C4 gene expression. (*J. Clin. Invest.* 1990. 85:943–949.) complement • mononuclear phagocytes • gene expression • lymphokines

Introduction

C4 is a three-chain, disulfide-linked glycoprotein which, together with the second component of complement (C2), forms the classical complement-activating C3 convertase (reviewed in reference 1). It is encoded by two loci, C4A and C4B, located in class-III region of the MHC on the short arm of chromosome 6 (2, 3). The two C4 genes lie ~ 30 kb downstream from the C2 and factor B genes, separated by 10 kb, and each has a cytochrome P-450 steroid 21-hydroxylase gene within 1.5 kb of the 3′ terminus (4, 5).

The complete nucleotide sequence of a few C4A and C4B genes has been determined (6, 7). There are only six nucleotide differences, resulting in four amino acid differences between the two genes. The variation in sequence is clustered around the active site, the thiol ester region of the C4 α chain, and thought to explain the differences in functional activity of the C4A and C4B genes. The product of the C4B gene reacts more effectively with hydroxyl groups and is two- to threefold more effective in complement hemolytic activity. C4A reacts more readily with amino groups, and is more effective in inhibiting the formation and in causing the dissolution of immune aggregates (8–10).

There are also two C4-like genes in the mouse (reviewed in reference 11). These two genes are similar to C4A and C4B in chromosomal localization, i.e., being encoded within the MHC, and in orientation with respect to the direction of transcription (12). The mouse C4-like proteins, Ss protein and Slp protein, are also characterized by a marked difference in specific hemolytic activity. Expression of the Slp gene is regulated by androgens (13).

The C4 protein is synthesized in liver as an ~ 185-kD single-chain precursor, pro-C4, and is converted into the functionally active three-chain disulfide-linked native protein during posttranslational transport/secretion. Approximately 80% of the C4 synthesized in human hepatoma HepG2 cells is C4A isotype (14). A marked increase in expression of C4 in HepG2 cells is mediated by IFN-γ. In HepG2 cells, which express both C4A and C4B, and in stable transfected mouse fibroblast cell lines, which express C4A or C4B alone, IFN-γ has a significantly greater and longer-lasting effect on the synthesis of C4A than that of C4B.

In order to further understand the significance of these differences in expression and regulation of the two human C4 genes and the molecular basis for deficiencies of C4 isotypes, we examined the possibility that C4 was synthesized in IFN-γ–activated monocytes from normal, C4A-, and C4B-null individuals.

Methods

Materials. Medium 199 (M199)[1] was obtained from Whittaker M.A. Bioproducts (Walkersville, MD). DME, DME without methionine, HBSS, L-glutamine, and penicillin-streptomycin were purchased from Washington University School of Medicine Tissue Culture Support Center. Human serum, type AB, was obtained from Sigma Chemical Co. (St. Louis, MO). Fetal bovine serum was purchased from Flow Laboratories, Inc. (McLean, VA). [^{35}S]Methionine was obtained from

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1. *Abbreviations used in this paper:* α_1-PI, α_1-proteinase inhibitor; M199, medium 199.

ICN Nutritional Biochemicals (Cleveland, OH). [^{32}P]CTP (specific radioactivity ~ 3,000 Ci/mmol) and ^{14}C-methylated protein standards were from Amersham Corp. (Arlington Heights, IL). Goat antihuman C4 and goat antihuman factor B were obtained from Atlantic Antibodies (Scarborough, ME). Rabbit antihuman α_1-proteinase inhibitor (α_1-PI) was from Dako Corp. (Santa Barbara, CA). Other reagents included IgG-Sorb from Enzyme Center (Cambridge, MA), cesium chloride from Boehringer Mannheim Biochemicals (Indianapolis, IN), guanidine-isothiocyanate from Fluka AG (Buchs, Switzerland), and sodium-*N*-lauroylsarcosine from Sigma Chemical Co. *Escherichia coli* serotype 0111:B4 LPS, prepared by Westphal phenolic extraction, was also purchased from Sigma Chemical Co. Recombinant-generated human IFN-γ was obtained from Biogen Research Corp. (Cambridge, MA) and diluted in M199 for use in tissue culture. Recombinant-generated human TNF-α was provided by Genentech Inc. (S. San Francisco, CA), recombinant IL-1β was provided by B. Joy (Monsanto Company, St. Louis, MO), and IL-6 by P. B. Sehgal (New York).

Cell culture. Confluent monolayers of human peripheral blood monocytes from blood donors were established by adherence of dextran-purified leukocytes (15) on Primaria® plates or dishes. The C4 allotype of each donor was determined by subjecting neuraminidase-treated EDTA plasma to agarose gel electrophoresis (16). Gels were then overlaid with anti-C4 for immunofixation. Cells at a density of 3.0×10^7/ml were allowed to adhere for 2 h at 37°C. Adherent monolayers were then rinsed vigorously and were either cultured further in M199 containing 15% human serum or used for experiments. Human hepatoma-derived HepG2 cells were cultured in DME containing 10% fetal bovine serum (14).

Metabolic labeling. Confluent monolayers of monocytes were incubated at 37°C in the presence of methionine-free medium containing 0.5 μCi/ml [^{35}S]methionine. In most experiments, synthesis of C4 was assessed by detection of newly synthesized radiolabeled pro-C4 in the cell lysate after a 1-h interval of pulse radiolabeling. In some experiments, the cells were pulse radiolabeled for 8 h, a time interval after which radiolabeled C4 α, β, and γ chains could be detected in the extracellular fluid. Methods for solubilization of cells and clarification of cell lysates have been described (14). Total protein synthesis was estimated by TCA precipitation of aliquots of cell lysates and culture fluids (17).

Immunoprecipitation and SDS-PAGE. Aliquots of cell lysate and culture fluid were incubated overnight at 4°C in 1% Triton X-100/1% SDS/0.4% deoxycholic acid, with excess antibody. Immune complexes were precipitated with excess formalin-fixed staphylococci-bearing protein A, washed, released by boiling in sample buffer, and applied to 6% SDS-PAGE under reducing conditions and described by Laemmli (18). ^{14}C-Methylated molecular size markers (200,000, 92,500, 68,000, 46,000, 30,000, and 17,000 mol wt) were included on all gels. After electrophoresis, gels were stained in Coomassie brilliant blue, destained, impregnated with 2,5-diphenoxylate (EN^{3}HANCE; New England Nuclear, Boston, MA), and dried for fluorography on XAR x-ray film (Eastman Kodak Co., Rochester, NY).

Ribonuclease protection assay. Total cellular RNA was isolated from monolayers of monocytes by quanidine isothiocyanate extraction and ethanol precipitation (19). RNA was quantified by absorbance at 260 μm and then subjected to a ribonuclease protection assay, based on the technique of Melton et al. (20). A 556-bp Pst I–Pst I fragment from the 3′ terminus of cDNA clone pC4AL1, which recognizes C4A and C4B mRNA (21), was cloned into the Pst I site of the PGEM 4 plasmid (Promega Biotec, Madison, WI), subjected to in vitro transcription with T7 polymerase, and the resulting ^{32}P-labeled cRNA used as probe. This probe was hybridized with 50 μg total cellular RNA at 55°C overnight and hybrids subjected to digestion with RNase A and RNase T1 at room temperature. Protected fragments were detected in a 6% acrylamide–8-M urea sequencing gel (22).

DNA blot analysis. Human genomic DNA was extracted from peripheral blood mononuclear cells (23). 10 μg of genomic DNA was digested to completion with restriction endonucleases Hind III and Bam HI, according to the manufacturer's recommendations, and subjected to agarose gel electrophoresis (24). A 0.9-kb Kpn I–Pst I genomic DNA fragment from the 5′ end of the C4A and C4B genes (25) was labeled with [^{32}P]dCTP and used as probe. This probe has been shown to recognize size polymorphisms of the C4A and C4B genes and to recognize a specific deletion of the C4A gene associated with the HLA-B8, DR3 extended haplotype and susceptibility to systemic lupus erythematosus (25). Hybridization with radiolabeled probes, washing, and autoradiography have been previously described (14).

Results

C4 is synthesized in human peripheral blood monocytes. After 2 h in culture, monolayers of monocytes were incubated for 24 h in serum-free control medium or medium supplemented with recombinant human IFN-γ. From these monolayers, total cellular RNA was isolated and subjected to RNase protection assay (Fig. 1 *a*). In IFN-γ–activated monocytes, a 556-bp fragment was protected. This fragment was present in monocytes under control conditions after a much longer autoradiographic exposure (data not shown). A fragment of identical apparent size was detected in HepG2 cells but none detected in the negative control, yeast tRNA. These results indicate that C4 mRNA is present in human monocytes and that IFN-γ mediates a marked increase in steady state levels of C4 mRNA in monocytes.

In order to determine whether the C4 protein is synthesized in the same cells, control and IFN-γ–activated monocytes were subjected to metabolic labeling and the resulting radiolabeled cell lysates subjected to immunoprecipitation and SDS-PAGE followed by fluorography (Fig. 1 *b*). An ~ 185-kD radiolabeled polypeptide corresponding to pro-C4 was detected in IFN-γ–treated monocytes. A radiolabeled polypeptide of identical electrophoretic mobility is detected in HepG2 cells. Although this polypeptide is not present in control

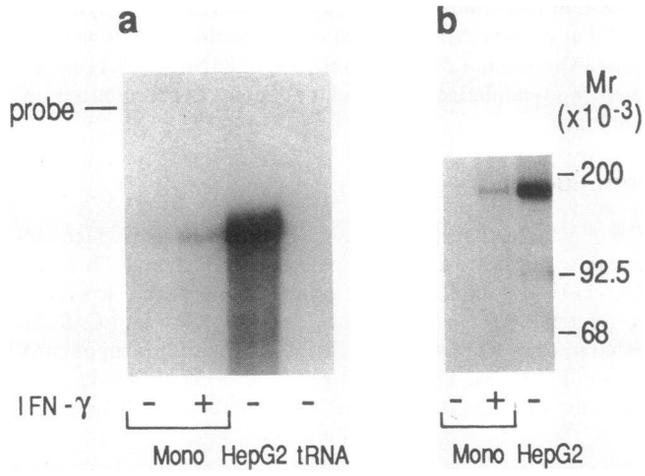

Figure 1. Detection of C4 mRNA and pro-C4 in peripheral blood monocytes and in HepG2 cells. Confluent monolayers of monocytes or HepG2 cells were incubated for 24 h in serum-free medium alone (−), and in recombinant human IFN-γ, 1,000 U/ml (+). (*a*) Total cellular RNA was isolated and subjected to RNAse protection assay as described in Methods. The relative electrophoretic mobility of the cRNA probe is indicated at the left margin. (*b*) Cells were subjected to pulse radiolabeling for 60 min. Pro-C4 was detected in cell lysates as described in Methods. Molecular mass markers are indicated at the right margin.

monocytes in this experiment, it is detected in control monocytes in many other experiments (see Figures below). Synthesis of C4 was also detected in human bronchoalveolar macrophages (data not shown).

IFN-γ regulates expression of C4 in human monocytes. Freshly isolated monocytes were incubated for 24 h in serum-free control medium and medium supplemented with IFN-γ in several different concentrations. Cells were then pulse radiolabeled and synthesis of pro C4 assayed (Fig. 2). IFN-γ mediates a concentration-dependent increase in synthesis of C4. The lowest effective concentration of IFN-γ is 10 U/ml. The effect of IFN-γ is time-dependent and specific in that there is no change in the synthesis of α_1-PI in these cells under the same conditions (data not shown).

In order to examine the synthesis and regulation of C4 at different times during the in vitro differentiation of monocytes, separate monolayers of monocytes were incubated with IFN-γ for 24 h after 2 h or 6 d in culture (Fig. 2). This experiment demonstrates a decrease in synthesis of C4 during in vitro differentiation of monocytes. The decrease in C4 synthesis is specific in that total protein synthesis and synthesis of other specific proteins (e.g., complement factor B) increases during the same time interval (26). The concentration-dependent effect of IFN-γ on C4 synthesis is still evident and similar in magnitude by day 7 in culture (Fig. 2).

The effect of IFN-γ on monocytes also resulted in an increase in C4 secreted into the extracellular fluid (Fig. 3). Radiolabeled C4 α and β chains and an α-β intermediate (27) were detected in the extracellular fluid of IFN-γ–activated monocytes. The C4 γ chain was also detected in other experiments (data not shown).

LPS and IFN-γ have counterregulatory effects on monocyte C4 synthesis. Since different types of macrophage activators, such as IFN-γ and LPS, are likely to be present at sites of tissue injury/inflammation, and since IFN-γ and LPS have been shown to elicit synergistic effects on the synthesis of another class-III MHC-linked complement protein, factor B (28), we examined the possibility of synergistic actions for IFN-γ and LPS in modulating monocyte C4 expression. First, we examined the effect of LPS on synthesis of C4. After 2 h in culture, monocytes were incubated in serum-free control medium or medium supplemented with LPS (Fig. 4). LPS mediated a concentration-dependent decrease in synthesis of C4. The effect was apparent at concentrations of LPS as low as 100 pg/ml. The effect was specific in that LPS mediated an increase in synthesis of factor B in the same experiment (data not shown).

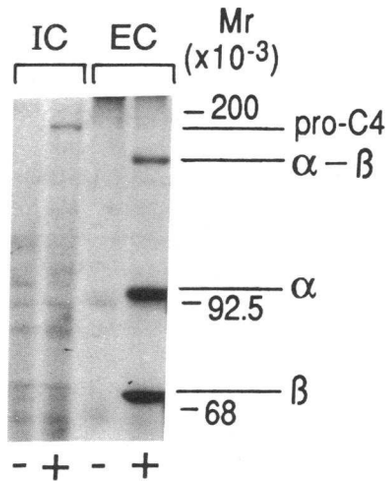

Figure 3. Effect of IFN-γ on synthesis and secretion of C4 in human monocytes. After 2-h adherence, monocytes were incubated for 16 h in serum-free medium alone (−) or in medium supplemented with IFN-γ, 1,000 U/ml (+). Cells were then subjected to pulse radiolabeling for 8 h. IFN-γ was present in the medium during the pulse radiolabeling of cells that had been preincubated with it. C4 was detected in cell lysates (IC) or culture fluid (EC). Molecular mass markers are indicated in the right margin.

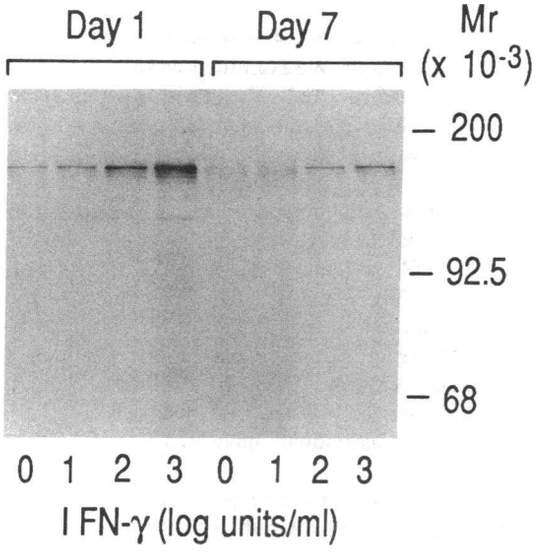

Figure 2. IFN-γ regulates the synthesis of C4 in human monocytes. After 2 h (*left*) or 6 d in culture (*right*) monolayers of monocytes were incubated for 24 h in serum-free medium alone (0) or in medium supplemented with IFN-γ in the specified concentrations. Cells were then subjected to pulse radiolabeling for 60 min and C4 was detected in cell lysates. Molecular mass markers are indicated in the right margin.

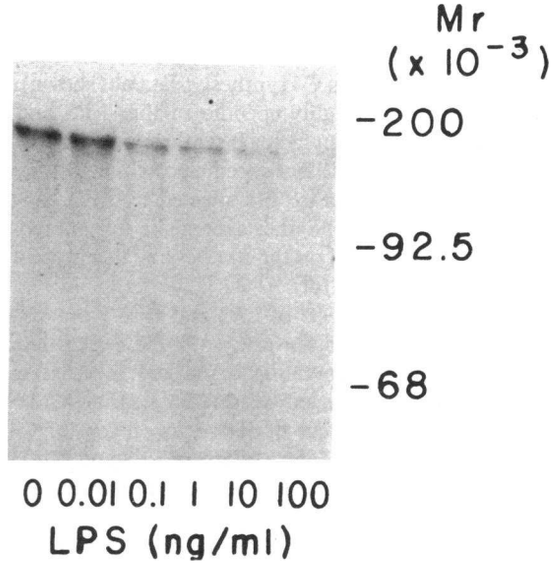

Figure 4. LPS decreases synthesis of C4 in monocytes. After 2 h in culture, monocytes were incubated for 24 h in serum-free control medium or medium supplemented with LPS in the specified concentrations. Synthesis of C4 was determined by methods described in the legend to Fig. 2. Molecular mass markers are indicated at the right margin.

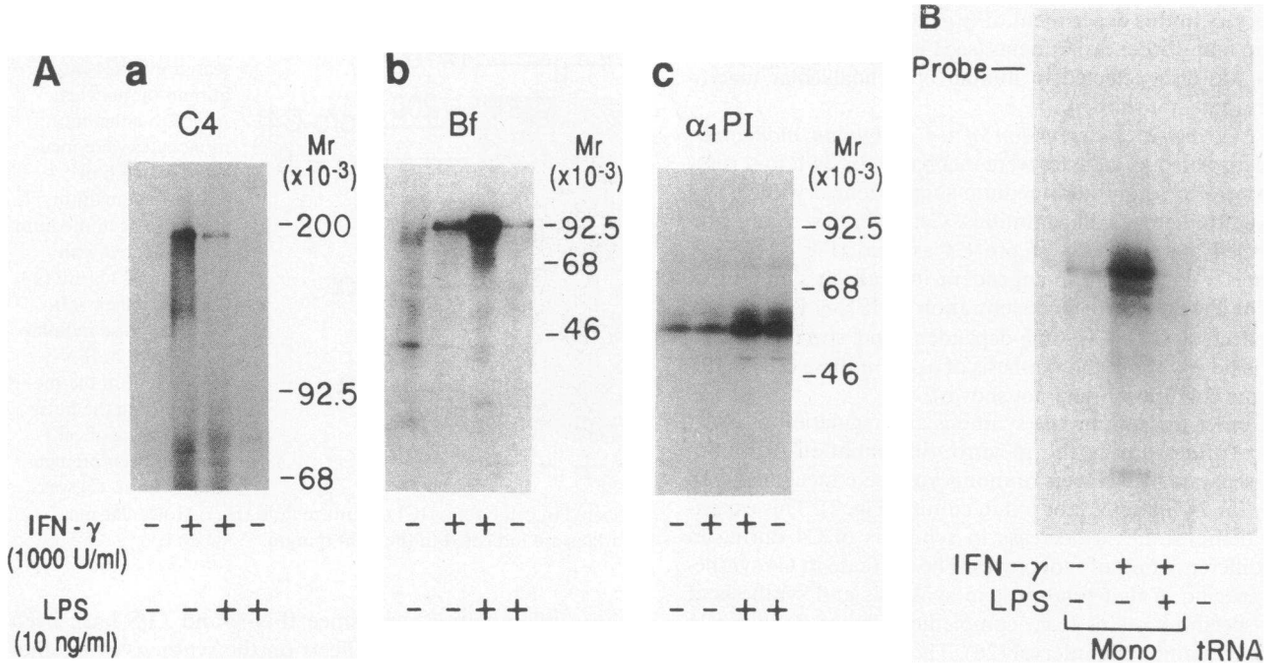

Figure 5. LPS abrogates the effect of IFN-γ on C4 synthesis in human monocytes. *A* After 2 h in culture, separate monolayers of monocytes were incubated for 24 h in serum-free control medium alone, medium supplemented with IFN-γ (1,000 U/ml), medium supplemented with LPS (10 ng/ml), or medium supplemented with both IFN-γ and LPS. Cells were rinsed and then subjected to pulse radiolabeling. Cell lysates were then immunoprecipitated with antibody to human C4, factor B, and α_1-PI. Molecular mass markers are indicated in the right margin. *B* After 2 h in culture, monocytes were incubated for 24 h in serum-free control medium alone, medium supplemented with IFN-γ (100 U/ml), or medium supplemented with IFN-γ (100 U/ml) and LPS (10 ng/ml). Cells were then rinsed, total cellular RNA isolated, and the RNA subjected to RNAse protection assay. The relative electrophoretic mobility of the cRNA probe is indicated at the left margin.

LPS also abrogated the effect of IFN-γ on monocyte C4 synthesis. In the experiment shown in Fig. 5 *A*, baseline synthesis of C4 is too low to determine whether LPS decreases synthesis of C4. However, LPS markedly decreases the positive regulatory effect of IFN-γ on C4 synthesis in monocytes coincubated with LPS and IFN-γ (*a*). At higher concentrations of LPS, or lower concentrations of IFN-γ, LPS completely abrogates the effect of IFN-γ on C4 synthesis (data not shown). This antagonistic effect is highly specific in that LPS and IFN-γ have synergistic effects on factor B synthesis in the same experiment (*b*). Synthesis of another specific protein, α_1-PI, by monocytes in this experiments is only affected by LPS, confirming previous studies (15). Neither synergistic nor antagonistic effects of IFN-γ and LPS on the synthesis of this protein are evident in this experiment (*c*).

The counterregulatory effects of LPS and IFN-γ are also evident in ribonuclease protection assays. In the experiment shown in Fig. 5 *B*, IFN-γ mediates a marked increase in steady-state levels of the protected C4 mRNA fragment. LPS completely abrogates the effect of IFN-γ on levels of C4 mRNA. Thus, the counterregulatory effects of LPS and IFN-γ are likely to involve transcriptional or posttranscriptional mechanisms.

In contrast to LPS itself, the LPS-inducible monokines IL-1β, TNF-α, and IL-6 do not have counterregulatory effects on monocyte C4 synthesis. In Fig. 6, LPS completely abrogates the effect of IFN-γ on C4 synthesis, but IL-1β, TNF-α, and IL-6 have no significant effect. All three of these monokines are biologically active in the same experiment as shown by increases in factor B synthesis for IL-1β and IL-6 and by increases in α_1-PI synthesis for IL-6 (data not shown). These results militate against, but do not exclude, the possibility that the effects of LPS on monocyte C4 synthesis are mediated through the induction of endogenous monokines IL-1β, TNF-α, or IL-6.

IFN-γ increases synthesis of C4 in monocytes from C4A-null and C4B-null individuals. We examined synthesis of C4 in monocytes from four different individuals with no detectable plasma C4B protein (A3BQO) and in monocytes from two different individuals with no detectable plasma C4A protein (AQOB1). For both C4A-null individuals, Southern blot analysis (not shown) indicated the presence of an ~ 30-kb deletion of the C4A gene similar to that which has been identified in HLA-B8 C4AQO, DR3 extended haplotype in several previous studies (25, 29, 30). The molecular mechanism for the C4B-null state was not determined in the four C4B-null individuals studied. However, Southern blot analysis indicated that it is a heterogeneous group. Hind III and Bam HI digest showed the presence of two C4 genes in all of these individuals. Three of the C4B-null individuals have two long C4 genes (data not shown).

In each case, synthesis of C4 and its regulation by IFN-γ and LPS in monocytes of the C4A-null or C4B-null individual was compared with that of a C4 A3 B1 homozygote (Figs. 7 and 8). There was no significant difference in synthesis of C4 in control or IFN-γ–activated monocytes of any of these individuals. LPS abrogated the effect of IFN-γ on synthesis of C4 in monocytes from all these individuals (data not shown). There was no significant difference in synthesis of a control protein factor B in these experiments (data not shown).

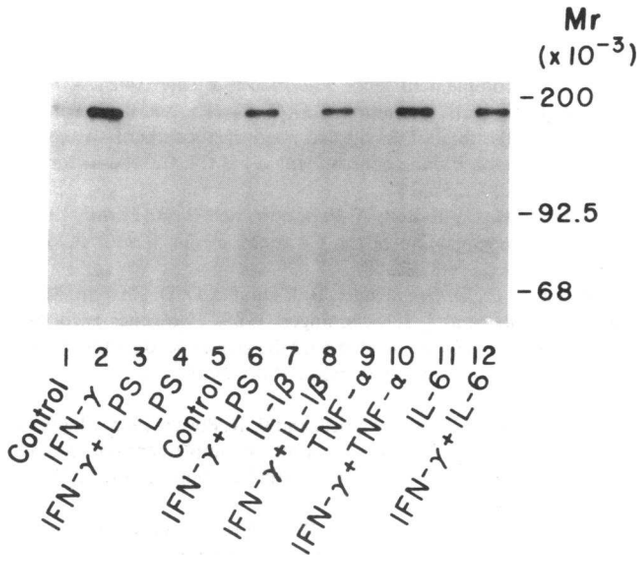

Figure 6. LPS-inducible monokines do not have counterregulatory effects on IFN-γ–regulated C4 synthesis. After 2 h in culture, monocytes were incubated for 24 h in serum-free control medium (lane *1*); medium supplemented with IFN-γ, 100 U/ml (lane *2*); IFN-γ plus LPS, 100 ng/ml (lane *3*); LPS alone (lane *4*); medium supplemented with polymyxin B, 10 μg/ml (lane *5*); IFN-γ plus LPS and polymyxin B (lane *6*); IL-1β, 100 ng/ml, plus polymyxin B (lane *7*); IFN-γ plus IL-1β and polymyxin B (lane *8*); TNF-α, 100 ng/ml (lane *9*); IFN-γ plus TNF-α and polymyxin B (lane *10*); IL-6, 100 ng/ml, plus polymyxin B (lane *11*); IFN-γ plus IL-6 and polymyxin B (lane *12*). Polymyxin B was included in lanes *5–12* in order to exclude the possibility that LPS contamination of cytokine preparations was responsible for any observed effects. The results show that polymyxin B in this concentration almost completely abrogates the counterregulatory effect of LPS (compare lanes *3* and *6*).

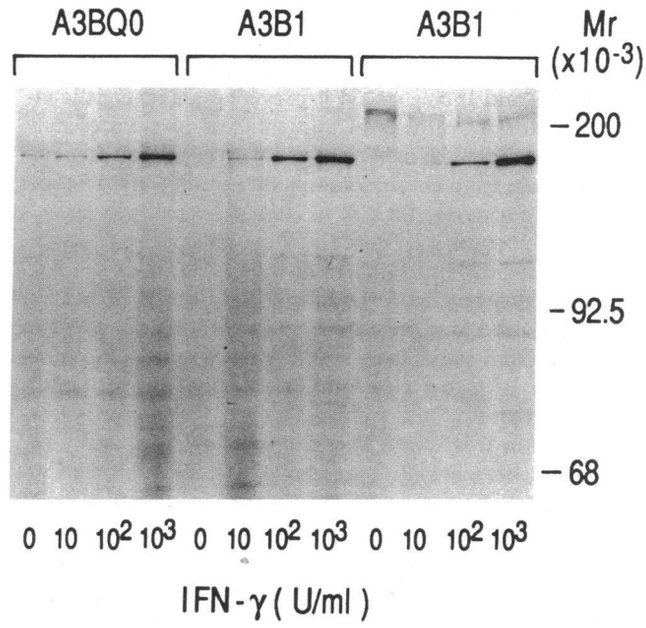

Figure 7. Effect of IFN-γ on the synthesis of C4 in monocytes from individuals having only C4A gene expressed (C4A3BQO allotype) or having both C4A and C4B genes expressed (C4A3B1). After 2-h adherence, monocytes were incubated for 24 h in medium alone (0) or in medium supplemented with IFN-γ in the specified concentrations. Cells were then subjected to pulse radiolabeling for 60 min. C4 was detected in cell lysates. Molecular mass markers are indicated in the right margin.

Discussion

Although several studies have suggested that C4 is produced by human mononuclear phagocytes (31, 32), technical limitations in analytical methods have prevented definitive identification of C4 biosynthesis in these cells. The results of these current experiments clearly demonstrate the biosynthesis of C4 in human monocytes. Expression of C4 decreases rapidly during the differentiation of monocytes in vitro despite increases in total cellular RNA content, total protein synthesis, and synthesis of other specific protein during the same time interval (26). Thus, expression of C4 in human monocytes is similar to that in murine peritoneal macrophages. In the latter cells, C4 synthesis rapidly decreases to undetectable levels within 24 h in culture (33, 34). In this respect, expression of C4 on human monocytes is also similar to that of class-II MHC gene product DR (35), but different from that of the other class-III MHC gene products, complement proteins C2 and factor B, expression of which increases during monocyte differentiation in vitro (26). There are several possible explanations for reduction in expression of C4 in monocytes during time in culture including the elaboration of a downregulating factor in vitro or the loss of an in vivo upregulating factor.

The results of these experiments also indicate that synthesis of C4 in mononuclear phagocytes is modulated by IFN-γ. The effect of IFN-γ on C4 gene expression is not tissue-specific in that IFN-γ increases synthesis of C4 in human hepatoma HepG2 cells and in murine fibroblasts transfected with the human C4 genes (14). IFN-γ also modulates the expression of the other class-III MHC complement genes C2 and factor B (36). However, the effect of IFN-γ on C4 probably involves a signal transduction pathway that is distinct from that for which the C2 and factor B genes are targets. This is best shown by experiments in which monocytes are co-incubated with LPS and IFN-γ; that is, LPS and IFN-γ have counterregulatory effects on C4 but synergistic effects on factor B synthesis. These results could not be explained by differences in IFN-γ

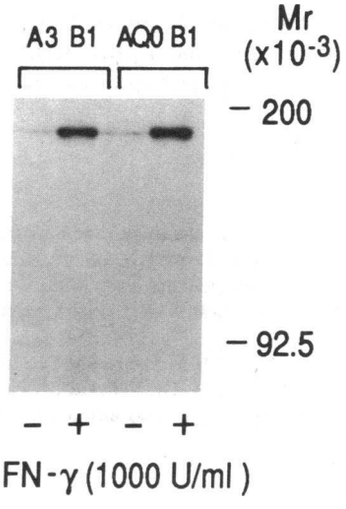

Figure 8. Effect of IFN-γ on synthesis of C4 in monocytes from an individual with only C4B gene expressed (C4AQOB1 allotype) or with both C4A and C4B gene expressed (C4A3B1). The experimental protocol was identical to that described in the legend for Fig. 5 *A*.

receptor expression or receptor occupancy and, therefore, implicate differences in steps distal to receptor activation within each signal transduction pathway. The counterregulatory effects of LPS and IFN-γ on C4 expression and synergistic effects on factor B synthesis in mononuclear phagocytes may indicate that the initial host response at local sites of bacterial infection shifts from classical complement pathway activation (the C4b2a convertase for C3) to alternative pathway activation (C3Bb convertase). Such a shift would also increase the host response by loop amplification.

Having demonstrated the biosynthesis of C4 in human monocytes, it became possible to examine synthesis of C4 in monocytes from individuals with C4A-null and C4B-null alleles. Results of such experiments indicate that synthesis of C4 in monocytes from these individuals is not different from that in monocytes from individuals expressing C4A and C4B. There is a relatively high frequency of null alleles at the C4A and C4B loci. The frequency of C4AQO is estimated to be 13–14% and C4BQO 15–16% among Caucasian populations (19). It is also estimated that more than half the Caucasian population carry a C4-null gene. Many of the C4 haplotypes carrying null alleles are due to deletion of the C4 gene (25, 29, 30, 37, 38). The most well characterized is an ~ 30-kb deletion of the C4A gene, extending from a position near its 5′ terminus to an analogous position in the C4B gene (25, 29, 30, 38). This deletion encompasses most of the C4A and all of the 21-hydroxylase–A pseudogene sequences. Such individuals presumably synthesize a C4 fusion protein recognized as C4B. Several haplotypes carrying null alleles are not associated with deletion of the C4 gene. Theoretically, this may result from a defect in expression of the C4 gene or expression of the same allotype at both C4 loci. The C4B-null phenotype of HLA haplotype B44DRw6 C4A3 C4BQO is probably an example of C4A3 expression at both C4 loci (39), presumably arising by deletion and duplication. These observations indicate that studies of gene structure, expression, and regulation of C4A and C4B may be necessary to completely elucidate the mechanisms of specific C4A- and C4B-null states. In any event, the results of our study indicate that expression and regulation of C4 isotypes can be studied in primary cultures of monocytes from the appropriate subjects.

Results of the current study also indicate that the relationship between the C4 haplotype, plasma concentrations of C4, and autoimmune disease is even more complex than previously conceptualized. This relationship must now take into consideration the structure of C4, specific C4 isotypes, hepatic and extrahepatic synthesis of C4, positive and negative regulation of synthesis, functional activity, and, perhaps, clearance/catabolism of C4.

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