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Voisin, Laure, Denis Breuillé, Benoît Ruot, Cécile Rallière, Fabienne Rambourdin, Michel Dalle, and Christiane Obled. Cytokine modulation by PX differently affects specific acute phase proteins during sepsis in rats. *Am. J. Physiol.* 275 (*Regulatory Integrative Comp. Physiol.* 44): R1412–R1419, 1998.—To explore the regulation of the acute phase response in vivo, the effects of pentoxifylline (PX) treatment (100 mg/kg ip 1 h before infection) were investigated in infected and pair-fed rats 2 and 6 days after an intravenous injection of live bacteria (*Escherichia coli*). PX treatment prevented the increase in plasma tumor necrosis factor (TNF)-α (peak 1.5 h after the infection) and resulted in an 84 and 61% inhibition of plasma interleukin (IL)-1β and IL-6, respectively (peaks at 3 h). Plasma corticosterone kinetics were not modified by the treatment. Infection increased α_1-acid glycoprotein (AGP), α_2-macroglobulin (A2M), and fibrinogen plasma concentrations and decreased albumin levels. PX significantly reduced AGP plasma concentration as early as *day 2* in infected animals but reduced A2M and fibrinogen plasma levels only at *day 6*. The treatment had no effect on the albumin plasma concentration. Hepatic AGP and fibrinogen mRNA levels increased in infected rats, whereas those of A2M were unchanged and those of albumin were decreased. Two days after infection, AGP and fibrinogen mRNA levels were reduced in treated infected animals. PX was ineffective in modifying those of A2M and albumin. These data demonstrate, in vivo, that different acute phase proteins are individually regulated in sepsis. The in vivo effects of PX treatment support the hypothesis that TNF-α plays an important role in the regulation of AGP production, whereas other factors seem to be involved in the regulation of A2M, fibrinogen, and albumin expression.

tumor necrosis factor; interleukin-1; interleukin-6

AMONG OTHER METABOLIC disturbances, sepsis causes a marked loss of weight and body proteins, muscle wasting, and a hepatic acute phase response. Total liver protein synthesis was markedly enhanced (11, 44), mainly due to an increased synthesis of exported proteins (acute phase proteins; see Ref. 44). Thus the liver response results in a concomitant increase in the circulating levels of these proteins. However, the levels of some plasma proteins such as albumin, named negative acute phase proteins, decrease (33, 42).

Cytokines, and especially tumor necrosis factor (TNF)-α (43) and interleukins-1 (IL-1; see Ref. 35) and 6 (IL-6; see Ref. 6), are considered to play key roles in the pathogenesis of sepsis. With respect to liver, administration of cytokines to healthy animals can reproduce the stimulation of protein synthesis (5, 13). The roles of cytokines in inducing individual acute phase protein changes have been studied extensively in various in vitro systems as hepatocyte primary cultures or hepatoma cell lines. Based on these studies, IL-6 appears to be the main cytokine regulating the expression of the majority of the acute phase protein genes, whereas IL-1β and TNF-α regulate a different set of genes (17, 29, 33). Maximal expression of several acute phase protein genes is dependent on the presence of glucocorticoids (29). However, for a particular protein, different responses can be obtained in various cell systems (29), and the regulatory processes involved in the in vivo acute phase response might be much more complex. Some studies have been reported in which the role of IL-1 (28, 37) and IL-6 (20, 31) on the acute phase induction was studied in vivo. However, a major difficulty in defining the roles of various cytokines in vivo is their ability to induce each other (35, 43). Another approach, poorly documented in sepsis, consists of inhibiting the production or action of individual cytokines (21, 32, 42).

TNF-α is the first cytokine to appear in the circulation after administration of endotoxin or living bacteria in various species (43). Thus TNF-α is thought to be a proximal mediator of the inflammatory response and most likely triggers the release of other secondary mediators, including other cytokines. Pentoxifylline (PX), a methylxanthine derivative, has been demonstrated both in vitro and in vivo to suppress lipopolysaccharide (LPS)-induced TNF-α secretion (12, 30, 39, 41). PX may also modulate other cytokines, but this effect is more controversial (30, 39, 41), and its effect on glucocorticoid level is unknown. Thus, in this study, we explored in vivo the effect of PX treatment on TNF-α, IL-1, IL-6, and corticosterone levels in a rat model of gram-negative sepsis. Furthermore, we examined whether inhibition of TNF-α production could modulate the expression and plasma appearance of individual acute phase proteins during the acute septic phase (2 days postinfection) and the chronic septic phase (6 days postinfection).

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (Iffa Credo, Saint-Germain sur l'Arbresle, France) were individually housed in

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wire-bottom cages in a temperature-controlled room (22–23°C) with a 12:12-h light-dark cycle. During a 6-day acclimatization period, all rats received a semisynthetic diet containing 12% protein distributed by an automatic device (12). Animals were weighed every morning, and food intake was measured every day.

At an initial body weight of 300 g, rats were divided into the following four groups: infected rats (INF) and their pair-fed control (PF) and infected rats treated with PX (PX-INF) and their pair-fed controls treated with PX (PX-PF). The INF group received saline intraperitoneally 1 h before injection of *Escherichia coli* (serotype 0153-K^--H^-; 7×10^8 colony-forming units) into a lateral tail vein. Control animals of the INF group (PF) received saline intraperitoneally 1 h before an intravenous saline injection; because infection dramatically decreases food intake (11), the animals were pair fed to the intake of infected rats. In the PX-INF group, PX (100 mg/kg) was injected intraperitoneally 1 h before bacteria administration. Control animals of the PX-INF group (PX-PF) received an equal volume of PX injected intraperitoneally 1 h before saline intravenous injection. Because PX treatment increases voluntary food consumption in infected rats, this control group (PX-PF) was pair fed to PX-INF animals. Pair feeding was conducted as previously described (11). Animals were weighed every day until the completion of the study. Animals of each group were studied at *days 2* and *6* after the infection, which represents, respectively, the acute and chronic septic phases previously described (11). In infected rats, the mortality was 7%, and there was no mortality in infected rats pretreated with PX. After anesthesia with pentobarbital sodium (6.0 mg/100 g body wt), gastrocnemius and soleus muscles were dissected and weighed. Blood samples were collected into EDTA tubes, and plasma was stored at −20°C for acute phase protein assays. Liver samples were taken by freeze clamping and kept at −80°C until analysis. The protocol was approved by the Ethics Committee of the Institute National de la Recherche Agronomique and was conducted in conformity with the guiding principles in the care and use of laboratory animals.

TNF-α-, IL-1β-, and IL-6-like bioactivity and corticosterone assays. A preliminary kinetic study of TNF-α, IL-1β, and IL-6 production in plasma from 1 to 24 h after the infection showed that maximal plasma levels occurred at 1.5 h for TNF-α and 3 h for IL-1β and IL-6 after bacteria administration (Fig. 1). Thus blood samples were collected in a lateral tail vein 1.5 and 3 h after the infection in heparinized tubes, and plasma was stored at −80°C for TNF-α and IL-1β and at −20°C for IL-6 assays. TNF-α and IL-1 plasma concentrations were measured by using ELISA kits, according to the manufacturer's instructions (Genzyme, Cambridge, MA, and Amersham, Bucks, UK, respectively). Biological activity of IL-6 was estimated in a bioassay using the B-9 hybridoma cell line (1). Briefly, B-9 cells (5,000/100 μl) were cultured in 96-well microtiter plates with serial dilutions of test samples (22). The IL-6 standard was human recombinant IL-6 (no. 89/548; National Institute for Biological Standards and Control, Hertfordshire, UK), which was serially diluted. After 48 h of incubation at 37°C with 5% CO_2, 20 μl of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (2 mg/ml) was added to each well in the presence of phenazine methosulfate and incubated for an additional 2 h to determine cell proliferation (16). The water-soluble formazan product was quantitated at 490 nm in an MR 700 microplate reader (Dynatech Laboratories, Guernsey, UK). Plasma corticosterone concentrations were assayed by RIA as described by Pradier and Dalle (34).

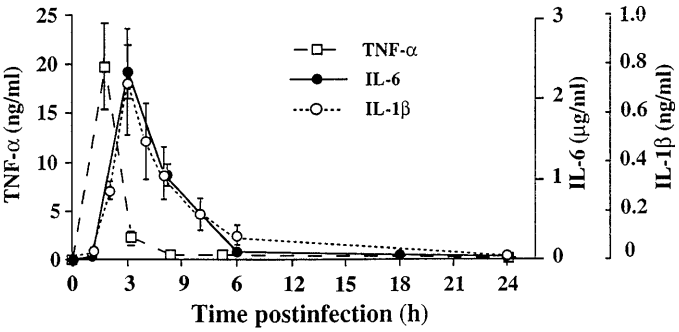

Fig. 1. Time course of plasma tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 concentrations within 24 h after infection. TNF-α and IL-1β were measured with the use of ELISA kits. Biological activity of IL-6 was estimated in a bioassay using the B-9 hybridoma cell line. Values are means ± SE for 6–8 animals.

Acute phase protein concentration. Fibrinogen, α_1-acid glycoprotein (AGP), α_2-macroglobulin (A2M), and albumin plasma levels were measured by single radial diffusion, using anti-rat fibrinogen and albumin from Cappel and anti-rat AGP and A2M produced in the laboratory.

Northern blot analysis. Total RNA was extracted from 0.2 g of liver by the method of Chomczynsky and Sacchi (14). Twenty micrograms of RNA were electrophoresed in formaldehyde agarose gels (1%) and transferred electrophoretically to nylon membranes (GeneScreen; NEN Research Products, Boston, MA). RNA was covalently bound to the membrane by ultraviolet cross-linking. Membranes were hybridized with cDNA probes AGP (36), A2M (no. 63099; American Type Culture Collection, Rockville, MD; see Ref. 19), α-fibrinogen (7), and albumin (25). Hybridizations were conducted overnight at 65°C with [^{32}P]cDNA fragments labeled by random priming. After washing at the same temperature, filters were autoradiographed at −80°C with intensifying screens on Hyperfilm-MP (Amersham). After stripping of the different probes, the filters were reprobed with a mouse 18S ribosomal probe (no. 63178; American Type Culture Collection). Autoradiographic signals were quantified by digital image processing and analysis (NIH Image 1.54) and normalized using the corresponding 18S rRNA signals to correct for uneven loading.

Statistics. All data are expressed as means ± SE. The significance of differences was analyzed by one-way ANOVA and by Student's *t*-test where appropriate. Differences among means were considered significant at $P < 0.05$.

RESULTS

Food intake, body weight, and muscle weight. Results presented in Figs. 2 and 3 show values obtained on rats studied for 6 days after the infection. Similar results were observed during the acute period in the group killed at *day 2*. Infection decreased food intake, especially during the acute phase period since rats ate only 5–15% of the preinfection intake (20–25 g). Thereafter, food intake of infected animals gradually increased to reach 75% of preinfection food consumption at the end of the study. Pretreatment of animals with PX before infection reduced anorexia, mainly at *days 2* to *4* after infection compared with untreated rats (Fig. 2). On *day 2* postinfection, the decrease of body weight observed in infected rats (INF) was significantly higher (27.5%) than in pair-fed animals (PF; Fig. 3), and the difference between these two groups strongly increased until 6 days after infection. By contrast, the body weight loss of

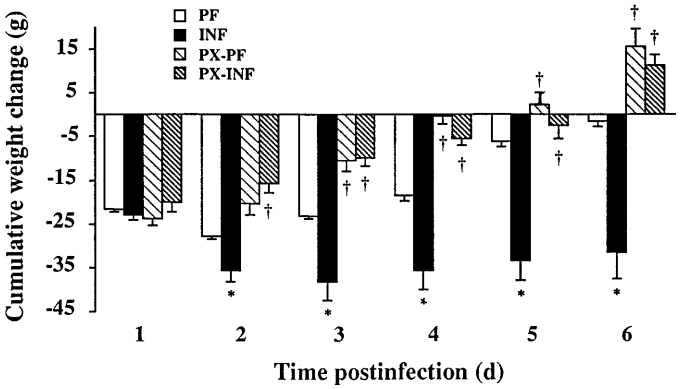

Fig. 2. Effect of pentoxifylline (PX) treatment on daily food intake within 6 days postinfection. Values are means ± SE for 6–11 animals. INF, infected rats; PF, pair-fed controls of INF rats; PX-INF, infected rats treated with PX; PX-PF, pair-fed controls treated with PX of PX-INF. *$P < 0.05$ vs. respective PF animals; d, days. †$P < 0.05$ vs. respective animals without PX treatment.

septic rats treated with PX (PX-INF) was always similar to that of their pair-fed controls (PX-PF). Moreover, PX-INF rats began to gain weight on *day 3* although INF rats continued to lose weight. Thus, 6 days after infection, septic rats had lost ~32 g, and infected rats treated with PX had regained ~10 g body weight compared with their initial body weight.

During the acute phase, the weight of the various muscles studied was significantly reduced in infected animals compared with control rats (15 and 13% for gastrocnemius and soleus, respectively; Table 1). In the same septic phase, similar decreases were observed for infected animals treated with PX compared with their pair-fed controls (~13%). Six days postinfection, the observed atrophy of gastrocnemius and soleus muscles in INF rats was more severe (30 and 17%, respectively; Table 1). By contrast, PX treatment reduced the atrophy of gastrocnemius (12% vs. respective pair-fed rats) and abolished the atrophy of soleus muscle.

TNF-α-, IL-1β-, and IL-6-like bioactivity and glucocorticoid plasma levels. Administration of PX 1 h before infection suppressed the rise of the plasma TNF-α level (by 99%; Table 2). PX treatment induced a reduction of 84 and 61% of plasma IL-1β- and IL-6-like bioactivity

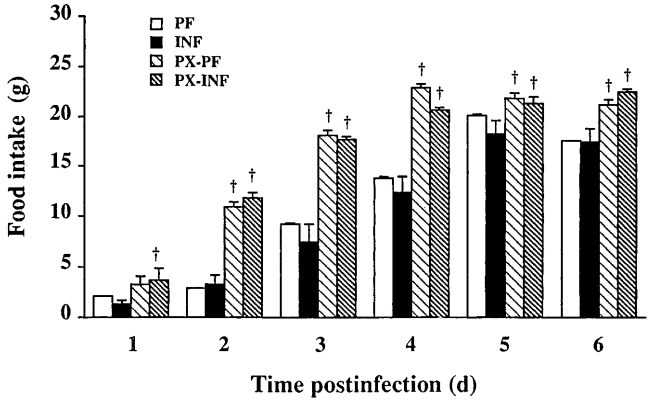

Fig. 3. Effect of PX treatment on cumulative weight change within 6 days postinfection. Values are means ± SE for 6–11 animals. †$P <$ 0.05 vs. respective animals without PX treatment.

concentrations, respectively, 3 h after infection (Table 2). In INF rats, plasma corticosterone levels were significantly higher 4 h after bacteria injection and were similar to normal values 24 and 48 h after the infectious stress (Fig. 4). PX treatment did not prevent the sepsis-induced increase in plasma corticosterone levels (Fig. 4).

Acute phase proteins. A2M, AGP, and fibrinogen concentrations were significantly increased 2 days after the infection (29-, 59-, and 2.3-fold, respectively) and 6 days after the infection (18-, 14-, and 2-fold, respectively) in INF rats compared with PF controls (Table 3). By contrast, albumin concentration was significantly reduced 2 and 6 days postinfection (45 and 54%, respectively). Infection significantly increased hepatic mRNA concentrations for AGP (19- and 8-fold, at *days 2* and *6*, respectively; Fig. 5) and fibrinogen (57 and 37% at *days 2* and *6*, respectively; Fig. 6). A2M mRNA levels increased significantly only on *day 6* postinfection but more moderately than those of AGP (55%; Fig. 5). By contrast, albumin mRNA levels were decreased by infection (50 and 57% at *days 2* and *6*, respectively; Fig. 6).

Administration of PX significantly decreased by 29% the rise in plasma AGP concentration in INF rats as early as 2 days after bacteria injection but did not significantly affect the increase in A2M and fibrinogen levels (Table 3). However, 6 days postinfection, the concentrations of these three positive acute phase proteins were significantly decreased in PX-INF rats compared with INF rats (65, 50, and 21%). No modification of the decrease of albumin concentration was observed with PX administration (Table 3). PX treatment reduced the increase in AGP and fibrinogen mRNA levels in PX-INF rats compared with nontreated infected rats 2 days after infection (Figs. 5 and 6), although they were not significantly different in PX-INF rats and nontreated infected rats on *day 6* postinfection. PX treatment did not induce any significant variation of the A2M and albumin mRNA levels 2 and 6 days after infection (Figs. 5 and 6).

DISCUSSION

PX has been shown to increase animal survival in lethal models of infection (38) and to block LPS fever (30). The beneficial effects of PX in sepsis have been attributed to its ability to inhibit the production of TNF-α in vitro (39, 41) and in vivo (12, 30, 38). TNF-α is the first cytokine to appear in the plasma after endotoxemia or infection, and it induces the synthesis and release of other mediators, such as IL-1 and IL-6, generating a cytokine cascade (43). The neutralization of endogenous TNF-α with anti-TNF-α antibodies greatly diminishes the increases in IL-1β and IL-6 in endotoxemia and sepsis (18, 42). Our results demonstrate that PX produced similar effects, decreasing IL-1β and IL-6 plasma levels 3 h after the infection. However, the effect of PX on the IL-1β plasma level was greater than on the IL-6 plasma level. IL-6 is produced later than TNF-α, and more or less simultaneously with IL-1β, both of which induced IL-6 (6, 22). Thus the

Table 1. *Effect of PX treatment on muscle weights 2 and 6 days postinfection*

	Days Postinfection							
	2				6			
	PF	INF	PX-PF	PX-INF	PF	INF	PX-PF	PX-INF
Gastrocnemius, g	1.69 ± 0.02	1.44 ± 0.02*	1.81 ± 0.03†	1.56 ± 0.03*†	1.83 ± 0.03	1.21 ± 0.04*	1.91 ± 0.04	1.68 ± 0.03*†
Soleus, mg	125 ± 3	109 ± 2*	128 ± 6	112 ± 4*	121 ± 11	97 ± 4*	124 ± 3	120 ± 2†

Values are means ± SE for 5 or 6 animals. INF, infected rats; PF, pair-fed control of INF rats; PX-INF, infected rats treated with pentoxifylline (PX); PX-PF pair-fed control treated with PX of PX-INF. *$P < 0.05$, one-way ANOVA vs. respective pair-fed controls. †$P < 0.05$, one-way ANOVA vs. respective infected animals.

remaining IL-1β can be sufficient to produce a large increase of IL-6 plasma level in PX-treated animals.

Plasma corticosterone levels were increased soon after administration of IL-1β (47) and LPS (22, 26), after cecum ligature and puncture in rats (23), or after bacteria injection as shown in our study. A novel result of this study is the inability of PX administration to modify glucocorticoid levels. Proinflammatory cytokines, like TNF-α and IL-1, were reported to increase glucocorticoid synthesis and release via the stimulation of the hypothalamic-pituitary axis (29). The lack of effect of PX treatment on corticosterone plasma level may be the result of remaining IL-1β and/or the presence of other factors sufficient to induce a maximum production of glucocorticoids, since pretreatment of turpentine-injected mice with anti-IL-1 receptor antagonist (ra) did not affect the increase in circulating corticosterone (21). Nevertheless, TNF-α did not appear to be the main mediator of corticosterone production in sepsis. Moreover, our results suggest that the mechanisms of PX action are independent of corticosterone levels or that corticosterone has only minor direct effects in sepsis.

PX administration reduced, but did not prevent, anorexia induced by infection, as previously observed in our laboratory (12) or by others using TNF-α monoclonal antibody pretreatment in endotoxemic rats (42). These data are in agreement with the view that TNF-α and IL-1β have anorexic effects (45). PX treatment suppressed body weight difference between INF rats and their PF control over the entire course of the study. Thus the effect of infection on body weight change completely disappeared in infected treated animals. Moreover, muscle atrophy linked to infection was significantly reduced by PX treatment and even abolished at *day 6* in soleus muscle. This rapid recovery of muscle weight of treated animals is consistent with the increase of protein synthesis (12, 15, 27) and the inhibition of proteolysis (46, 48) reported previously with inhibitors of TNF-α secretion or action and IL-1ra.

Table 2. *Effect of PX treatment on plasma TNF-α (measured 90 min after infection)-, IL-1β-, and IL-6-like bioactivity (measured 3 h after infection)*

	INF	PX-INF
TNF-α, ng/ml	71.9 ± 4.6	0.6 ± 0.1*
IL-1β, ng/ml	1.38 ± 0.48	0.22 ± 0.05*
IL-6, μg/ml	5.09 ± 0.56	1.98 ± 0.40*

Values are means ± SE for 12–14 animals. TNF-α, tumor necrosis factor-α; IL, interleukin. *$P < 0.05$ vs. infected animals.

The acute phase response associated with sepsis is accompanied by changes of the plasma concentration of a large number of proteins. Increased rates of incorporation of radiolabeled amino acids into proteins suggest, at least in part, that the increase of the plasma concentration of acute phase proteins during inflammation or sepsis is due to increased rates of their synthesis (40). The regulation of protein synthesis may occur at different levels, including various steps in the transcriptional and translational events. The analysis of mRNA levels and the measurement of transcription activity have provided evidence that transcriptional mechanisms play a central role in the regulation of expression of many acute phase proteins (9, 31, 36). Our results are in agreement with this view for AGP and fibrinogen, since mRNA levels were increased 2 and 6 days after the infection. By contrast, we found no coordinated changes between mRNA levels and plasma concentration of A2M, suggesting that the genes of these three proteins may be partially regulated by different mechanisms during inflammation. However, the increase in A2M mRNA level could be achieved before 48 h (19). Moreover, hepatic mRNA levels for A2M and AGP were found to increase to a maximum 24 h after LPS injection, with normal levels at 48 h for A2M but 10-fold over control values for AGP (42). Regulation of A2M may also occur on posttranscriptional levels, since Andus et al. (4) demonstrated that IL-6 markedly accelerated the secretion of A2M in hepatocyte primary cultures. For albumin, hepatic mRNA level and plasma concentration were diminished by ~50% 2 and also 6 days after infection. Such correlated decreases, suggesting that hypoalbuminemia is due to a decreased synthesis of the protein, have been described 24–48 h after LPS and turpentine injections, but they were followed by recovery over the next 48–72 h (2, 42). These results underline the long-lasting perturbations associated with our model, as described previously (11).

Cytokines have been implicated in the induction of the acute phase response. Most studies devoted to the exploration of the role of cytokines and hormones in inducing acute phase proteins have been carried out in cell culture systems and have shown direct cytokine effects. Prominent stimulatory functions have been ascribed to TNF-α, IL-1β, IL-6, and glucocorticoids (17, 29, 33). Two types of acute phase genes with different

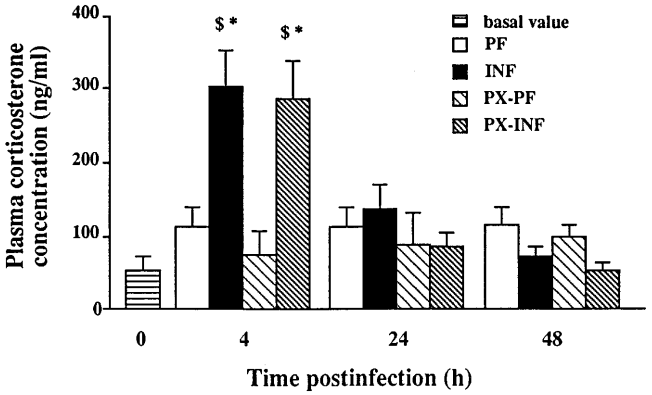

Fig. 4. Effect of PX treatment on plasma corticosterone concentration 4, 24, and 48 h after infection. Plasma corticosterone levels were assayed by RIA. Values are means ± SE for 6–11 animals. $\$P < 0.05$ vs. basal value. $*P < 0.05$ vs. respective pair-fed animals.

cytokine controls have been described as follows: those that respond to IL-1β and TNF-α such as AGP and those that respond to IL-6 such as A2M and fibrinogen for the rat (8, 29, 33). Moreover, to achieve maximally regulated expression of some proteins, a combination of cytokines and glucocorticoids is often required.

The qualitative pattern of the regulated positive acute phase proteins observed with PX treatment during the acute phase in our study was characteristic of that generally described in vitro. The first protein affected both at the transcriptional level and plasma concentration by the treatment was AGP, which is recognized to be regulated mainly by TNF-α and IL-1β (8, 29, 33). These results were consistent with the findings of Sharma et al. (42) using TNF-α monoclonal antibody treatment of endotoxemia in rats. Glucocorticoids alone are able to induce AGP (31), but there was no difference in the corticosterone level in treated and nontreated infected rats. Although AGP is minimally affected by IL-6 alone (31), some synergistic action with TNF-α and IL-1 can occur (8).

By contrast, A2M and fibrinogen plasma levels were not initially decreased by PX treatment, which did not modify A2M mRNA levels but slightly decreased those of fibrinogen. Because IL-6 secretion was not abolished by PX, these results are in agreement with numerous studies attributing a predominant role of IL-6 in inducing these two proteins (3, 31, 37). Moreover, in vitro data have shown that TNF-α and IL-1 did not alter either the expression or the synthesis of A2M (4). The regulation of fibrinogen gene expression seems more complex, since IL-1 inhibits its stimulation and this inhibitory effect is reversed by endogenous IL-1ra (37). Moreover, glucocorticoids are required to achieved a maximal IL-6 response for A2M but not for fibrinogen (31).

The decline in plasma albumin and mRNA levels appears not to be affected by PX treatment of septic rats, as shown with anti-TNF-α antibody treatment of endotoxemic rats (42). Anti-IL-1 receptor antibody administration before turpentine injection in mice failed to restore albumin plasma concentration (21). However, in vivo administration of TNF-α, IL-1β, or IL-6 is able to decrease albumin synthesis (5, 10, 20), and the combination of these cytokines, especially IL-1β and IL-6, resulted in an additive downregulation of albumin synthesis in vitro (4). On the other hand, glucocorticoids are known to increase albumin synthesis (24) and could partially antagonize the inhibitory effect of cytokines. Taken together, these data suggest that a small amount of any cytokine is enough to inhibit albumin synthesis and/or that unknown mediators or mechanisms play a predominant role in determining hypoalbuminemia.

During the chronic phase, the levels of AGP, A2M, and fibrinogen were significantly decreased in infected rats treated with PX compared with nontreated animals, indicating perhaps the rapid recovery of treated animals. No evidence of return to normal levels of albumin appeared at the end of the study, showing that albumin constitutes a poor index of outcome. However, mRNA levels tend to increase at the end of the experiment. The function of the decreased plasma level of albumin, and more generally of the negative acute phase proteins, is not yet clear and deserves further studies (2).

In summary, in a rat model of long-lasting sepsis, the administration of PX before infection inhibited circulating TNF-α, depressed plasma IL-1β and IL-6 levels, but had no effect on corticosterone levels. Moreover, PX treatment reduced anorexia and body weight loss, suppressed muscle protein wasting, and modulated the acute phase response. Our results suggest that glucocorticoids exert their action not directly, but mainly in

Table 3. *Effect of PX treatment on α_1-acid glycoprotein, α_2-macroglobulin, fibrinogen, and albumin levels 2 and 6 days postinfection*

	Days Postinfection							
	2				6			
	PF	INF	PX-PF	PX-INF	PF	INF	PX-PF	PX-INF
α_1-Acid glycoprotein, μg/ml	10 ± 1	586 ± 69*	16 ± 2	394 ± 48*†	20 ± 3	283 ± 74*	14 ± 1	79 ± 14*†
α_2-Macroglobulin, μg/ml	40 ± 3	1,140 ± 131*	90 ± 12	995 ± 106*	47 ± 5	833 ± 160*	66 ± 9	348 ± 30*†
Fibrinogen, mg/ml	2.7 ± 0.2	6.3 ± 0.4*	3.1 ± 0.1	6.3 ± 0.5*	3.2 ± 0.2	6.1 ± 0.4*	3.6 ± 0.1	4.7 ± 0.4*†
Albumin, mg/ml	16.4 ± 0.5	9.1 ± 0.3*	15.2 ± 0.4	10.0 ± 0.5*	16.1 ± 0.3	7.4 ± 0.3*	17.5 ± 0.7	8.9 ± 0.7*

Values are means ± SE for 4–6 animals. $*P < 0.05$, one-way ANOVA vs. respective pair-fed controls. $\dagger P < 0.05$, one-way ANOVA vs. respective infected animals.

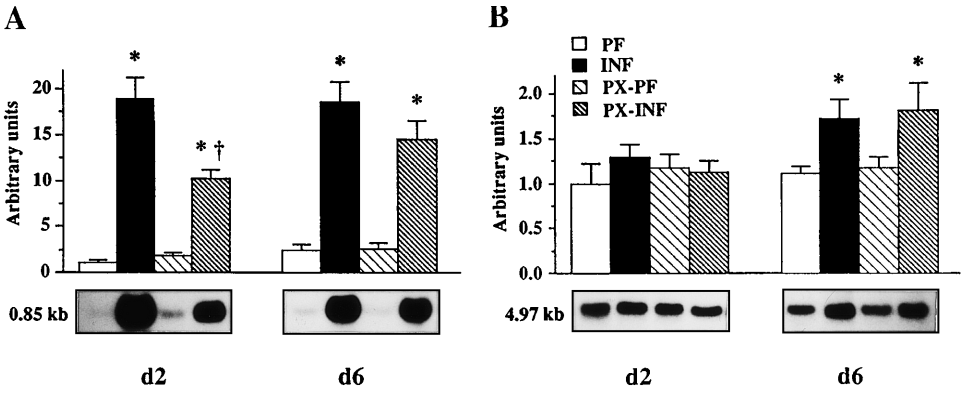

Fig. 5. Effect of PX treatment on mRNA levels for α_1-acid glycoprotein (*A*) and α_2-macroglobulin (*B*). RNA was extracted from liver of infected rats (INF) and their pair-fed controls (PF) and of infected rats treated with PX (PX-INF) and their pair-fed control treated with PX (PX-PF). Samples (20 μg) were electrophoresed, transferred to nylon membranes, and hybridized with [^{32}P]cDNAs encoding α_1-acid glycoprotein and α_2-macroglobulin. After stripping of the probes, blots were rehybridized with an 18S ribosomal oligonucleotide. Data were corrected for 18S rRNA abundance to take into account variations in RNA loading. Values are means ± SE for 4 or 5 rats. All data were referred to pair-fed *day 2* value, which was arbitrarily chosen to equal 1. Representative Northern blots are also shown. d2, 2 days postinfection; d6, 6 days postinfection. *$P < 0.05$ vs. respective PF animals. †$P < 0.05$ vs. respective animals without PX treatment.

combination with, other mediators. Moreover, our results underline in vivo that regulation of acute phase protein expression can occur at different levels according to the protein (17, 33).

The effects of PX treatment of infected rats shown in this study support the hypothesis of an important role of TNF-α in the regulation of protein metabolism during sepsis. However, further experiments are needed to understand the link between the very early and transient TNF-α secretion and the long-lasting effects observed in muscle and liver protein metabolism. Despite the complexity of the cytokine and hormonal network, the present study demonstrates in vivo that individual mediators have specific effects on particular acute phase proteins, making AGP a better index of recovery after PX treatment than A2M. This emphasizes again that acute phase proteins do not always respond in unison in disease states. Clearly, the role of the various acute phase proteins and the regulation of the acute phase response have to be evaluated in detail in various diseases before acute phase proteins become a practical clinical diagnostic and prognostic tool.

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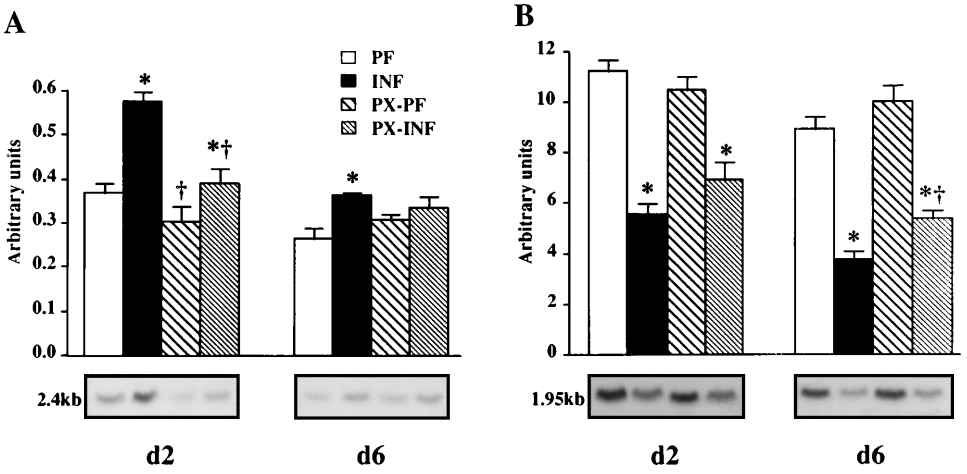

Fig. 6. Effect of PX treatment on mRNA levels for fibrinogen (*A*) and albumin (*B*). RNA was extracted from liver of infected rats (INF) and their pair-fed controls (PF) and of infected rats treated with PX (PX-INF) and their pair-fed control treated with PX (PX-PF). Samples (20 μg) were electrophoresed, transferred to nylon membranes, and hybridized with [^{32}P]cDNAs encoding fibrinogen and albumin. After stripping of the probes, blots were rehybridized with an 18S ribosomal oligonucleotide. Data were corrected for 18S rRNA abundance to take into account variations in RNA loading. Values are means ± SE for 4 or 5 rats. All data were referred to pair-fed *day 2* value, which was arbitrarily chosen to equal 1. Representative Northern blots are also shown. *$P < 0.05$ vs. respective PF animals. †$P < 0.05$ vs. respective animals without PX treatment.

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