Dysregulation of In Vitro Cytokine Production by Monocytes during Sepsis

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Abstract

The production by monocytes of interleukin-1α (IL-1α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNFα) in intensive care unit (ICU) patients with sepsis syndrome ($n = 23$) or noninfectious shock ($n = 6$) is reported. Plasma cytokines, cell-associated cytokines within freshly isolated monocytes and LPS-induced in vitro cytokine production were assessed at admission and at regular intervals during ICU stay. TNFα and IL-6 were the most frequently detected circulating cytokines. Despite the fact that IL-1α is the main cytokine found within monocytes upon in vitro activation of cells from healthy individuals, it was very rarely detected within freshly isolated monocytes from septic patients, and levels of cell-associated IL-1β were lower than those of TNFα. Cell-associated IL-1β and TNFα were not correlated with corresponding levels in plasma. Upon LPS stimulation, we observed a profound decrease of in vitro IL-1α production by monocytes in all patients, and of IL-1β, IL-6, and TNFα in septic patients. This reduced LPS-induced production of cytokines was most pronounced in patients with gram-negative infections. Finally, monocytes from survival patients, but not from nonsurvival ones recovered their capacity to produce normal amounts of cytokines upon LPS stimulation. In conclusion, our data indicate an in vivo activation of circulating monocytes during sepsis as well as in noninfectious shock and suggest that complex regulatory mechanisms can downregulate the production of cytokines by monocytes during severe infections. (*J. Clin. Invest.* 1991. 88:1747–1754.) Key words: interleukin-1 • interleukin-6 • tumor necrosis factor • endotoxin • infection

Introduction

Cytokines are essential for hematopoiesis and immune responses, and play a key role in the defense against infectious diseases. In animal models, circulating IL-1, IL-6, and tumor necrosis factor (TNF)[1] have been documented after injection of whole bacteria or endotoxins prepared from gram-negative bacteria or toxins derived from gram-positive bacteria (1–7). Similarly in human, bolus injection of LPS induces a transient

1. Abbreviations used in this paper: ARDS, adult respiratory distress syndrome; SAPS, simplified acute physiological score; TNF, tumor necrosis factor.

appearance of IL-1, TNFα, and IL-6 in plasma (8–10). The presence of circulating cytokines has been often reported in the plasma of infected patients (10–19). Most of the deleterious effects of microbial toxins, especially endotoxins, can be mimicked by IL-1 and TNF. This was demonstrated in case of pulmonary injury (20, 21), adult respiratory distress syndrome (ARDS) (22, 23), hemodynamic, and cardiovascular dysfunctions (24–26), intravascular coagulation (27, 28), and tissue lesions (29, 30). IL-6, together with IL-1 and TNF, plays a major role in the production of acute phase proteins by hepatocytes (31–33) and in fever (34, 35). In addition, these cytokines are able to induce each other via cascade events (36–40), and potentiation of pathologic effects are observed consecutively to the synergistic action of these mediators together (41–44), or with bacterial products (45). Levels of TNF have been associated with the severity of illness (18) and amounts of circulating TNF and IL-6 have been reported to correlate with the prognosis of the disease (10–13, 17–19). During infection, the major cytokine production is very probably consecutive to a nonspecific activation of monocytes/macrophages by microbial-derived products. The exact nature of the cells involved in the process leading to the presence of circulating cytokines has been poorly studied. We had previously shown in hemodialyzed patients that the presence of IL-1 activity in cell lysates could reflect the in vivo monocyte activation (46). Such an in vivo activation has also been reported by Haeffner-Cavaillon et al. (47) who observed the presence of IL-1β in cell lysates from monocytes of patients undergoing cardiopulmonary bypass. Thus, in order to characterize the origin of the circulating cytokines, we measured IL-1, TNFα, and IL-6 production by monocytes from patients with sepsis. Because cytokines, mainly IL-1, can be accumulated within the cells, we investigated the content of freshly isolated monocytes and we studied the capacity of these cells to release in vitro these cytokines in the absence of exogeneous stimulus as well as after endotoxin stimulation.

Methods

Patients. 29 patients admitted to the Intensive Care Unit (ICU) were studied (Table I). 23 (15 males, 8 females; mean age±SD = 65±15; range, 32–81) fulfilled the criteria for the sepsis syndrome as described by Bone et al. (48), including evidence of an infectious site and a sign of inadequate organ perfusion (mental dysfunction, hypoxemia, elevated plasma lactate, or oliguria). Of these, 14 met the criteria for septic shock (sepsis syndrome plus a systolic BP < 90 mmHg or a decrease from baseline in systolic BP > 40 mmHg), and seven met the criteria for ARDS (bilateral alveolar infiltrates, pulmonary wedged pressure < 18 mmHg, and PaO_2/FiO_2 < 150 with positive expiratory pressure ≥ 5 cm H_2O). The infectious conditions included pneumonia ($n = 8$), peritonitis ($n = 4$), catheter-related sepsis ($n = 3$), pyelonephritis ($n = 2$), infectious aortic aneurism ($n = 1$), aortic graft sepsis ($n = 1$), cholangitis ($n = 1$), cellulitis ($n = 1$), meningitis ($n = 1$), and meningococcal arthritis ($n = 1$). The pathogenic agents were gram-negative bacilli (GNB) in nine cases, gram-positive cocci (GPC) in seven, association of GNB and GPC in six, and *Mycobacterium tuberculosis* in one. 10 patients were bacteremic. The simplified acute physiologic score (49) on admis-

Table I. Clinical Parameters of the ICU Patients

Infections	Patients	Infection site or type of shock	Outcome*	Shock	ARDS[‡]	Initial SAPS	Bacteremia
Gram-negative bacteria	1	Pneumonia	R	yes	no	12	yes
	2	Infect. aortic aneurism	D	yes	no	10	yes
	3	Pneumonia	R	yes	no	9	no
	4	Pyelonephritis	R	yes	no	13	yes
	5	Pneumonia	R	yes	no	18	no
	6	Cholangitis	D	yes	yes	23	yes
	7	Peritonitis	D	yes	no	15	no
	8	Pneumonia	D	yes	yes	18	no
	9	Septicemia arthritis	R	yes	no	11	yes
Combination of gram-negative	10	Meningitis	R	yes	no	19	yes
and gram-positive bacteria	11	Peritonitis	,D	yes	yes	15	no
	12	Peritonitis	R	no	no	16	no
	13	Pneumonia	R	yes	yes	9	no
	14	Intravasc. catheter	R	yes	no	12	no
	15	Pyelonephritis	R	no	no	13	no
Gram-positive bacteria	16	Aortic graft	D	no	yes	14	no
	17	Intravasc. catheter	R	no	yes	14	yes
	18	Endocarditis	R	no	no	5	yes
	19	Intravasc. catheter	R	no	yes	9	yes
	20	Pneumonia	R	no	no	8	yes
	21	Pneumonia	R	no	no	8	no
	22	Cellulitis	R	yes	no	13	no
Tuberculosis	23	Tubercul. pneumonia	D	no	no	13	no
Mean			30% D	61% shock	30% ARDS	12.9±4.2	43% bacteremia
No infection	24	Cardiogenic	D	yes	yes	15	no
	25	Hypovolaemic	R	yes	no	7	no
	26	Cardiogenic	D	yes	no	14	no
	27	Cardiogenic	D	yes	yes	12	no
	28	Hemorrhagic	R	yes	no	9	no
	29	Hemorrhagic	R	yes	no	19	no
Mean			50% D	100% shock	33% ARDS	12.7±4.3	none

* R = recovery; D = death. ‡ Adult respiratory distress syndrome. § Simplified acute physiological score.

sion was 12.9±4.2 (mean±SD) for the 23 septic patients. All infected patients (mean weight±SD = 66±14 kg; range, 33–90) received parenteral feeding (glucids + lipids = 1,650±605 kCal/d, nitrogen 9.5±5.5 g/d). Treatment of infected patients included antibiotics ($n = 23$), morphinics and benzodiazepin ($n = 15$), heparin ($n = 19$), catecholamines ($n = 12$), corticosteroides ($n = 3$; 0.5–1 mg/kg per d), insulin ($n = 7$), and cyclooxygenase inhibitors ($n = 2$).

The six remaining patients (five males, one female; mean age = 64±18; range, 31–78) were admitted because of cardiogenic ($n = 3$), hemorrhagic ($n = 2$), or hypovolemic shocks ($n = 1$). The simplified acute physiologic score on admission was 12.7±4.3. All patients with noninfectious shock (mean weight = 71±17 kg; range, 56–100) received parenteral feeding (glucids + lipids = 1,465±525 KCal/d; nitrogen = 13 g/d). Their treatment included antibiotics ($n = 3$), morphinics ($n = 5$), benzodiazepin ($n = 4$), cyclooxygenase inhibitors ($n = 1$), catecholamines ($n = 3$), heparin ($n = 4$), and insuline ($n = 2$).

10 patients died in the ICU. Healthy controls ($n = 14$) were donors of either sex from the blood bank and research workers from our laboratory.

Collection of blood samples. Blood was collected within 2 d after admission of the patients in the ICU, daily during the 3 following days,

and then weekly until day 14 or 21 if the patients were still in the ICU. Last measurements were performed on day 14±1.5 (range, 5–21) for survival patients and on day 7±2.5 (range, 1–21) for nonsurvival patients.

Plasma. 20 ml of blood were drawn on EDTA. Blood was immediately placed in ice and processed within 2 h. 200 µl of aprotinin (a protease inhibitor; 0.67 TUI/ml of blood) (Sigma Chemical Co., St. Louis, MO) were added and tubes were centrifuged at 400 g and 4°C for 10 min. Plasma was transferred in eppendorf tubes and further centrifuged at 10,000 g and 4°C for 5 min. Plasma was then aliquoted and kept at −30°C.

Monocytes. Cell pellet was resuspended to 25 ml in RPMI-1640 medium (Gibco, Grand Island, NY) and centrifuged over ficoll (Milieu de Separation des Lymphocytes, Eurobio, Les Ul, France) for 25 min at 150 g at room temperature. After washing peripheral blood mononuclear cells were numerated and nonspecific esterase (NSE) staining performed (50). 5×10^5 NSE$^+$ cells/0.5 ml RPMI medium/well (24-well multidish plates, Falcon Labware, Oxnard, CA) were let to adhere for 1 h as previously described (51).

Collection of cell-associated cytokine content. Immediately after adherence, nonadherent cells were removed, adherent cells were washed,

and 0.5 ml of fresh RPMI medium were added. The plates were then frozen as previously described (52). Three cycles of freezing-thawing were performed before harvesting the cell lysate which was then centrifuged and kept at $-30°C$ before cytokine content assessments.

In vitro cultures. In vitro cultures were performed as previously described (50, 51). Briefly, after washing, adherent cells were cultured for 24 h in 0.5 ml RPMI-1640 medium supplemented with antibiotics, 1 μg/ml indomethacin, and 0.2% normal human serum, in the absence (spontaneous cytokine production) or in the presence of 2 μg/ml *Neisseria meningitidis* LPS kindly provided by Dr. M. Caroff (Institut Biochimie, Orsay, France). This LPS was specially chosen for its potency to induce cytokine production (53). At the end of the culture, supernatants were harvested, and cell lysates prepared as described above.

IL-6 bioassay. IL-6 activity was determined by using the specific 7TD1 IL-6-dependent cell line (54) kindly provided by Dr. J. Van Snick (Ludwig Institute for Cancer Research, Brussels). Cells were cultured at a density of 1,200 cells/well (96-well multidish plates, Falcon Labware, Oxnard, CA) in 100 μl of RPMI medium supplemented with antibiotics, 2-mercaptoethanol (5×10^{-5} M) and 10% FCS, in the presence of serial dilutions of cell supernatants or heat inactivated (30 min, $56°C$) plasma. After 4 d of culture at $37°C$, the proliferation was monitored by a dye method (55). Briefly, 125 μg of tetrazolium salts (MTT) were added to each well and after 1–2 h of incubation at $37°C$, the test was then stopped with 100 μl/well of an extraction buffer (20% SDS, 50% DMF in H_2O, 2.5% HCl 1 N, 2.5% of an 80% acetic acid solution, pH 7.4). After an overnight incubation at $37°C$, the optical densities were measured at 540 nm using an automated microELISA autoreader. 1 U of IL-6 corresponds to half-maximum growth of the hybridoma cells. IL-6 activity detected in supernatants of LPS-stimulated monocytes and in plasma was completely abolished by the addition of 10 μg rabbit polyclonal anti-human IL6 antibodies (Genzyme Corp., Boston, MA).

TNFα RIA. The TNFα RIA was based on a competitive inhibition assay for TNFα as developed by Van der Meer et al. (56), and modified in our laboratory. In every assay, 10 standards of TNFα (Rhône Poulenc, France) containing 0, 40, 80, 150, 300, 600, 1,250, 2,500, 5,000, and 10,000 pg/ml were employed. All standards and cell supernatant or cell lysate samples were diluted in a BSA buffer. TNFα in plasma was assessed in undiluted samples. On day 1, 100 μl of a rabbit anti-TNFα antiserum (a kind gift of Catherine Rougeot, Institut Pasteur) diluted 1:10,000 to precipitate 35% of the radiolabeled TNF was added to 100 μl of standards or samples. To determine the nonspecific binding, 100 μl of BSA buffer was added to a tube instead of sample. Then 300 μl of BSA buffer were added to each tube. After vortexing, the tubes were incubated for 24 h at room temperature. On day 2, 100 μl of ^{125}I-TNFα solution (30 μCi/μg, New England Nuclear, Boston, MA) containing $\sim$ 10,000 cpm was added to each tube, and after vortexing left for a further incubation at room temperature for 24 h. On day 3, 500 μl of BSA buffer containing 6% of polyethylene glycol 8000 (Sigma Chemical Co.), 1% of horse anti–rabbit IgG (C. Rougeot, Institut Pasteur) and 0.1% of normal rabbit serum were added. The tubes were vortexed and incubated for 1 h at room temperature. The tubes were then centrifuged at 1,500 g for 15 min at room temperature. Thereafter, the supernatants were discarded and the tubes were kept inverted for 30 min and drained on absorbant paper. Tubes were counted in a gamma counter and the value for nonspecific binding was subtracted. All standards and samples were expressed as percentage of the standard containing no TNFα (zero standard). The concentrations of TNFα in pg/ml on the logarithmic x-axis were plotted against the binding percentage on a logarithmic y-axis. The standard curve obtained was used to determine the concentrations of TNFα in samples. The detection limit was 70 pg/ml (set binding of 95% of zero standard).

IL-1β RIA. To remove putative factors present in plasma that interfere with IL-1β RIA, a chloroform extraction was performed as described by Cannon et al. (57). 1 ml of chloroform was transfered in 1.5 ml eppendorf tube containing 500 μl of plasma. Each tube was mixed vigorously for 10 min and centrifuged at 10,000 g for 10 min at $4°C$. This operation was repeated twice. The tube was left undisturbed for 20 min at room temperature. The aqueous phase was collected and stored at $-30°C$ until assaying. A similar RIA protocol as that used for TNFα measurement was employed to determine the concentration of IL-1β. Standard IL-1β was obtained from Rhône Poulenc (France), rabbit anti-IL-1β antiserum diluted (1:100) was purchased from Genzyme Corp. and ^{125}I-IL-1β (126–253 μCi/μg) from New England Nuclear. On day 3, 500 μl of BSA buffer containing 6% polyethylene glycol 8000, 1% of sheep anti–rabbit IgG (Sigma Chemical Co.) and 0.05% normal rabbit serum were added to each tube. Determination of IL-1β concentrations in plasma and samples were calculated as described above. The detection limit was 70 pg/ml.

IL-1α ELISA. The IL-1α concentrations were determined by a specific ELISA method developed in our laboratory using two anti-IL-1α monoclonal antibodies which have been obtained in collaboration with J.-C. Mazié (Hybridolab, Institut Pasteur). After immunization of Balb/c mice with recombinant human interleukin-1α (hrIL-1α) kindly provided by Rhône Poulenc, spleen cells were fused with X63-Ag8.653 mouse myeloma cells (58). Hybridoma cells were then tested for antibody production against IL-1α by ELISA. Cells from positive wells were cloned by limiting dilutions in microtiter plates. Immunoglobulin subclass determinations were performed using MAb isotyping from Amersham International, Amersham, UK). Hybridomas were expanded for antibody production by intraperitoneal injection into pristane-primed mice. Two antibodies were selected: one monoclonal (28-9) is an IgG1, and the other (2-12) is an IgG2b. They do not recognize the same epitope on IL-1α molecule as judged by competition ELISA and do not bind to IL-1β. Both inhibit the biological activity of IL-1α in the thymocyte comitogenic assay.

On day 1, Luxlon ELISA microtiter plates were coated with 100 μl of monoclonal mouse IgG1 anti-rhIL-1α (10 μg/ml in carbonate buffer) and incubated 2 h at $37°C$. The plates were washed three times with 0.1% tween-20 PBS. Standards (0, 10, 30, 100, 300, 1,000, 3,000, and 10,000 pg/ml rhIL-1α) or cell supernatant or cell lysate samples diluted in 1% BSA, 0.1% Tween-20 PBS were added to coated wells and incubated overnight at $4°C$. On day 2, the plates were washed three times and 100 μl of the second monoclonal mouse anti-rhIL-1α (IgG2b) (1:2,000 in BSA/Tween/PBS) were added into each well. Plates were incubated for 3 h at $37°C$. After washing, 100 μl of peroxydase conjugate anti–mouse IgG2b (1:1,000) (ICN Biomedicals, Inc., Irvine, CA) were added to each well and the plates were left for 1 h at $37°C$. After washing, enzymatic activity was detected with a phosphate citrate buffer containing 1 mg/ml O-phenylenediamine dihydroxychloride (Sigma Chemical Co.) and hydrogen peroxyde (0.06%). The reaction was stopped with 50 μl of HCl 3 N and the absorbance was read at 492 nm in a microplate reader (Titertek multiskan MC340; Flow Laboratories, Inc., McLean, VA). The levels of IL-1α in the samples were calculated by reference to the standard curve. The detection limit of IL-1α was 30 pg/ml.

All cytokine measurements have been assessed using the international standards (National Institute for Biological Standards and Controls, Herfordshire, UK).

Statistical analysis. Statistical analyses were carried out using the paired or unpaired student t test.

Results

Circulating cytokines. Cytokines were under detection limits in plasma of healthy controls. The levels of plasma TNFα, IL-6, and IL-1β at admission and the maximum levels of circulating cytokines during longitudinal survey are plotted in Table II. No IL-1α could be detected. Circulating TNFα could be detected in 86% of the patients, IL-6 in 83%, and IL-1β in only 57% of the patients. Maximum levels of TNFα and IL-1β were significantly higher than the corresponding levels at admission. Significant amounts of circulating cytokines were also observed in patients with nonseptic shock. At the end of the survey, only two among 16 survival infected patients still had detectable

Table II. Mean of Plasma Cytokines Levels Measured within 2 d after Patients Admission in the ICU Compared to the Maximum Values Observed during the Longitudinal Study of Septic Patients and Patients with Nonseptic Shock

	TNFα	P*	IL-1β	P	IL-6	P
	pg/ml		pg/ml		U/ml	
First determination	133±23		188±51		1079±396	
Range (% patients)	0–420 (66%)		0–1630 (54%)		0–11800 (77%)	
Maximum observation	197±20	<0.001	305±74	0.01	4172±2011	0.08
Range (% patients)	0–470 (86%)		0–1630 (57%)		0–46000 (83%)	

* Comparisons between first determination at admission in ICU and the maximum observation, paired Student t test.

plasma TNFα, whereas six of seven nonsurvival patients had circulating TNFα.

Cell-associated cytokines in freshly isolated monocytes. Cell-associated IL-1α and IL-1β were never detected in lysates of freshly isolated monocytes from healthy donors, and most controls had no TNFα (50±29 pg/ml). Maximum levels of cell-associated IL-1α, IL-1β, and TNFα in monocytes of patients with sepsis syndrome are shown in Fig. 1. Data at admission were not significantly different from the maximum levels observed during the longitudinal survey. IL-1α was usually absent in cell lysates and only found in 35% of septic patients. IL-6 was not found in monocytes lysates. 56% of the septic patients had cell-associated IL-1β at any time of the evolution (mean±SEM = 206±73 pg/ml), and 91% of the patients had cell associated TNFα (625±126 pg/ml). Patients with nonseptic shock had also significant amounts of cell-associated cytokines (data not shown). No differences were noticed between the levels of cell-associated IL-1β and TNFα in monocytes of survival or nonsurvival patients. No correlation was found between plasmatic and cell-associated levels of IL-1β and TNFα. Furthermore, although at the end of the longitudinal study, plasma levels of TNFα were under detection limits in 14 of 16 survival infected patients, significant levels of cell-associated TNFα were still detectable in 14 of 16 patients.

In vitro cytokine production upon LPS stimulation. At admission, a decreased in vitro cytokine production by mono-

cytes upon stimulation by LPS was noticed among infected patients as compared to healthy controls (Fig. 2). The initial levels were not significantly different between survival and nonsurvival patients. During the longitudinal survey, minimum levels of released IL-1α, IL-1β, TNFα, and IL-6 and cell-associated IL-1α and IL-1β were reached. Comparisons to the levels of cytokine productions by monocytes from the healthy control group, are shown in Table III. Significant decreased levels of released and cell-associated IL-1α and IL-1β and released IL-6 and TNFα were noted in all ICU infected patients. The patients with gram-negative infections had lower cytokine production than patients with gram-positive ones. This last group had IL-1β and IL-6 productions close to controls. In patients with a nonseptic shock only IL-1α production was significantly lowered compared to controls.

A restoration of IL-1α and IL-1β (Fig. 3), IL-6 and TNFα (Fig. 4) was found in septic patients who survived. After LPS stimulation, levels close to normal or even slightly higher for IL-6 and TNFα were obtained. On the contrary, in septic patients who died, a lowered production of cytokines by monocytes upon LPS stimulation persisted until the last determination before death.

No correlation was found between the levels of cytokine produced by monocytes upon LPS stimulation and the severity score, the drugs which were used, or the nutritional status of the patients.
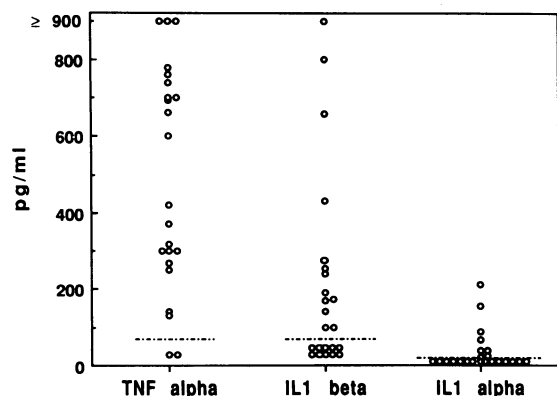

Figure 1. Maximum amounts of cell-associated cytokines found within freshly isolated monocytes from septic patients. (Mean±SEM of TNFα = 625±126 pg/ml; mean±SEM of IL-1β = 206±73 pg/ml; P = 0.005). Dotted lines represent the detection limits for TNFα, IL-1β, and IL-1α, respectively.
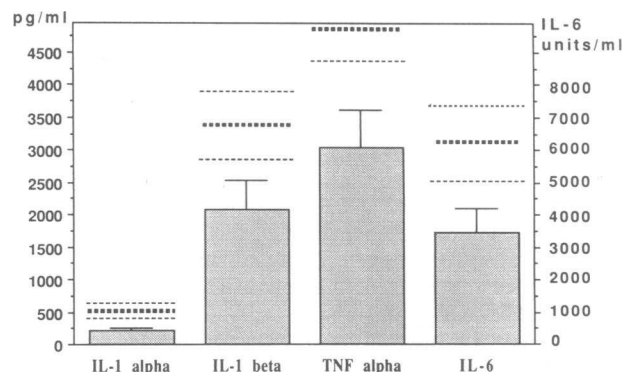

Figure 2. In vitro cytokine release upon N. meningitidis LPS activation of adherent monocytes from infected patients at admission in the ICU. Thick dashed lines represent the mean values of healthy controls and thin dashed lines represent±SEM. (Statistical analysis between controls and infected ICU patients: P < 0.01, P = 0.09, P < 0.05, and P = 0.06 for IL-1α, IL-1β, TNFα, and IL-6, respectively).

Table III. Levels of Cytokines Production by Monocytes Triggered by 1 µg Neisseria meningitidis LPS, in Healthy Controls, Patients with Sepsis Syndrome and Nonseptic Shock (Minimal Levels during ICU Stay)

	Controls ($n = 11-19$)	All infections ($n = 23$)*	Gram − and −/+ ($n = 15$)	Gram + ($n = 7$)	Nonseptic shock ($n = 6$)
Released IL-1α‡	520±109§	149±38	81±21	316±93	106±61
P‖		0.001	<0.001	0.24	0.03
Cell-associated IL-1α	6799±1119	1823±401	1114±380	3548±702	3023±576
P		<0.001	<0.001	0.07	0.05
Released IL-1β	3375±528	1523±348	1023±251	2795±857	3153±1210
P		0.005	<0.001	0.55	0.85
Cell associated IL-1β	1921±354	1102±185	1012±255	1428±209	2016±380
P		0.03	0.04	0.42	0.75
IL-6	6245±1250	2958±878	2477±1127	4380±1530	4465±963
P		0.03	0.04	0.42	0.45
TNFα	4852±539	1759±271	1614±282	2177±662	4000±1257
P		<0.001	<0.001	0.008	0.47

* Including one patient with tuberculosis; ‡ IL-1α, IL-1β, and TNFα measurements are expressed as pg/ml and IL-6 as U/ml;
§ mean±SEM; ‖ comparisons with values from healthy controls (Student t test).

Discussion

Significant amounts of circulating cytokines in septic patients have been well documented since 1987 (10–18). In animal models half-life of i.v. injected TNFα is ~ 6–7 min (59). A biphasic plasma clearance has been described for IL-1β (60) and IL-6 (61) with an initial rapid disappearance of 3 min and a subsequent slower one (1–4 h). In this context, the fact that cytokines might be detectable in plasma over a long period of time as demonstrated in this study (61a) as well as in several other ones (17, 62) suggests an intensive and continuous production. The cellular origin of these circulating cytokines remains to be fully elucidated. In the literature, little information is available on cell-associated cytokines in freshly isolated monocytes. As it is well known that IL-1 can be accumulated within activated monocytes, investigations have been mainly

concentrated on this cytokine (46, 47). IL-1α is the main cell associated form of IL-1 in LPS-stimulated normal human monocytes (63, 64). Thus, it was surprising to find very rarely IL-1α within the adherent cells isolated from septic patients. Cell-associated IL-1β was found in 56% of septic patients as well as in monocytes from patients with nonseptic shock. In our mind the discovery of frequent (90% patients) cell-associated TNFα in freshly isolated monocytes is interesting. Furthermore, cell-associated TNFα levels at admission (267±55 pg/ml) or maximum levels during ICU stay (625±126 pg/ml) were particularly high compared to those which could be detected in in vitro LPS-activated monocytes from healthy volunteers (i.e., 98±43 pg/ml) (Munoz, C., unpublished results). It is noteworthy that TNFα appears as the most frequent cytokine in plasma and within monocytes. Although we found cell-associated TNFα and IL-1β, their presence and amounts did not
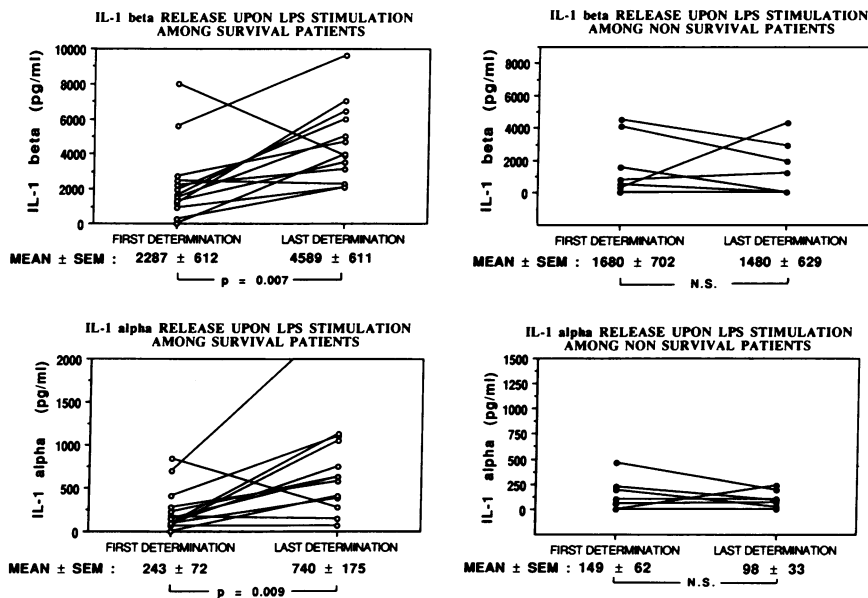

Figure 3. Comparison between first determinations (performed within 2 d after admission of the patients in the ICU) and last measurements (performed at the end of the followup of the patients) of LPS-induced IL-1α and IL-1β release by monocytes from survival ($n = 13$) and nonsurvival ($n = 7$) septic patients. The mean day of the last determination was 14±7 and 10±5 for the survival and the nonsurvival groups, respectively).
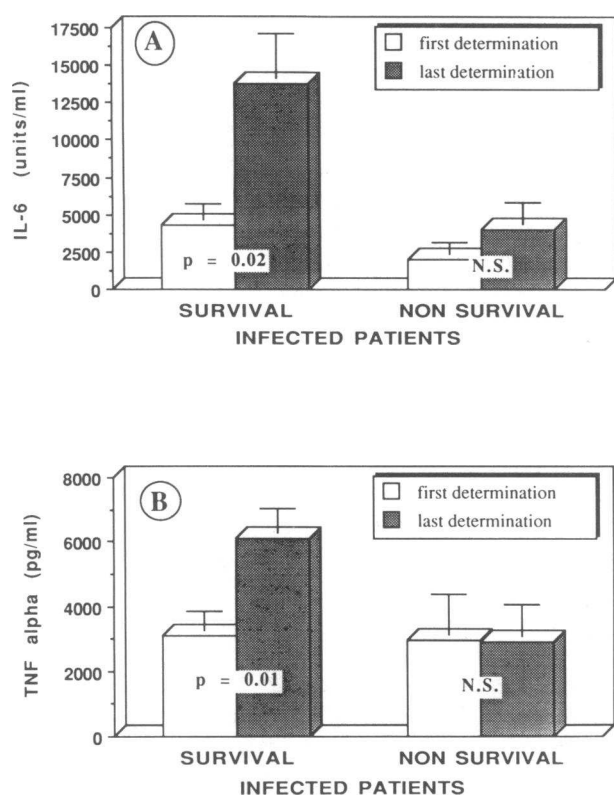
Figure 4. Comparison between first and last determinations of LPS-induced IL-6 (A) and TNFα (B) in vitro production by monocytes from survival and nonsurvival septic patients.

correlate with the levels of the corresponding circulating cytokines. However, it is likely that measurable circulating cytokines represent the excess of produced cytokines. One could assume that in vivo "reasonable" production would not be detected in plasma because all cytokines would be trapped by their specific high-affinity receptors on host cells. Indeed in the last determinations performed in survival patients, TNFα was often detected within freshly isolated monocytes, whereas plasma TNFα levels were back under detection limit values. Furthermore, the absence of correlation between plasma and cell-associated levels might also suggest that circulating monocytes are not the only sources of circulating cytokines and that other cells, like Kupffer cells or alveolar macrophages, could also participate to this production.

When monocytes were cultured in the absence of any stimuli, spontaneous release of TNFα and IL-1β were not significantly higher than in controls (data not shown). In this study we have investigated the behavior of isolated monocytes to respond in vitro to an LPS-induced activation. In septic patients, we found a profound impairment of LPS-induced IL-1α, IL-1β, TNFα, and IL-6 productions which were dramatically decreased. We have chosen optimal culture conditions (i.e., in the presence of indomethacin and 0.2% normal human serum) for which we established the levels of IL-1α, IL-1β, TNFα, and IL-6 produced by monocytes from normal healthy donors. Among peripheral blood mononuclear cells, a rather higher number of nonspecific esterase positive (NSE+) cells was obtained in the beginning of the follow up of the septic

patients. As a constant number of input NSE+ cells were cultured, a comparison between different cultures was possible. We did not observe any differences in adherence properties of monocytes from normal donors or septic patients. In the group of patients with noninfectious shock, a reduced production of IL-1α was also noticed, whereas this was not the case for IL-1β, IL-6, and TNFα. This observation confirms the fact that the regulation of IL-1α and IL-1β productions are under distinct pathways (65). This downregulation of cytokine production was especially significant for patients with gram-negative infections. In agreement with our findings, a recent case report indicates that IL-1 activity produced by monocytes from two children with Haemophilus influenza meningitis upon LPS stimulation was significantly reduced (66). On the contrary Fieren et al. (67) reported that peritoneal macrophages from patients on continuous ambulatory peritoneal dialysis with infectious peritonitis, had an increased IL-1β release upon in vitro challenge with LPS. In this study, only one of eight patients had a gram-negative infection.

Interestingly, the observed down production of cytokine production upon LPS stimulation was a transient event in survival patients. At the end of the survey, they recovered levels of in vitro cytokine productions close to those of healthy controls. Although this finding was expected, it ensured that despite a well-known interindividual heterogeneity in cytokine production, comparison could be made between groups.

The mechanisms underlying these phenomena remain to be fully elucidated and further experiments should address the question whether this dysregulation is a transcriptional or a posttranscriptional event, as shown in the rabbit model (68). Different parameters can be suggested to explain this dysregulation. Stress is known to generate a decrease immune responsiveness. However, we did not find any correlation between the severity scores and the levels of in vitro cytokine productions. Differences were found in IL-1β, IL-6, and TNFα production between patients with sepsis syndrome and patients with noninfectious shock although they had rather similar severity scores. Furthermore, the reduced production was not predictive for the outcome (recovery or death). All patients received different drugs; although none was directly associated with the observed phenomenom, one cannot exclude that an association between some of them may modify the in vitro cytokine production. However, the recovery of normal levels of cytokine productions among survival patients was observed while patients were still receiving most of these drugs. The nutritional status is also a factor which could influence the in vitro cytokine production. However, infected and noninfected groups had rather similar nutritional status. Because the phenomenon was mainly associated with gram-negative infection, we can hypothesize that our observation might account for the well-described endotoxin tolerance (69) which is accompanied by a decreased LPS-induced TNFα production (68, 70, 71). This is currently investigated using other stimuli than LPS to know whether this downregulation is specific for this activator. Indeed, Takayama et al. (72), using gamma interferon and muramyl dipeptide as inducers, showed that patients with sepsis had a greater TNFα production, as compared to normal controls.

The presence of cell-associated cytokine in circulating monocytes and the down regulation observed upon a further in vitro activation suggest the existence of complex regulatory mechanisms, and further experiments are required to under-

stand the relative roles of the different populations of mononuclear phagocytes.

Acknowledgments

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