Pretreatment with Ibuprofen Augments Circulating Tumor Necrosis Factor-α, Interleukin-6, and Elastase during Acute Endotoxinemia

Giatgen A. Spinas, Daniel Bloesch, Ulrich Keller, W. Zimmerli, and Salvatore Cammisuli

Departments of Internal Medicine and Research, University Hospital, and Preclinical Research, Sandoz Ltd., Basel, Switzerland

Plasma levels of tumor necrosis factor-α (TNFα), interleukin-1 (IL-1), and interleukin-6 (IL-6) were monitored after intravenous administration of *Escherichia coli* endotoxin with or without ibuprofen pretreatment to healthy volunteers. Intravenous endotoxin ($n = 7$) resulted in elevated plasma TNFα concentrations with maximal levels at 90 min (369 ± 44 pg/ml, $P < .001$ vs. saline controls, $n = 7$). The rise in TNF-α was followed by a rise in plasma IL-6 (27 ± 12.8 ng/ml), peaking 30–90 min thereafter. Pretreatment with ibuprofen ($n = 6$) caused a significant augmentation and temporal shift in cytokine elaboration with maximal TNFα levels (627 ± 136 pg/ml) at 120 min and IL-6 peaks (113 ± 66 ng/ml) at 180 min. In ibuprofen-treated volunteers, the additional increase in TNFα was paralleled by increased levels of circulating elastase. In vitro experiments suggest a causal relationship between these events. Thus, the cyclooxygenase inhibitor ibuprofen blunts the clinical response to endotoxin but augments circulating cytokine levels and leukocyte degranulation.

Acute endotoxinemia is accompanied by a variety of metabolic changes such as fever, activation of hepatic protein synthesis, and increased numbers of circulating leukocytes. These changes, referred to as acute-phase response, are mediated by a number of monocyte/macrophage-derived cytokines [1]. Among these, interleukin-1 (IL-1) and tumor necrosis factor-α (TNFα), both elaborated by stimulated macrophages, are considered to play a central role in mediating many of the beneficial and adverse effects of the host's response to endotoxinemia [2–4]. Furthermore, TNFα has been implicated as an essential factor in the pathogenesis of septic shock [4, 5].

Evidence is accumulating that several of the biologic effects ascribed to IL-1 and TNFα are mediated by the induction of interleukin-6 (IL-6) [6]. Like IL-1, IL-6 is a pleiotropic cytokine with a wide range of biologic activities including the property to induce acute-phase proteins [7, 8]. However, the exact role of IL-6 in the systemic response to tissue injury remains to be elucidated.

A number of studies have shown that cytokines can be detected in the circulation of septicemic patients [9–12] or after experimental endotoxin administration [13–15]. However, since the different cytokines are released sequentially, the relative serum levels of cytokines differ depending on the time of blood sampling. Furthermore, there appears to be a correlation between the level of circulating cytokines and the severity of sepsis, with the highest IL-1, TNFα, and IL-6 concentrations being found in patients with fatal illness [9–12].

There is abundant evidence that arachidonic acid metabolites act as endogenous regulators of cytokine production [16, 17]. In vitro prostaglandin E_2 (PGE_2) was found to exert a negative feedback on the release of IL-1 and TNFα [18, 19], while inhibition of prostaglandin synthesis by indomethacin augmented lipopolysaccharide (LPS)-induced TNFα release [20]. It is likely that in vivo pharmacologic manipulation of arachidonic acid metabolism interferes with cytokine regulation.

We monitored the pattern of circulating cytokines in temporal relation to the systemic response after endotoxin administration to normal volunteers and investigated whether inhibition of the cyclooxygenase pathway of arachidonic acid metabolism by ibuprofen affected cytokine levels.

Methods

Subjects and study procedure. The volunteers were 20 men aged 20–42 years. The investigation was done simultaneously with an evaluation of endotoxin effects on protein and glucose turnover. (Those data will be published elsewhere.) Before the study, a complete medical history was taken and physical examination, electrocardiography, and routine blood and urine analysis were done. The volunteers were randomly assigned to receive endotoxin alone, endotoxin plus ibuprofen, or 0.9% saline (controls).

The subjects were admitted at 7 A.M. after a 10-h (overnight) fast. A Teflon cannula was placed into a left forearm vein, and 0.9% saline (0.7 ml/kg body weight/h) was infused. A butterfly needle was inserted retrograde into a dorsal vein of the right hand, which was kept in a warming chamber (60°C) to arterialize blood. After a 2-h resting period, *Escherichia coli* endotoxin (US ref., lot EC-5, Bureau of Biologics, US Food and Drug Administration, Bethesda, MD) with a specific activity of 5 units/ng or an equivalent volume of 0.9%

Received 15 March 1990; revised 23 July 1990.

Participants gave written informed consent; the study was approved by the local Ethics Committee.

Financial support: Swiss National Research Foundation (3.888–0.86 and 3.876–0.88).

Reprints or correspondence: Dr. Giatgen A. Spinas, Department of Internal Medicine, Kantonsspital, Petersgraben 4, CH-4031 Basel, Switzerland.

The Journal of Infectious Diseases 1991;163:89–95

0022-1899/91/6301-0016$01.00

saline was injected intravenously. Endotoxin was reconstituted from the lyophylisate just before use. A fellow was continuously present, and a senior physician was in attendance during the entire experiment. Emergency equipment was always within reach. Six randomly selected volunteers ingested a first dose of ibuprofen (800 mg, Boots) 120 min before and a second dose immediately before the endotoxin injection.

The subjects remained supine for 6 h after the injection. Pulse rate, systolic and diastolic blood pressure, and rectal temperature were monitored at 30-min intervals from 30 min before until 6 h after endotoxin or saline injection. Blood samples were obtained 30 min before and at 0, 30, 60, 90, 120, 180, 240, 300, and 360 min for measurement (or count) of hemoglobin, white blood cells, thrombocytes, and fibrinogen. Plasma was obtained rapidly and stored at −70°C for later assay of IL-1α, IL-1β, TNFα, and IL-6. For the determination of IL-1, plasma samples were extracted with chloroform as described by Cannon et al. [21].

In vitro studies. Polymorphonuclear leukocytes (PMNL) were purified on a Percoll gradient, as previously described [22]. Purified C5a (gift of C. Dahinden, Bern, Switzerland) was used as degranulation stimulus. PMNL (5×10^6) were preincubated with 5 μg/ml cytochalasin B in the presence or absence of TNFα (Boehringer, Mannheim, FRG) in a shaking water bath (37°C). After 5 min, C5a was added, and the incubation continued for 15 min more. The reaction was stopped on ice, the tubes were centrifuged at 14,000 *g* for 5 min, and primary granule (β-glucuronidase) and secondary granule (B12-binding protein) exocytosis was determined in the Triton-X 100 (0.1%)-lysed pellet [23]. Results were obtained as percentage of total cellular granule content as follows: [100 − (granules in PMNL after assay/granules in PMNL before assay)] × 100.

Plasma Analyses. Plasma IL-6 was determined according to Aarden et al. [24] using the IL-6-dependent mouse hybridoma cell line, B13-29. Briefly, 5000 cells were cultured in triplicate for 48 h in synthetic medium [25] in 200-μl wells of a 96-well flat-bottom microtiter plate in the presence of graded dilutions of the plasma samples to be tested. Plasma samples were heat-inactivated (30 min at 56°C) before assay. Recombinant human IL-6 was used as the internal standard. The cell cultures were pulsed with 1 μCi of [^{3}H]thymidine (2 Ci/mmol; Amersham Ltd., Amersham, UK) during the final 6 h of incubation. Cells were then harvested and the incorporated radioactivity measured with a liquid scintillation counter. The amount of radioactivity in triplicate cultures was expressed as disintegrations per minute. IL-6 concentrations were extrapolated from recombinant IL-6 internal standards. The detection limit of the assay was 1 pg/ml.

Plasma TNFα was measured with a TNFα-specific ELISA (Endogen, Boston) with a detection limit of 10 pg/ml. As a control, seven plasma samples obtained at peak TNFα elaboration were assessed for TNFα bioactivity using the WEHI 164 cell line as described previously [26]. Biologically active TNFα correlated well with the immunologic TNFα concentrations ($r = .86$, $P = .01$).

Plasma IL-1 concentrations were measured in untreated and chloroform-extracted samples [21] using an IL-1α-specific ELISA (Endogen) with a detection limit of 50 pg/ml and an IL-1β-specific RIA (Cistron Biotechnologies, Pine Brook, NJ) with a sensitivity of 50 pg/ml.

Elastase-α_1-proteinase inhibitor was determined in plasma samples by an enzyme immunoassay (Merck, Darmstadt, FRG) as previously described [27].

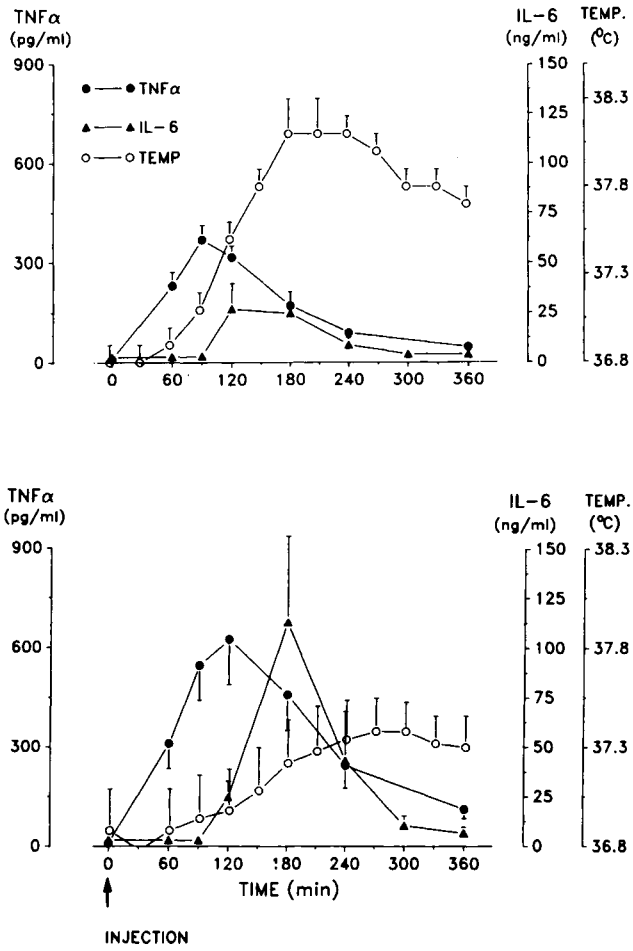

Figure 1. Plasma concentrations of tumor necrosis factor-α (TNFα), interleukin-6 (IL-6), and body temperature after endotoxin injection without (top, $n = 7$) or with ibuprofen pretreatment (bottom, $n = 6$). Pretreatment with ibuprofen caused significant increase and temporal shift in plasma levels of both TNFα ($P = .003$ vs. endotoxin alone) and IL-6 ($P = .05$ vs. endotoxin alone by analysis of variance).

Data analysis. Standard statistical software (BMDP Statistical Software, San Francisco) was used. Analysis of variance with repeated measures (ANOVA), paired *t* tests, the Wilcoxon test for matched pairs, and least-squares regression analysis were done as indicated, and 5% was chosen as the level of significance. Data are expressed as mean ± SE if not otherwise stated.

Results

Cytokine responses. Figure 1 (top) illustrates the changes in circulating cytokine concentrations in temporal relation to fever in volunteers receiving endotoxin. Plasma concentrations of TNFα rose from <10 pg/ml at baseline to a maximum of 369 ± 44 pg/ml at 90 min and returned to values slightly above baseline at 360 min. In six of seven subjects, peak TNFα concentration was seen after 90 min; in the other,

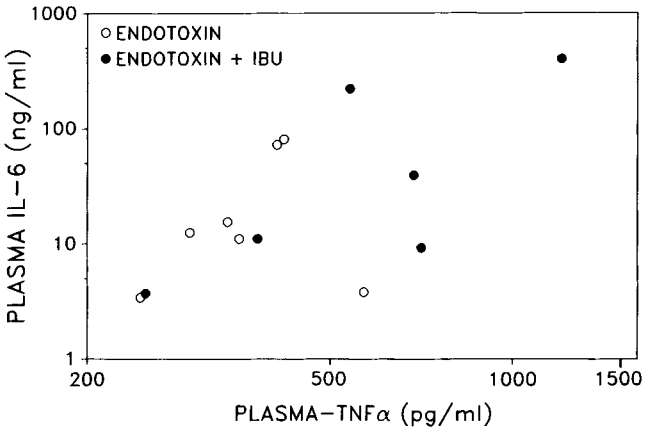

Figure 2. Relation between peak plasma levels of tumor necrosis factor-α (TNFα) and interleukin-6 (IL-6) in subjects receiving endotoxin without (n = 7) or with (n = 6) ibuprofen (IBU) pretreatment (r = .77, $P < .01$ by regression analysis).

TNFα peaked at 120 min. Individual peak TNFα concentrations ranged from 245 to 570 pg/ml.

Plasma IL-6 remained at baseline levels up to 90 min after endotoxin injection, were increased 10-fold at 120 and 180 min, and returned to values slightly above baseline at 360 min. In all cases, peak IL-6 levels occurred at 120 (n = 5) or 180 (n = 2) min and followed the TNFα peak by 30–90 min (P = .03, Wilcoxon test for matched pairs).

Body temperature increased steadily after 90 min, when TNFα levels peaked, but clearly before IL-6 became detectable. Maximal body temperature was seen at 218 ± 14 min, significantly later than maximal TNFα (P = .015) and IL-6 (P = .03) elaboration.

Effects of ibuprofen pretreatment on plasma cytokine concentrations. Treatment with ibuprofen before endotoxin administration resulted in a significant increase and temporal shift of cytokine elaboration compared with endotoxin alone (figure 1, bottom). Maximal TNFα concentrations (627 ± 136 vs. 369 ± 44 pg/ml without ibuprofen) were observed at 120 min ($P < .003$). Similarly, maximal IL-6 concentrations were significantly higher (113 ± 66 vs. 27 ± 12 ng/ml) and occurred later (180 vs. 120 min) when ibuprofen was given before endotoxin (P = .05 vs. endotoxin without ibuprofen by ANOVA).

As expected, the increase in body temperature was significantly blunted in subjects pretreated with ibuprofen ($P <$ .001 vs. endotoxin alone).

In figure 2 individual maximal values of TNFα are plotted against the corresponding IL-6 levels. When the data of volunteers without and with ibuprofen pretreatment were pooled (n = 13), there was a significant correlation between peak TNFα and peak IL-6 levels (r = .77, $P < .01$).

Plasma IL-1α and IL-1β concentrations. No significant changes in plasma IL-1α and IL-1β levels were found after endotoxin administration. The values for IL-1β ranged from

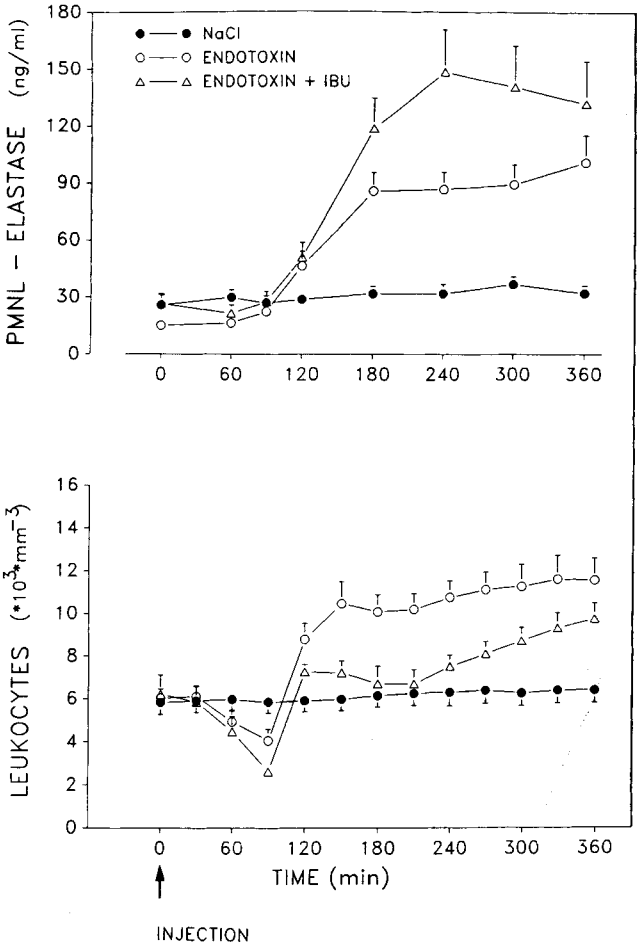

Figure 3. Polymorphonuclear leukocyte (PMNL)–elastase levels and leukocyte counts in subjects receiving endotoxin without (n = 7) or with pretreatment with ibuprofen (IBU; n = 6) or saline (n = 7). Pretreatment with ibuprofen resulted in increased PMNL-elastase levels (P = .001 vs. endotoxin alone by analysis of variance (ANOVA) despite less pronounced leukocytosis ($P < .01$ vs. endotoxin alone by ANOVA).

below the detection limit of the assay (50 pg/ml) to 70 pg/ml (in endotoxin recipients) and 65 pg/ml (in saline recipients) in plasma diluted 1:2. IL-1α levels were below the detection limit in all cases.

Systemic effects of endotoxin without and with ibuprofen pretreatment. Symptoms such as headache, myalgia, nausea, and chills occurred 60–90 min after endotoxin injection in all untreated subjects; the severity ranged from mild (four subjects) to moderate (three). Subjects pretreated with ibuprofen remained asymptomatic after receiving endotoxin.

Figure 3 shows PMNL-elastase concentrations and leukocyte counts in the three treatment groups. PMNL elastase increased to higher levels in those who received ibuprofen than in those who did not ($P < .001$) despite less pronounced leukocytosis (P = .01 vs. endotoxin alone). No changes in leukocyte counts and PMNL elastase were observed in the

Table 1. Differentiated circulating leukocyte counts in subjects receiving endotoxin without or with ibuprofen (Ibu) pretreatment.

	Endotoxin (n = 7)		Endotoxin + Ibu (n = 6)		Controls, NaCl (n = 7)	
	Before	After	Before	After	Before	After
Leukocyte counts ($\times 10^3/\mu l$)	6.3 ± 0.7	11.9 ± 1.3*	5.2 ± 0.3	9.4 ± 0.8*	5.9 ± 0.6	6.4 ± 0.6
% neutrophils	53.7 ± 1.6	83.3 ± 2.8†	57.1 ± 4.9	87.9 ± 1.1*	54.9 ± 3.0	53.4 ± 2.4
% lymphocytes	33.7 ± 1.7	8.9 ± 2.2†	31.0 ± 3.4	6.0 ± 1.1*	33.8 ± 2.9	35.7 ± 2.2
% monocytes	6.9 ± 0.6	5.6 ± 0.7	7.3 ± 1.5	4.5 ± 0.5	6.5 ± 1.2	6.5 ± 0.8
% eosinophils	2.4 ± 0.6	0.7 ± 0.1‡	1.6 ± 0.3	0.8 ± 0.2‡	2.5 ± 0.8	2.6 ± 0.7
% basophils	1.3 ± 0.3	0.4 ± 0.0‡	0.9 ± 0.1	0.4 ± 0.0*	0.7 ± 0.2	0.5 ± 0.0

* $P < .01$; † $P < .001$; ‡ $P < .05$ (Student's t test).

saline-treated (control) group. The increase in circulating leukocytes was characterized by a significant relative increase in PMNL and a relative decrease in lymphocytes, eosinophils, and basophils (table 1).

As depicted in figure 4 (top) endotoxin administration caused an increase in heart rate, with maximal values after 3–5 h ($P < .001$ vs. controls). Ibuprofen pretreatment had no significant influence on this endotoxin effect. No changes were observed in control subjects.

Systolic blood pressure did not change after endotoxin injection (figure 4, bottom), while diastolic pressure showed a transient rise at 60 and 90 min ($P < .05$). This was not seen in ibuprofen-pretreated subjects (data not shown).

Effect of TNFα on PMNL degranulation in vitro. Ibuprofen does not increase PMNL degranulation in vitro (data not shown). In consequence, the higher elastase concentrations in volunteers pretreated with ibuprofen could not be explained by a direct effect of this drug on PMNL. We therefore studied whether TNFα would prime C5a-induced PMNL degranulation when tested at concentrations similar to those measured in our volunteers (table 2). TNFα indeed primed PMNL for degranulation of primary and secondary granules in a dose-dependent manner. The same concentrations of TNFα by itself did not induce degranulation (data not shown).

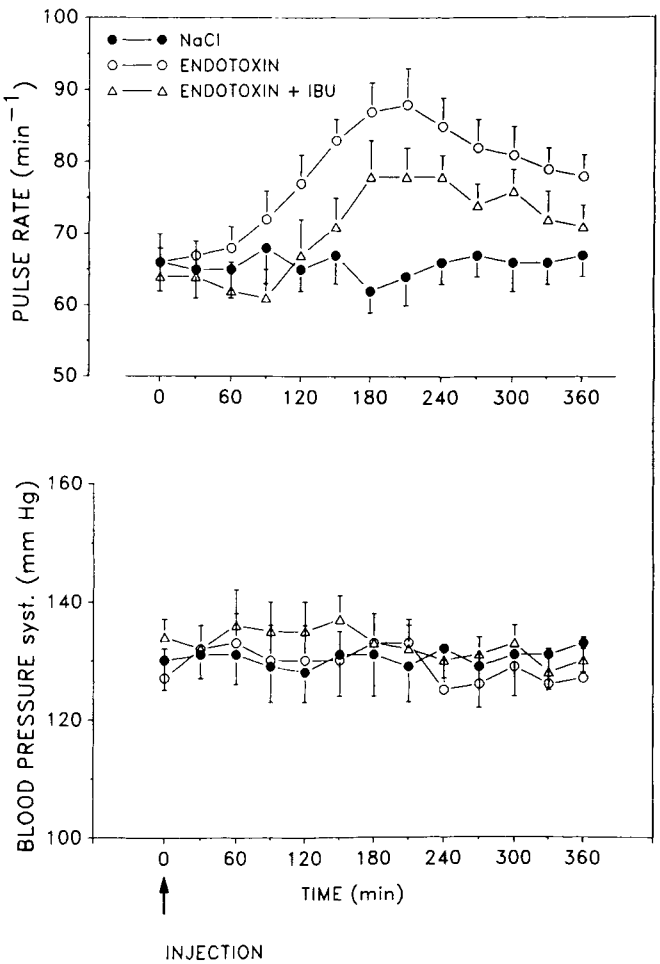

Figure 4. Cardiovascular responses in subjects receiving endotoxin without (n = 7) or with pretreatment with ibuprofen (IBU; n = 6) or saline (n = 7). Heart rate increased after endotoxin administration ($P < .0001$ vs. controls) and was not significantly blunted by ibuprofen. Systolic blood pressure remained unchanged.

Discussion

In this study, intravenous administration of endotoxin to healthy human volunteers elicited a typical pattern in circulating cytokines characterized by sequential pulses of TNFα and IL-6. The concomitant changes in body temperature, pulse rate, and leukocyte counts coincided with the increase in circulating TNFα but clearly preceded the appearance of IL-6 in the circulation. These observations are in line with a recent report showing in humans that the host's response to endotoxin in terms of fever, leukocytosis, and hormonal changes was associated with elevated plasma levels of TNFα [13]. Plasma IL-6 was not measured in that study. In eight patients with septic shock, serial blood sampling revealed that in five peak TNFα concentrations occurred earlier in the course of the disease than did IL-6, suggesting that TNFα may induce the release of IL-6 [11]. In the present study, the appearances of TNFα and IL-6 in the circulation in acute endotoxinemia were indeed temporally related, the rise in TNFα preceding the appearance of IL-6 by 30–90 min.

Table 2. Effect of tumor necrosis factor-α (TNFα) on C5a-induced degranulation by neutrophils.

Incubation mixture	Enzyme release (% of total content) β-glucuronidase	B_{12}-binding protein
Buffer	5 ± 5.5 (6)	5 ± 5 (4)
C5a (10^{-8} *M*)	47 ± 15 (9)	54 ± 21 (6)
TNFα (100 pg/ml) + C5a (10^{-8} *M*)	49 ± 17 (9)	61 ± 17 (6)
TNFα (1000 pg/ml) + C5a (10^{-8} *M*)	56 ± 11 (9)	66 ± 16 (6)

NOTE. Incubation conditions are described in Methods; data are mean ± SD of (*n*) experiments. NS = not significant. * $P < .05$, † $P < .001$, ‡ $P < .01$ (Student's *t* test).

In vitro TNFα has been shown to induce both IL-6 activity and mRNA in cultured human cell lines such as fibroblasts, endothelial cells, and osteosarcoma cells [28, 29]. In vivo, systemic administration of recombinant TNFα to cancer patients resulted in a transient increase of circulating IL-6 levels [30]. Furthermore, induction of IL-6 activity has been observed in two patients treated with human recombinant IL-2 [30]. In these cases the increase in IL-6 activity was preceded by a rise in endogenous TNFα levels, indicating that the release of IL-6 may be secondary to a release in TNFα. Our data suggest that also after an endotoxin challenge, IL-6 elaboration depends on prior production of TNFα. The present data do not permit us to dissect the relative direct or indirect contributions of IL-6, IL-1, and TNFα in mediating the host's response to endotoxin, but IL-6 seems not to be primarily involved in inducing fever and leukopenia, as these systemic signs occurred before IL-6 became detectable in circulation. In contrast, the appearance of TNFα in the plasma was temporally associated with the onset of clinical symptoms (rise of body temperature, tachycardia, and leukopenia). Since the detection limit for IL-6 was 10-fold lower (1 pg/ml) than for TNFα, the later appearance of IL-6 in circulation was not due to differences in the sensitivity of the assays used. However, as circulating levels of cytokines only roughly reflect fixed-tissue elaboration, it cannot be excluded that IL-6 produced locally by cells in the hypothalamus, such as microglial cells, astrocytes [31], or endothelial cells [32], may have contributed to the thermoregulatory response.

The peak levels of TNFα in the present study were considerably higher than those commonly detected in patients with sepsis [9–11]. Comparing plasma TNF concentrations in patients with experimental endotoxinemia and septic shock, Cannon et al. [33] found that volunteers receiving endotoxin exhibited higher TNFα levels (> 500 pg/ml) than did septic patients (100–150 pg/ml). Furthermore, intravenous administration of recombinant human TNFα to volunteers resulted in transient TNFα elevations > 4000 pg/ml [34]. Thus, it appears that healthy individuals tolerate very high TNFα concentrations for a short time with no serious consequences, whereas in critically ill patients, sustained release of TNFα in concert with the synergistic action of other cytokines [35, 36], increased leukocyte degranulation (see below), and additional aggravating factors [10] will finally determine the clinical outcome.

We failed, as did others [13], to detect significant changes in circulating IL-1 after endotoxin challenge. It has been suggested that this may be due to limitations of the available assays [37] or to serum factors interfering with the radioimmunologic measurement [21]. In fact, using a bioassay, Hesse et al. [38] could detect low levels of IL-1 activity 2–4 h after administration of endotoxin to human volunteers.

An immunologically important and possibly clinically relevant observation of the present study is the finding that pretreatment with ibuprofen, an inhibitor of the cyclooxygenase pathway of arachidonic acid metabolism, enhanced circulating TNFα and IL-6 levels after endotoxin challenge. The predominant role of arachidonate metabolism in the context of immunologic responses lies in restructuring of cell membrane [16] with a minor proportion of the arachidonate being metabolized into prostaglandins or leukotrienes, depending on the local cellular environment. Prostaglandins, in particular prostaglandins of the E series, serve mainly as local mediators of inflammatory processes (e.g., vasodilatation, increased vascular permeability, sensitization of pain receptors) [16]. In addition, they play an important role in the regulation of the immune response by acting as local feedback inhibitors of both cellular and humoral immunity [16, 39, 40]. In vitro PGE_2 has been shown to suppress LPS-induced TNFα release from macrophages [19, 41], while inhibition of this negative feedback action by addition of indomethacin resulted in greater lymphokine production in lymphocyte cultures [42] and LPS-induced TNFα production by macrophages [19, 20]. The present study demonstrated for the first time that also in humans inhibition of the cyclooxygenase pathway of arachidonic acid metabolism results in increased levels of circulating cytokines in endotoxinemic subjects.

The mechanism responsible for the observed increase in cytokine elaboration is unknown. However, considering the body of in vitro evidence, it is likely that interruption of the negative feedback action of PGE_2 on cytokine release by ibuprofen plays a significant part. Whether in addition changes in leukotriene production due to, for example, diversion of arachidonic acid metabolism from cyclooxygenase to lipoxygenase pathways also affects cytokine regulation is still in ques-

tion [16, 43]. In preliminary experiments, we observed no changes in circulating leukotrienes in volunteers receiving endotoxin regardless of whether they were pretreated with ibuprofen (unpublished data).

Besides affecting cytokine levels, pretreatment with ibuprofen resulted in significantly increased PMNL-elastase concentrations. The increased elastase levels cannot be attributed to a higher number of circulating leukocytes, because in ibuprofen-treated volunteers leukocytosis was less pronounced after endotoxin challenge. Our in vitro degranulation experiments demonstrated that the increase of a primary granule marker of PMNL may be a consequence of the concomitantly increased TNFα levels in these subjects. Others have also shown that TNFα stimulates degranulation and respiratory burst of PMNL [44], which in turn leads to increased surface expression of the C3bi-receptor/adherence glycoproteins, thereby favoring adherence of PMNL to endothelial cells [45].

Several authors have demonstrated an association between high serum concentrations of cytokines and fatality of septic patients. As ibuprofen therapy increased plasma levels of TNFα, IL-6, and consequently PMNL degranulation during endotoxinemia, this potential adverse effect should be remembered when administrating cyclooxygenase inhibitors to patients with sepsis. It is conceivable that the increased intravascular degranulation is harmful and contributes to fatality in patients with high serum concentrations of TNFα.

Acknowledgment

We thank H. Achermann, Central Laboratory, University Hospital Basel, for performing the elastase assay; H. R. Frey, Hoffmann-La Roche, for performing the TNF bioassay; I. Sklenar, Sandoz, for providing the endotoxin; and M. Hagen for preparing the manuscript.

References

1. Dinarello CA. Interleukin-1. Rev Infect Dis **1984**;6:51-94.
2. Dinarello CA, Cannon JC, Mier JW, et al. Multiple biological activities of human recombinant interleukin-1. J Clin Invest **1986**;77:1734-9.
3. Dinarello CA. Interleukin-1: amino acid sequences, multiple biological activities and comparison with tumor necrosis factor (cachectin). Year Immunol **1986**;2:68-89.
4. Beutler B, Milsark IW, Cerami AC. Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. Science **1985**;229:869-75.
5. Cerami A, Beutler B. The role of cachectin/TNF in endotoxin shock and cachexia. Immunol Today **1988**;9:28-31.
6. Gauldie JC, Richards D, Harnish P, Lansdorp P, Baumann H. Interferon β2/B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. Proc Natl Acad Sci USA **1987**;84:7251-5.
7. Wong GC, Clark SC. Multiple actions of interleukin-6 within a cytokine network. Immunol Today **1988**;9:137-9.
8. Kishimoto T. The biology of interleukin-6. Blood **1989**;74:1-10.
9. Waage A, Halstensen A, Espevik T. Association between tumor necrosis factor in serum and fatal outcome in patients with meningococcal disease. Lancet **1987**;1:355-7.
10. Girardin E, Grau GE, Dayer JM, Roux-Lombard P, the J5 Study Group, Lambert PH. Tumor necrosis factor and interleukin 1 in the serum of children with severe infectious purpura. N Engl J Med **1988**;319: 397-400.
11. Waage A, Brandtzaeg P, Halstensen A, Kierluf P, Esperik T. The complex pattern of cytokines in serum from patients with meningococcal septic shock. J Exp Med **1989**;169:333-8.
12. Hack CE, De-Groot ER, Felt-Bersma RJ, et al. Increased plasma levels of interleukin-6 in sepsis. Blood **1989**;74:1704-10.
13. Michie HR, Manogue KR, Spriggs DR, et al. Detection of circulating tumor necrosis factor after endotoxin administration. N Engl J Med **1988**;318:1481-6.
14. Beutler BA, Milsark IW, Cerami A. Cachectin/tumor necrosis factor: production, distribution and metabolic fate in vivo. J Immunol **1985**;135:3972-7.
15. Fong Y, Moldawer LL, Marano M, et al. Endotoxemia elicits increased circulating β-2-IFN/IL-6 in man. J Immunol **1989**;142:2321-4.
16. Morley J. Role of prostaglandins secreted by macrophages in the inflammatory process. Lymphokines **1981**;4:377-94.
17. Chouaib S, Bertoglio JH. Prostaglandins E as modulators of the immune response. Lymphokine Res **1988**;7:237-45.
18. Knudsen PJ, Dinarello CA, Strom TB. Prostaglandins posttranscriptionally inhibit monocyte expression of interleukin-1 activity by increasing intracellular cyclic adenosine monophosphate. J Immunol **1986**; 137:3189-94.
19. Kunkel SL, Spengler M, May MA, Spengler R, Larrick J, Remick D. Prostaglandin E_2 regulates macrophage-derived tumor necrosis factor gene expression. J Biol Chem **1988**;263:5380-4.
20. Spengler RN, Spengler ML, Strieter RM, Remick DG, Larrick JW, Kunkel SL. Modulation of tumor necrosis factor alpha gene expression. J Immunol **1989**;142:4346-50.
21. Cannon JG, van der Meer JWM, Kwiatowski D, et al. Interleukin-1 beta in human plasma: optimization of blood collection, plasma extraction, and radioimmunoassay methods. Lymphokine Res **1988**;7:457-67.
22. Zimmerli W, Seligman B, Gallin JI. Exsudation primes human and guinea pig neutrophils for subsequent responsiveness to the chemotactic peptide *N*-formylmethionylleucylphenylalanine and increases complement component C3bi receptor expression. J Clin Invest **1986**;77:925-33.
23. Metcalf JA, Gallin JI, Nauseef WM, Root RK. Laboratory manual of neutrophil function. New York: Raven Press, **1986**;1-191.
24. Aarden LA, De Groot ER, Schaap OL, Lansdorp PM. Production of hybridoma growth factor by human monocytes. Eur J Immunol **1987**;17:1411-6.
25. Iscove NN, Guilbert LJ, Weyman C. Complete replacement of serum in primary cultures of erythropoietin-dependent rat cell precursors (CFU-E) by albumin, transferrin, iron, unsaturated fatty acid, lecithin, and cholesterol. Exp Cell Res **1980**;126:121-6.
26. Peck R, Brockhaus M, Frey JR. Cell surface tumor necrosis factor (TNF) accounts for monocyte- and lymphocyte-mediated killing of TNF-resistant target cells. Cell Immunol **1989**;122:1-10.
27. Neuman S, Gunzer G, Heinrich N, Lang H. "PMN-elastase": enzyme immunoassay for human polymorphonuclear elastase complexed with α_1-proteinase inhibitor. J Clin Chem Clin Biochem **1984**;22:693-7.
28. Zilberstein A, Ruggieri R, Korn JH, Revel W. Structure and expression of cDNA and genes for human interferon beta-2, a distinct species inducible by growth stimulatory cytokines. EMBO J **1986**;5:2529-37.
29. Van Damme J, Opdenakker G, Simpson RJ, et al. Identification of the human 26-kD protein, interferon β2 (IFN-β2), as a B-cell hybridoma/plasmacytoma growth factor induced by interleukin-1 and tumor necrosis factor. J Exp Med **1987**;165:914-9.
30. Jablons DM, Mulé JJ, McIntosh JK, et al. IL-6/IFN-β-2 as a circulating hormone. Induction by cytokine administration in humans. J Immunol **1989**;142:1542-7.

31. Frei K, Malipiero UV, Leist TP, Zinkernagel RM, Schwab ME, Fontana A. On the cellular source and function of interleukin-6 produced in the central nervous system in viral diseases. Eur J Immunol **1989**;19:689–94.
32. Jirik FR, Podor TJ, Hirano T, et al. Bacterial lipopolysaccharide and inflammatory mediators augment IL-6 secretion by human endothelial cells. J Immunol **1989**;142:144–7.
33. Cannon JG, Tompkins RS, Gelfand JF, et al. Circulating interleukin 1 and tumor necrosis factor in septic shock and experimental endotoxin fever. J Infect Dis **1990**;161:70–84.
34. Van der Poll I, Büller HR, ten Cate H, et al. Activation of coagulation after administration of tumor necrosis factor to normal subjects. N Engl J Med **1990**;322:1622–7.
35. Aggarwal BB, Eessalu TE, Hass PE. Characterization of receptors for human tumor necrosis factors and their regulation by gamma-interferon. Nature **1985**;318:665–7.
36. Ruggiero V, Baglioni C. Synergistic anti-proliferative activity of interleukin 1 and tumor necrosis factor. J Immunol **1987**;138:661–3.
37. Dinarello CA. Biology of interleukin 1. FASEB J **1988**;2:108–15.
38. Hesse DG, Tracey KJ, Fong Y, et al. Cytokine appearance in human endotoxemia and primate bacteremia. Surg Gynecol Obstet **1988**; 166:147–53.
39. Goodwin JS, Webb DR. Regulation of the immune response by prostaglandins. Clin Immunol Immunopathol **1980**;15:106–22.
40. Schultz RM, Parlidis NA, Stylos WA, Chirigos MA. Regulation of macrophage tumoricidal function: a role for prostaglandin of the E series. Science **1978**;202:320–1.
41. Renz H, Gong JG, Schmidt A, Nain M, Gemsa D. Release of tumor necrosis factor alpha from macrophages. Enhancement and suppression are dose-dependently regulated by prostaglandin E_2 and cyclic nucleotides. J Immunol **1988**;41:2388–93.
42. Gordon D, Bray M, Morley J. Control of lymphokine secretion by prostaglandins. Nature **1976**;262:401–2.
43. Dinarello CA, Bishai I, Rosenwasser LY, Coceani F. The influence of lipooxygenase inhibitors on the in vitro production of human leukocytic pyrogen and lymphocyte activating factor (interleukin-l). Int J Immunopharmacol **1984**;6:43–50.
44. Klebanoff SJ, Vadas MA, Harlan JM, et al. Stimulation of neutrophils by tumor necrosis factor. J Immunol **1986**;136:4220–5.
45. Gamble JR, Harlan JM, Klebanoff SJ, Vadas MA. Stimulation of the adherence of neutrophils to umbilical vein endothelium by human recombinant tumor necrosis factor. Proc Natl Acad Sci USA **1985**;82:8666–71.