A Cytokine-selective Defect in Interleukin-1β-mediated Acute Phase Gene Expression in a Subclone of the Human Hepatoma Cell Line (HEPG2)*

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Several well-differentiated human hepatoma cell lines (HepG2, Hep3B) have been used to identify factors which regulate hepatic gene expression during the host response to inflammation/tissue injury (acute phase response). Studies in these cell lines, as well as in primary cultures of rat, rabbit, and mouse hepatocytes, have demonstrated that interleukin-1β (IL-1β), tumor necrosis factor (TNF-α), and interferon-β (IFN-β2) each mediate changes in expression of several hepatic acute phase genes. In this study we identify a subclone of the HepG2 cell line in which there is a selective defect in IL-1β-mediated acute phase gene expression. Recombinant human IL-1β mediates an increase in synthesis of the positive acute phase complement protein factor B and a decrease in synthesis of negative acute phase protein albumin in the parent uncloned HepG2 cell line (HG2Y), but not in the subclone HG2N. Recombinant human IFN-β2 and TNF-α, however, regulate acute phase protein synthesis in the subclone HG2N; i.e., IFN-β2 and TNF-α increase synthesis of factor B and decrease synthesis of albumin in both HG2Y and HG2N cells. Equilibrium binding analysis with [125I]-rIL-1β at 4°C showed that both HG2N and HG2Y cells bind IL-1β specifically and saturably. HG2N and HG2Y possess 3.8 and 4.0 x 10^7 plasma membrane receptors/cell with affinities of 0.96 and 1.07 x 10^7 M, respectively. Thus, the defect in this subclone of the HepG2 cell line is likely to involve the signal transduction pathway for the biological activity of IL-1β and will be useful in elucidation of this signal transduction pathway.

The host response to inflammation or tissue injury is characterized by a coordinated series of metabolic reactions that constitute the acute phase response. These reactions include fever, muscle proteolysis, leukocytosis, alteration in fat, carbohydrate and trace mineral metabolism, and marked changes in plasma concentrations of many liver-derived glycoproteins known as the acute phase plasma proteins, or acute phase reactants. Plasma concentrations of some acute phase reactants increase (C-reactive proteins, serum amyloid A, fibrinogen, α1-antitrypsin, complement proteins factor B and C3) while others decrease (albumin, transferrin). The changes in absolute concentration are primarily due to changes in synthetic rate, rather than in rate of catabolism. Thus, this acute phase response involves a sequential and orderly activation of multiple genes.

Due to limitations in the availability and long-term cultivation of human hepatocytes, and ethical limitations in studies of humans in vivo, it has been difficult to specifically study the response of human liver during acute inflammation. However, the acute phase response has been recently studied in human hepatoma cell lines (HepG2, Hep3B) using well-defined and highly purified mediators. These studies show that changes in expression of hepatic genes characteristic of the acute phase response may be elicited in these hepatoma cell lines by recombinant human IL-1α,1 (1–3), TNF-α, (2, 3) IFN-β2 (4–7), and IFN-γ (8). IL-1β, TNF-α, IFN-β2 also mediate hepatic acute phase gene expression in primary hepatocyte cultures from mouse and rat (5, 9, 10). Several hepatic acute phase genes are selectively affected by one of these cytokines; e.g., fibrinogen and α1-antitrypsin are only affected by IFN-β2 (2, 4, 7). Other hepatic acute phase genes are affected by different cytokines; e.g. albumin expression decreases in the presence of IL-1β, TNF-α, or IFN-β2. It is now known that IL-1β, TNF-α, and IFN-β2 each bind to unique cell surface receptors (11–14). Thus, the possibility that overlapping signal transduction mechanisms are triggered by these cytokines, once engaged by receptor, needs to be examined. In this study we identify a subclone of one of the human hepatoma cell lines which is defective in the signal transduction pathway for IL-1β, but not for IFN-β2, TNF-α, of IFN-γ. This subclone will therefore facilitate further delineation of signal transduction mechanisms for hepatic acute phase gene expression.

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1 The abbreviations used are: IL, interleukin; TNF-α, tumor necrosis factor-α; INF, interferon; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; Hpes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DMEM, Dulbecco's modified Eagle's medium.
EXPERIMENTAL PROCEDURES

Materials

DMEM and MEM lacking methionine were purchased from Gibco. Hank's balanced salt solution and medium 199 (M199) were purchased from Microbiological Associates, Walkersville, MD. Fetal calf serum, L-glutamine, and penicillin-streptomycin were from Flow Laboratories, Inc., McLean, VA. [35S]Methionine was purchased from ICN Radiochemicals; Bolton-Hunter reagent, [32P]deoxyctydine triphosphate (specific radioactivity ~3000 Ci/mmol), and [3H]-methylated protein standards were obtained from Amersham Corp., Arlington Heights, IL. Other reagents included IgG-Sorb from Enzyme Technology, Pine Brook, NJ. Preparation of Escherichia coli-derived human IFN-β2 and rabbit polyclonal antiserum to E. coli-derived human IFN-β2 has been previously described (15). Recombinant human TNF-α was obtained from Genentech, South San Francisco, CA, and recombinant human IFN-γ was obtained from Biogen, Boston, MA.

Methods

Cell Culture—HepG2 and Hep3B cells, originally provided by Drs. B. B. Knowles and D. P. Aden (Wistar Institute, Philadelphia, PA) (16), were maintained in culture as previously described (17). The a16 subclone of HepG2 was generated from the parent cell line independently by limiting dilution (18). It has been maintained in culture for 7 years. The a16 subclone is referred to in this paper as HG2N to distinguish it from the parent uncloned cell line HG2Y.

Detailed chromosome analysis was made on the parent HepG2 cell line (19) and on the HG2N subclone in 1982 by Dr. Barbara B. Knowles and during these studies by Dr. Michael Watson (Washington University School of Medicine, St. Louis, MO). Techniques for these analyses have been described (19). These analyses show that there are no discernible karyotypic differences between the parent and subclone cell lines. The parent HepG2 cell line is characterized by trisomy or tetrasomy of chromosome 2. The only difference in subclone HG2N is that a higher proportion of cells exhibit trisomy of chromosome 2, leading to a median number of chromosomes of 52 as compared to 54 in the parent cell line. The karyotypes of the parent and subclone cell lines have not changed over 7 years.

Metabolic Labeling—Confluent monolayers were rinsed and incubated at 37 °C in the presence of methionine-free medium containing [35S]methionine, 250 μCi/ml (pulse period). To determine the net synthesis of factor B or albumin, cells were subjected to a short pulse interval (20 min), and radiolabeled proteins were detected in the cell lysate alone. Methods for solubilization of cells and clarification of cell lysates after labeling have been described (17). Total protein synthesis was estimated by trichloroacetic acid precipitation of aliquots of cell lysates (20).

Immunoprecipitation and Analytical Gel Electrophoresis—Aliquots of cell lysate or medium were incubated overnight at 4 °C in 1% Triton X-100, 1.0% SDS, 0.5% 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 9.0% SDS-PAGE under reducing conditions (21). [3H]-Methylated molecular size markers (200,000; 95,500; 68,000; 46,000; 30,000; and 17,000) were included on all gels. After electrophoresis, gels were stained in Coomassie Brilliant Blue, destained, impregnated with 2,5-diphenyloxazole (ENHANCE, Du Pont-New England Nuclear), and dried for fluorography on XAR x-ray film (Eastman Kodak Co., Rochester, NY). Laser densitometer 2222 ultrascan XL from LKB Instruments, Inc., Houston, TX, was used for scanning of fluorograms.

Detection of RNA by RNA Blot Analysis—Total cellular RNA was isolated from adherent monolayers of hepatoma cells by guanidine isothiocyanate from Fluka AG, Buchs, Switzerland. Recombinant human IL-1β was kindly provided by Dr. Bill Joy (Monsanto, St. Louis, MO), and polyclonal antiserum to recombinant human IL-1β was purchased from Cistron Center, Cambridge, MA, and guanidine isothiocyanate from Fluka AG, Buchs, Switzerland. Other reagents included IgG-Sorb from Enzyme Technology, Pine Brook, NJ. Preparation of Escherichia coli-derived human IFN-β2 and rabbit polyclonal antiserum to E. coli-derived human IFN-β2 has been previously described (15). Recombinant human TNF-α was obtained from Genentech, South San Francisco, CA, and recombinant human IFN-γ was obtained from Biogen, Boston, MA.

RESULTS

IL-1β and IFN-β2 Have Independent Regulatory Effects on Expression of the Factor B Gene in Hepatoma Cells—HG2Y cells were incubated with recombinant human IL-1β or IFN-β2 at submaximal concentrations (Fig. 1). Separate monolayers were incubated with each cytokine in the absence or presence of antibody to recombinant human IL-1β, IFN-β2, or Ig. Cell monolayers were radiolabeled with [35S]methionine for 20 min and newly synthesized factor B in cell lysates assayed by immunoprecipitation, SDS-PAGE followed by fluorography. IL-1β and IFN-β2 each mediate an increase in synthesis of factor B. The effect of IL-1β is completely blocked by antibody to IL-1β but not by anti-IFN-β2 or anti-Ig. The effect of IFN-β2 is only blocked by anti-IFN-β2 and not by anti-IL-1β or anti-Ig. Antibodies to IL-1β, IFN-β2, or Ig do not affect the constitutive synthesis of factor B. IL-1β and IFN-β2 also have independent effects on factor B synthesis in Hep3B cells (data not shown).

IL-1β Regulates Factor B and Albumin Gene Expression in HG2Y but Not in HG2N Cells—Separate monolayers of parent uncloned HG2Y cells and subclone HG2N cells were incubated in medium supplemented with IL-1β or IFN-β2 (Fig. 1).
FIG. 2. Effect of IL-1β and IFN-β2 on factor B (a) and albumin (b) synthesis in HG2Y and HG2N cells. Separate monolayers of hepatoma cells were incubated for 18 h in control medium, medium supplemented with IL-1β or medium supplemented with IFN-β2 in the specified concentrations. Cells were then rinsed thoroughly and pulse radiolabeled as described in the legend to Fig. 1. Clarified cell lysates were subjected to sequential immunoprecipitation with goat anti-human factor B and then goat anti-human albumin. Immunoprecipitates were subjected to SDS-PAGE followed by fluorography. Molecular mass markers are indicated at the right.

2). Each cytokine mediates a concentration-dependent increase in factor B synthesis in HG2Y cells (Fig. 2a, left panel). IFN-β2 also mediates a concentration-dependent increase in factor B synthesis in HG2N cells, but IL-1β has no effect on factor B in these cells (Fig. 2a, right panel). IL-1β and IFN-β2 each mediate a decrease in albumin synthesis in HG2Y cells (Fig. 2b, left panel). IFN-β2 also decreases albumin synthesis in HG2N cells but IL-1β is completely defective in down-regulation of albumin synthesis in this subclone (Fig. 2b, right panel).

The difference in response to IL-1β is not due to differences in kinetics as shown in the following experiment. HG2Y and HG2N cells were separately incubated with IL-1β (100 ng/ml) for 2, 5, 8, 16, and 24 h. Synthesis of factor B in HG2Y cells increases within 2 h, continues to increase from 8 to 16 h, and reaches a plateau between 16 and 24 h. Synthesis of factor B does not increase in HG2N cells at any interval up to 24 h (data not shown).

Although data in Fig. 2a shows a greater effect for IFN-β2 in HG2N cells than in HG2Y cells, data from five separate experiments in each cell line indicate that there is no significant difference in the effect of IFN-β2 on factor B synthesis in these cell lines. Densitometric scanning of fluorograms from these experiments demonstrate increases in factor B synthesis of 3.09 ± 0.98-fold in HG2Y cells and of 2.93 ± 0.99-fold in HG2N cells.

Similar results are observed in RNA blot analysis (Fig. 3). Steady-state levels of factor B mRNA increase in HG2Y cells incubated with IL-1β or IFN-β2 and in HG2N cells incubated with IFN-β2. However, there is no change in factor B mRNA levels in HG2N cells incubated with IL-1β.

TNF-α, another cytokine with positive regulatory effects on factor B synthesis and negative regulatory effects on albumin synthesis in uncloned HepG2 cells (3), is effective in regulating gene expression in HG2N cells. Recombinant TNF-α mediates a concentration-dependent decrease in synthesis of albumin (Fig. 4a) and an increase in synthesis of factor B (data not shown) in HG2N cells. Furthermore, the effect of IFN-γ on synthesis of C4 in uncloned HepG2 cells (8) is also evident in HG2N cells (Fig. 4b). These data indicate that the defect in IL-1β activity in HG2N cells is cytokine-specific.

One possible explanation for the absence of IL-1β biologic activity in HG2N cells is occupation of IL-1β receptors by endogenous IL-1β in this subclone. This possibility was excluded by subjecting lysates of radiolabeled HG2N cells to immunoprecipitation with anti-IL-1β (Fig. 5a). This antibody recognizes 31- and 27-kDa intracellular precursors of IL-1β and recognizes the trace amount of 17-kDa mature IL-1β present in lysates of lipopolysaccharide-activated monocytes (last lane). No IL-1β polypeptides are detected in HG2N cells, or in HG2Y cells, under control, IL-1β-, or IFN-β2-stimulated conditions.

FIG. 3. Effect of IL-1β and IFN-β2 on steady-state levels of factor B mRNA in HG2Y and HG2N cells. Hepatoma cells were incubated for 18 h in control medium or medium supplemented with IL-1β (100 ng/ml) or IFN-β2 (100 ng/ml). Total cellular RNA was then isolated in guanidine isothiocyanate, purified, and subjected to RNA blot analysis with radiolabeled factor B cDNA as probe. There was no difference in ethidium bromide-stained ribosomal RNA bands on these blots. 28 and 18 S ribosomal RNA markers are indicated at the right.
We also excluded the possibility that the biological activity of IL-1β in the parent uncloned HG2Y cells depends on the induction of endogenous IFN-β2. Lysates of radiolabeled HG2Y and HG2N cells were subjected to immunoprecipitation with anti-IFN-β2 (Fig. 5b). This antibody recognizes the five forms of IFN-β2 of apparent molecular mass in the range of 23–30 kDa in lysates of lipopolysaccharide-activated human monocytes (Fig. 5b, last lane, and Ref. 15). Within the limits of detection of this assay system, no IFN-β2 polypeptides are detected in HG2Y or HG2N cells under control, IL-1β-, or IFN-β2-stimulated conditions.

**HG2N and HG2Y Cells Bind Iodinated IL-1β**—In order to examine whether the defective response in HG2N cells is due to a deficiency in cell surface receptor for IL-1β, separate monolayers of HG2N and HG2Y cells were subjected to direct binding assays with radiolabeled IL-1β (Fig. 6). There is specific, saturable binding of IL-1β in each case. Scatchard plot analysis predicts 3.8 \times 10^3 and 4.0 \times 10^3 plasma membrane receptors/cell in HG2N and HG2Y cells, respectively. The k_d for IL-1β binding is 9.6 \times 10^{-9} M in HG2N and 1.07 \times 10^{-9} M in HG2Y cells.

**IL-1β and IFN-β2 Have an Additive Effect on Synthesis of Factor B** in Both HG2Y and HG2N Cells—Separate monolayers of HG2Y and HG2N cells were incubated for 18 h with IL-1β alone, IFN-β2 alone, or both IL-1β and IFN-β2 (Fig. 7). Synthesis of factor B was determined by the method described above and is shown graphically on the basis of densitometric scanning of fluorograms for four separate experiments. In these experiments, synthesis of factor B in HG2Y cells increases 2.31 ± 0.45-fold in the presence of IL-1β alone, 2.42 ± 0.67-fold in the presence of IFN-β2 alone, and 4.10 ± 1.39-fold in the presence of both cytokines. Synthesis of factor B in HG2N cells does not increase in the presence of IL-1β alone but increases 2.57 ± 0.41-fold in the presence of IFN-β2 alone and 5.20 ± 0.91-fold in the presence of both IL-1β and IFN-β2.

**DISCUSSION**

These data demonstrate a defect in the signal transduction pathway for biological activity of IL-1β in a subclone of the human hepatoma cell line HepG2 (HG2N). Previous studies have shown that IL-1β mediates a specific increase in expression of the factor B gene and a decrease in expression of...
including one responsible for its discrete action and one  

eral different signal transduction pathways in HG2Y cells  

that the effect of IFN-P2 is permissive for transduction of IL- 

cells even though IL-1p has no effect by itself. This may mean  

binding of IL-1 with a  

duction pathway for the discrete biological activity of IL-lp.  

have a similar additive effect on factor B synthesis in HG2N  

effect on factor B synthesis in HG2Y cells; IL-1P and IFN-P2  

B synthesis in HG2N cells: IL-lp and IFN-P2 have an additive  

when together with IFN-@2, is capable of modulating factor  

possibility that IL-1p binding to HG2N cells is defective.  

several examples of this type of receptor defect have been  

results in defective cellular responses despite normal levels of  

receptor binding (33, 34). Nevertheless, any step within the  

signal transduction pathway distal to receptor occupancy  

could be affected in the HG2N cell line. It has been shown that  

IL-1β does not stimulate phosphatidylinositol turnover or  

increases in cytosolic-free calcium concentration in T- 

lymphocytes (35), but there is little additional data on signal  

transduction mechanisms for its diverse biological activities.  

Although there are no major differences between the two cell  

lines by cytogenetic analysis (see "Methods"), we cannot  

exclude the possibility of one or more subtle genetic  

differences. The control experiments emphasize, however, that  

such differences are specific for the transduction pathway  

activated by IL-1β, one of a group of cytokines which have been  

shown to modulate acute phase gene expression in human hepatoma  

cells. Comparisons of the HG2N subclone and HG2Y un-  

cloned parental cell line will therefore provide an opportunity  

for identification of the mechanisms and mechanisms necessary  

for IL-1β activity in liver cells.  

Results of these experiments also indicate that IL-1β, IFN- 

β2, and TNF-α have direct and independent effects on acute  

phase proteins, factor B and albumin. Expression of other  

acute phase proteins in HepG2 cells, such as fibrinogen (6)  

and α1-antitrypsin (9), is regulated by IFN-β2 but not by IL- 

β or TNF-α. Still another hepatic acute phase gene, comple-  

ment protein C4, is regulated only by IFN-γ (10). Initial  

results suggest that a combination of IL-1β and IFN-β2 may  

be necessary to induce expression of C-reactive protein and  

serum amyloid A in human hepatoma cell lines (6). These  

data, together with the implication from the current study,  

indicate that discrete and overlapping signal transduction  

pathways may be elicited by individual cytokines.  

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Fig. 7. IL-1β and IFN-β2 have an additive effect on synthesis  

of factor B in HG2Y and HG2N cells. Hepatoma cells were  

incubated for 18 h in serum-free control medium, medium  

supplemented with IL-1β alone, IFN-β2 alone, or both (100 ng/ml). Cells  

were then rinsed thoroughly, pulse radiolabeled, and cell lysates  

subjected to the analytical system described above. Fluorograms from  
four separate experiments were subjected to densitometric scanning  
to determine fold increase over control. Hatched bars represent mean  

values and error bars represent 1 standard deviation.
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