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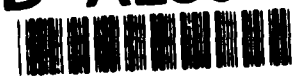

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INCREASED FIBRINOGEN SYNTHESIS IN MICE DURING THE ACUTE PHASE RESPONSE: CO-OPERATIVE INTERACTION OF INTERLEUKIN 1, INTERLEUKIN 6, AND INTERLEUKIN 1 RECEPTOR ANTAGONIST

Hanna Rokita,* Ruta Neta,† Jean D. Sipe‡

Interleukin 6 (IL-6) stimulates fibrinogen (Fg) gene expression both in vivo and in vitro; while interleukin 1 (IL-1) paradoxically stimulates in vivo, yet inhibits in vitro, Fg synthesis. The naturally occurring interleukin 1 receptor antagonist (IL-1ra) and passive immunization with anti-IL-6 antiserum were used to study the in vivo mechanism of action of IL-1 on Fg gene expression. Changes in plasma Fg and hepatic Fg mRNA concentrations were measured following administration of exogenous IL-1ra together with IL-6 or IL-1 to CD2F1 mice. Our results suggest that in vivo, IL-1 per se inhibits Fg production since when IL-1ra was co-administered with IL-6, greater concentrations of Fg were observed than when IL-6 was administered alone. The data suggest that IL-1 stimulates Fg production through intermediate production of IL-6, since stimulation was abrogated when either IL-1ra or anti-IL-6 antiserum was co-administered with IL-1. An in vivo role for IL-1ra in the stimulation of Fg by IL-1 was supported by the observation that within 1 h of IL-1 administration to mice, IL-1ra mRNA was detectable in liver. It appears that IL-1, an early mediator of inflammation, inhibits constitutive expression of Fg genes and stimulates the IL-1ra and IL-6 genes. The inhibitory effect of IL-1 is reversed by endogenous IL-1ra and by the direct stimulation of Fg gene expression by IL-6.

Changes in hepatic protein synthesis are a major manifestation of the acute phase response and are mainly regulated by the mononuclear phagocyte system through secretion of cytokines such as interleukin 6 (IL-6) and interleukin 1 (IL-1).[1-6] IL-6 and, to a lesser extent, IL-1 have been implicated as modulators of hepatic serum amyloid A (SAA) and fibrinogen (Fg) mRNA and/or protein synthesis in humans and mice.[4-7] Treatment of mice with endotoxin and the two lipopolysaccharide-induced cytokines, IL-1 and TNF, caused an increase in plasma Fg concentration.[8] IL-1 is an early and pluripotent mediator of the acute phase response, with capacity to induce itself, IL-6 and a structurally related receptor ligand with antagonist activity, termed IL-1 receptor antagonist (IL-1ra). Recently, recombinant generated preparations of IL-1ra, a 17 kDa protein with 26–30% homology to IL-1 beta and 19% to IL-1 alpha have been used to distinguish physiological roles of IL-1 from closely related cytokines such as IL-6.[9-17] Since several in vitro studies[5,18-21] indicate that constitutive and IL-6 stimulated Fg gene expression in hepatoma cell lines is inhibited by IL-1, the present study was undertaken to investigate the basis of the in vivo stimulatory effect of IL-1 on Fg gene expression.

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RESULTS

IL-1ra Inhibits IL-1, but Enhances IL-6, Stimulation of Plasma Fibrinogen

In vivo administration of exogenous cytokines demonstrates biological activity and permits com-

parison of dose response and sensitivity to individual cytokines. Although IL-1 alpha and IL-6 both stimulated a concentration-related elevation in Fg, IL-1 was more effective on a weight basis than IL-6 (Table 1).

TABLE 1. Stimulation of in vivo fibrinogen production by cytokines.

	Treatment			Fibrinogen (% control)
	IL-1 (ng)	IL-6 (μg)	IL-1ra (μg)	
1.	–	–	–	100
2.	10		–	147 ± 15
3.	300	–	–	264 ± 5
4.	–	0.25	–	146
5.	–	1.25	–	157 ± 20
6.	300	–	100	115 ± 15
7.	–	–	75	110
8.	–	1.25	10	167 ± 7
9.	–	1.25	75	194 ± 7
10.	–	1.25	100	213 ± 23

Mice were bled 24 h after injections. Values are means and standard errors of two to three experiments, each three or four mice per group. Exceptions are numbers 4 and 7 where data from one experiment were available.

The stimulatory effect of IL-1 on Fg production was blocked by the co-administration of IL-1ra with IL-1. In contrast, the stimulation of Fg by IL-6 was enhanced by co-administration of IL-1ra and IL-6.

Endogenous IL-1ra Production in Response to IL-1 but Not IL-6

Endogenous expression of IL-1ra mRNA in liver was detectable 1 and 1.5 h after administration of IL-1 alone and in combination with IL-6, but not after IL-6 alone (Fig. 1). The polyadenylated RNA enriched

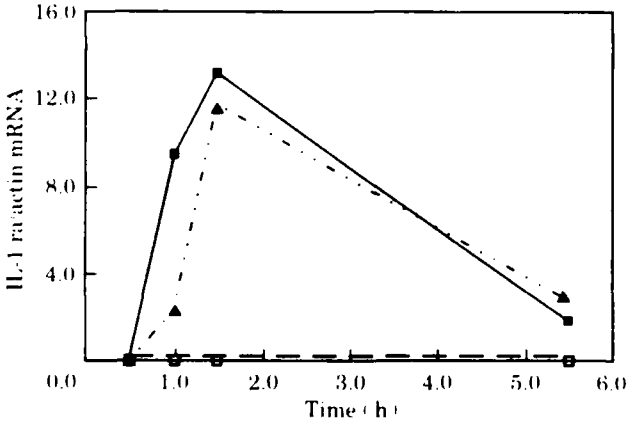

Figure 1. Kinetics of IL-1ra mRNA, relative to beta-actin mRNA in livers of mice.

Results are given 0, 1, 1.5 and 5 h after administration of IL-1 (- - —▲— - -; 300 ng), IL-6 (— -□- —; 1.25 μg) alone or in combination (—■—) to mice. Northern blots were prepared from mouse liver RNA enriched in polyadenylated mRNA, 3–5 μg/lane. Results are representative of four experiments.

fractions contained varying amounts of ribosomal RNA, leading to fluctuation in the relative abundance of beta-actin mRNA. Therefore the results shown in Fig. 1 are normalized for the content of beta actin in each preparation; the quantities of beta-actin and GAPDH mRNA were comparable. IL-1ra mRNA was not detectable at 30 min, appeared to reach maximal levels at 2–3 h and was expressed at a diminished level by 5.5 h after IL-1 administration.

IL-1 Stimulates Fg and SAA Production by Induction of IL-6

IL-6 has been shown to mediate other in vivo responses to IL-1;[22,23] the role of IL-6 in IL-1 stimulated Fg gene expression was indicated by the reduction in stimulation of Fg after administration of 20 ng IL-1 to mice passively immunized with anti-IL-6 antiserum 16 h earlier (Table 2) as compared with Fg

TABLE 2. Passive immunization with anti-IL-6 antibodies blocks stimulation of fibrinogen by IL-1.

	Fibrinogen		
Pretreatment	Protein (% control)	mRNA (Fg/Actin mRNA)	SAA (μg/ml)
None	176 ± 15	1.0	248 ± 25
Anti-IL-6	118 ± 17	0.6	93 ± 4
Rat Ig Control	310 ± 15	nd	196 ± 2
G113, Ab Control	182 ± 9	1.2	220 ± 16

Each of three mice per group were injected with 600 μg of each Ig 16–20 h prior to stimulation with 20 ng of IL-1. Values are mean and standard errors of individual plasma samples obtained 24 h after administration of IL-1. nd = not done. Results are representative of two to four experiments.

concentration in mice that received no pretreatment or received control immunoglobulins. The ratio of hepatic Fg mRNA to hepatic actin mRNA 1 h after administration of IL-1 to mice pretreated with anti-IL-6 antibodies was 50% of that in mice treated with control immunoglobulins or in mice with no pretreatment (Fig. 2). The in vivo production of IL-6 following IL-1 was further demonstrated by reduction in plasma SAA (Table 2) which is known to be produced in response to the synergistic action of IL-1 and IL-6.[24] We used a hybridization probe for gamma-Fg in these studies; expression of alpha, beta and gamma chains has been shown to proceed essentially coordinately[25] and in our study hybridization with either gamma-Fg or alpha-Fg specific cDNA probes revealed similar kinetics (Figs 1, 2).

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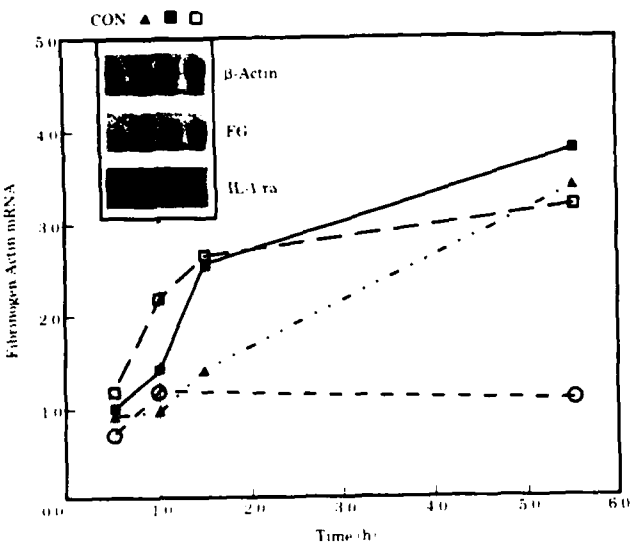

Figure 2. Kinetics of Fg and IL-1ra (mRNA, relative to beta-actin mRNA in livers of mice.

Results are given 0, 1, 1.5 and 5 h after administration of IL-1 (20 ng), IL-6 (1.25 μg) alone and in combination to mice that received anti-IL-6 antibodies (600 μg), control immunoglobulins (600 μg) or no treatment 16 h earlier. - -—▲—- -, IL-1; — -+— -, IL-6; —■—, IL-1 + IL-6; — -+— -, IL-1 + anti IL-6. Northern blots were prepared from mouse liver RNA enriched in polyadenylated mRNA. 3–5 μg/lane. Insert shows the Northern hybridization signal for the 1 h time point. Results are representative of four experiments.

DISCUSSION

This study has addressed the conflicting observations that IL-1 suppresses Fg gene expression in hepatoma cell lines, yet enhances Fg gene expression in mice.[1,2,5] The basis for increased concentrations of plasma Fg during the acute phase response, has, for the first time, been investigated by combining an analysis of plasma protein changes with changes in mRNA expression in liver. Using IL-1ra together with IL-6 we can confirm in the in vivo situation the previously reported in vitro inhibition of Fg gene expression by IL-1. Our results also provide strong evidence that, in vivo, IL-1 counteracts its direct inhibitory effect on Fg gene expression by stimulation of IL-1ra and IL-6 gene expression. In turn, IL-6 directly stimulates Fg gene expression and IL-1ra blocks inhibition of Fg gene expression by IL-1 leading to increased Fg production in the presence of IL-6.

The observation of IL-1ra mRNA expression in mice following IL-1 but not IL-6 administration (Fig. 2) suggests that IL-1ra exerts both temporal and bidirectional quantitative regulation of acute phase changes in liver protein synthesis by counteracting both the synergistic interaction of IL-1 with IL-6 required for SAA induction and its antagonistic effect on Fg induction by IL-6.[5,18–22,24] IL-1 has been shown to influence the effects of IL-6 on plasma proteins other than Fg. For example, IL-1 acts synergistically with IL-6 to enhance SAA gene expression by Hep 3B and Hep G2 cells.[24] IL-1 has been shown to decrease the stimulatory effect on IL-6 on alpha-2-macroglobulin as well as Fg in Hep G2 cells.[5] These reports together with our findings in this paper indicate that IL-1 and IL-6 interactions may be one of the factors that contribute to the unique kinetic changes that individual acute phase reactants undergo during the acute phase response.

The mechanism by which IL-1 reduces constitutive Fg production and IL-6 induced Fg gene upregulation is not understood. The Fg genes are expressed constitutively in liver and are further stimulated by dexamethasone and IL-6 and indirectly by IL-1 and TNF.[1,3,22,26] The studies of Huber and coworkers demonstrated the interplay between IL-6 and glucocorticoid responsive elements in the promoter region of Fg genes.[26] Fluctuation in receptor ratios and/or occupancy may play a role in the inhibitory effect of IL-1 on Fg gene expression. The recent studies of Nesbitt and Fuller[27] indicate that IL-1 and, to a lesser extent, IL-6, may elicit a transient reduction in the number of IL-6 receptors in rat hepatocytes, whereas IL-6 and IL-1 increased IL-6 receptor mRNA in human primary hepatocytes.[28] Other explanations may lie in the selective interaction of transcription factors. Thus, while our study has resolved the paradox of the conflicting in vivo and in vitro effects of IL-1 on Fg gene expression, the question as to how IL-1 down-regulates Fg remains.

MATERIALS AND METHODS

Experimental Animals

Female CD2F1-mice, 6–8 weeks of age, free from overt disease, were employed. All animal handling procedures were performed as described[29] in compliance with guidelines from the National Research Council and the Armed Forces Radiobiology Research Institute. At various time points, citrated plasma was collected and livers were removed and snap-frozen in liquid nitrogen and stored at −85°C for subsequent mRNA analysis.

Reagents

Recombinant human IL-1 alpha was the gift of Dr Peter Lomedico of Hoffman-LaRoche, Nutley, NJ. The preparation, lot IL-1 2/88 was stored in aliquots at −20°C until use. Recombinant human interleukin 6, lot PPG9001 was provided by Dr E. Liehl, Sandoz Pharme AG, Basel.

Switzerland. The IL-1ra was provided by Dr Robert Thompson, Synergen, Boulder, CO. Rat monoclonal antibody to recombinant mouse IL-6 (MP5 20F3) was prepared using partially purified Cos-7 mouse IL-6 as immunogen (22). Rat monoclonal antibody to beta-galactosidase was used as an isotype control.[23]

Protein Assays

Fibrinogen in citrated plasma was measured as the rate of conversion of fibrinogen to fibrin in the presence of excess thrombin using the Sigma Diagnostic Kit (Sigma Chemical Co., St Louis, MO) as calibrator to express the data as milligrams of fibrinogen per 100 ml plasma. Measurements of fibrin clot formation were performed on a fibrometer (Becton-Dickinson Co., Fairleigh, N.J.). Mouse SAA was measured by ELISA (Hemagen Diagnostics, Waltham, MA).

RNA Extraction and Northern Blot Hybridization Analysis

Polyadenylated RNA was extracted from individual and pooled livers using Mini RiboSep mRNA isolation kits (Collaborative Research, Bedford, MA) according to the manufacturer's instructions. RNA was denatured by glyoxylation and size-fractionated by electrophoresis through 1.5% agarose, and transferred to Genescreen Plus nylon membranes (Dupont NEN, Boston, MA). The quantity of IL-1ra,[16] fibrinogen,[30] actin[31] and GAPDH[32] mRNA in each lane was determined by hybridization with the corresponding cDNA probes labelled with alpha-32P-dCTP (Dupont NEN, Boston, MA) using a random priming oligonucleotide labelling kit (Bethesda Research Laboratories, Gaithersburg, MD). Unincorporated cCTP was removed from the reaction mixture through the use of Nensorb columns (Dupont NEN). Following overnight hybridization at 42°C, filters were washed as previously described[33] and exposed to X-ray film at −70°C for 2–24 h. The relative abundance of SAA mRNA was measured relative to beta actin by scanning densitometry of autoradiographs following Northern blot hybridization.

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