Comparative Study of Cytokine Content in the Plasma and Wound Exudate from Children with Severe Burns

E. V. Mikhal'chik*,, J. A. Piterskaya***, L. Y. Budkevich***, L. Yu. Pen'kov***, A. Facchiano**, C. De Luca**, G. A. Ibragimova*, and L. G. Korkina*,****

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 148, No. 11, pp. 524-528, November, 2009
Original article submitted April 8, 2009

The content of 27 cytokines was measured in blood plasma from 19 children with severe uncomplicated burns (group 1) and complicated burns (septic toxemia, toxemia, and pneumonia; group 2). Before surgical treatment (day 4 (±2) after burn), significant differences were found in the concentrations of interleukin-1 receptor antagonist, interleukin-6, interleukin-8, interleukin-10, tumor necrosis factor-α, interferon-γ, MCP-1, and granulocyte colony-stimulating factor. Cytokine concentration in group 2 patients was much higher than in group 1 patients and healthy children. The concentrations of interleukin-6, interleukin-8, and MCP-1 in group 1 patients significantly surpassed the normal level. Cytokine concentration in the plasma and wound exudates and myeloperoxidase activity in wound exudates from 4 patients of group 2 were measured over 18 days after burn. The inflammatory response was characterized by an increase in the content of interleukin-1β, interleukin-8, MCP-1, tumor necrosis factor-α, MIP-1α, and granulocyte-macrophage colony-stimulating factor in the wound (as compared to that in the plasma). Activity of myeloperoxidase in all patients was shown to correlate with the amount of MIP-1α (r=0.47), tumor necrosis factor-α (r=0.47), and granulocyte-macrophage colony-stimulating factor (r=0.55, p<0.05). Interleukin-8 concentration was beyond the limits of calibration. No correlation was found between the concentration of any of 27 cytokines in blood plasma and exudate. Our results indicate that during active surgical treatment, the wound serves as the source of inflammatory cytokines. Cytokines play a role in the systemic response and increase the degree of local inflammation, which modulates the number and activity of wound neutrophils.

Key Words: *severe burns; cytokines; wound exudate; myeloperoxidase*

Severe burns induce the inflammatory response, which is accompanied by the release of various cytokines. This reaction is particularly pronounced during the development of serious complications (*e.g.*, sepsis) [5]. Proinflammatory cytokines interleukin-1 (IL-1), IL-2, IL-6, IL-8, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) can potentiate the respiratory burst in neutrophils and macrophages. The results of previous studies suggest that overproduction of radicals by leukocytes is accompanied by oxidative tissue damage and polyorgan failure [10].

The wound is the major source of postburn inflammation. Macrophages, lymphocytes, fibroblasts, keratinocytes, and endothelial cells are involved in wound healing. Activity of these cells is regulated by various interleukins and growth factors. IL-1α, IL-1β,

*Russian State Medical University, Moscow; **Italian Dermatology Institute, Rome; ***G. N. Speranskii Children's Municipal Clinical Hospital No. 9, Moscow, Russia. ***Address for correspondence:*** lemik2007@yandex.ru. E. V. Mikhal'chik

0007-4888/09/1485-0771

IL-6, IL-8, EGF, bFGF, and TGF-β were found in the exudate of burn blisters [9].

The course of severe wounds in patients can be complicated by secondary necrosis. Activated neutrophils generate oxygen radicals and proteases, which is followed by spontaneous deepening of the wound. The burnt skin can serve as the source of IL-8. This cytokine is a potent chemoattractant and stimulator of neutrophils [6]. Our previous studies showed that intact neutrophils are present in the wound of burn patients even 3 weeks after injury [2]. Surgical cleaning of the burn wound and skin autografting can accelerate wound healing and prevent secondary necrosis [8].

The regulation of neutrophil activity is an important effector component of inflammation and recovery. Hyperactivation and impairment of neutrophil apoptosis in the wound can be followed by deceleration of wound healing. Hyperactivation of circulating neutrophils may cause a variety of systemic complications. It cannot be excluded that removal of burn scab is accompanied by the release of wound cytokines into the circulation [7].

Here we compared the cytokine profile of blood plasma and wound exudate in children with severe burns during active surgical treatment.

MATERIALS AND METHODS

We examined 19 children with grade IIIB-IV burn injuries. Serious complications (sepsis and pneumonia) occurred in 9 of 19 patients (group 2) over 2 weeks after injury. Surgical treatment of burn wounds was conducted in combination with anti-inflammatory and antibacterial therapy.

Cytokine content in patients was measured before surgical treatment (days 2-5 after injury). The concentrations of cytokines in blood plasma and wound exudate from 4 patients of group 2 were measured over 18 days after admittance to the burn center.

Plasma samples were obtained by centrifugation of the peripheral blood, which contained an anticoagulant heparin. The wound exudate was collected by means of extraction through paper filters.

Cytokine content was measured by enzyme immunoassay (Bioplex technology, Bio-Rad Laboratories Inc.) according to manufacturer's recommendations. We studied the expression of the following 27 cytokines: IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, eotaxin, FGF-β, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN-γ, IP-10, MCP-1, MIP-1α, MIP-1β, RANTES, PDGFbb, TNF-α, and VEGF. The samples of blood plasma and wound exudate were incubated with anticytokine antibody-labeled microbeads for 30 min. After incubation, these samples were washed. The beads were incubated with a mixture of peroxidase-labeled antibodies against cytokines. After washing, the beads were treated with a solution of streptavidin and phycoerythrin. The concentration of cytokines was estimated using a laser detector (Bio-Rad) and expressed in pg/ml. The estimated values were within the limits of calibration. To compare the amount of cytokines in blood plasma and exudate, the concentrations were calculated per protein content (method of Lowry).

Myeloperoxidase (MPO) activity in wound exudate was measured spectrophotometrically [3].

Plasma samples from 8 healthy children were assayed to derive the standard values.

The results were analyzed by Statistica 6.0 software and expressed as $M \pm m$ (M, mean value; m, standard deviation). The data were analyzed by means of nonparametric descriptive statistics. The significance of between-group differences was estimated by Mann–Whitney test. The significance of differences between the samples was estimated by Wilcoxon test. The differences were significant at $p<0.05$. The correlation coefficients were calculated by Pearson's test (significance level $p<0.05$).

RESULTS

Table 1 shows the comparative characteristics of patients from two groups. Intergroup differences were found in the area of deep burns, which required skin autografting. Toxemia or septic toxemia (6 patients), pneumonia (2 patients), and combination of these disorders (1 patient) were revealed in group 2 patients (days 3-11 after injury).

The concentration of 27 cytokines in blood plasma from burn patients was measured on day 4 (±2) after injury (before surgical treatment). The content of 8 cytokines in group 2 patients (IL-1ra, IL-6, IL-8, IL-10, TNF-α, IFN-γ, MCP-1, and G-CSF) and 3 cytokines in group 1 patients (IL-6, IL-8, and MCP-1) significantly exceeded the normal level (Table 2). It should be emphasized that the amount of IL-6, IL-8, and MCP-1 in group 2 patients was higher than in group 1 patients (statistically significant differences for IL-6 and IL-8).

High concentration of proinflammatory cytokines in blood plasma from patients with serious complications probably contributes to an increase in the radical-producing activity of circulating neutrophils (estimated from whole-blood chemiluminescence) [1]. All cytokines (except for IL-1ra and IL-10; Table 2) cause prestimulation of neutrophils and potentiate the respiratory burst.

Simultaneous study of the plasma and wound exudate from 4 patients of group 2 was performed over

TABLE 1. Characteristics of Patients with Burn Injury

Parameter	Group	
	1, 10 patients	2, 9 patients
Age, years	9.8±3.0	8.3±4.0
Sex, male/female	6/3	5/4
Total burn area, % of body surface	33.0±16.4	46.1±17.8
Deep burn area, % of body surface	15.0±1.1	40.6±19.9*
Start of surgical treatment, days	4±2	4±2

Note. *p<0.05 compared to group 1.

18 days after admittance to the hospital. The measurements were conducted at 1-3-day intervals. Significant variations in study parameters were found during surgical treatment. These data are presented as 25% and 75% percentiles (Table 3). However, significant differences between the plasma and wound exudate were revealed by the analysis of mean values (Table 3).

IL-8 concentration in wound exudate was beyond the upper limit of calibration, which made it impossible to perform statistical analysis and correlation analysis. Our findings indicate that IL-8 concentration in wound exudate exceeds that in the plasma, which is consistent with published data [6,8,9].

The concentrations of IL-1β, IL-1ra, IL-8, MCP-1, MIP-1α, TNF-α, and GM-CSF in the exudate were higher than in blood plasma at all periods of the study.

By contrast, the concentrations of IL-2, IL-6, IL-10, G-CSF, IFN-γ, PDGFbb, and VEGF in blood plasma were higher than in wound exudate. No correlation was found between the contents of these cytokines in the plasma and exudate from 4 patients. Similar results for surgical wounds were obtained previously [4]. These data illustrate the specific features of the local inflammatory response.

Our results indicate that during active surgical treatment of patients with severe burns, the wound serves as the source of proinflammatory (IL-1β, IL-8, MCP-1, TNF-α, MIP-1α, and GM-CSF) and anti-inflammatory cytokines (IL-1ra). The majority of these cytokines (IL-1β, IL-8, MCP-1, TNF-α, MIP-1α, and GM-CSF) are prestimulatory factors for neutrophils and macrophages.

It should be emphasized that the increase in the content of IL-8, MCP-1, and TNF-α in blood plasma is associated with the risk of serious complications (Table 2). These cytokines probably cause hyperstimulation of radical production by neutrophils, which results in oxidative damage to various organs and development of complications.

The influx of cytokines into wounds is probably related to high concentration of potent chemoattractants in the exudate (*e.g.*, IL-8, MCP-1, and MIP-1α). This assumption is confirmed by the presence

TABLE 2. Concentration of Some Cytokines (pg/ml) in the Plasma from Burn Patients before the Start of Surgical Treatment (Day 4 (±2) after Injury)

Cytokines	Normal (healthy children)	Group 1	Group 2
TNF-α	2.2±0.7	6.6±0.7	13.7±1.3*
IFN-γ	13.7±2.4	17.6±3.3	44.9±6.8**$^{++}$
IL-6	2.5±0.2	22.4±4.0**	41.3±2.7**$^{+}$
IL-8	6.7±1.9	21.6±2.2*	143.5±15.9^{*+++}
MCP-1	10.3±2.4	123.1±11.3**	235.3±21.7**^{o}
G-CSF	25.2±7.7	43.3±4.7	112.8±11.0*$^{+}$
IL-10	1.2±0.6	1.3±0.1	8.3±0.7*$^{+}$
IL-1ra	129.7±12.6	124.4±8.6	431.2±37.6*$^{+}$

Note. *p<0.05 and **p<0.01 compared to normal (healthy children); ^{o}p=0.05, ^{+}p<0.05, and ^{++}p<0.01 compared to group 1.

TABLE 3. Average Content of Some Cytokines (pg/mg Protein) in the Plasma and Wound Exudate from Burn Patients

Cytokines	Plasma (39 measurements)			Wound exudate (39 measurements)			*p*
	percentiles		median	percentiles		median	
	25	75		25	75		
IL-1β	4.2	12.3	6.3	107.2	972.9	276.3	**
IL-1ra	462.6	1367.4	904.7	2506.8	37,266.0	11,266.8	**
IL-2	9.5	73.5	15.5	0.4	7.9	3.4	**
IL-4	1.2	2.9	1.9	1.6	4.7	2.2	0.067
IL-6	101.0	477.4	163.9	5.7	55.6	21.6	**
IL-7	4.6	28.2	14.3	2.8	15.7	6.3	0.118
IL-8	224.6	674.9	371.0	>2000	>2000	>2000	
IL-9	34.3	119.3	54.3	26.1	61.8	39.3	0.051
IL-10	10.9	32.3	15.5	1.3	2.8	1.9	**
IL-12	0.00	10.5	2.2	1.7	10.1	3.1	0.133
IL-13	0.00	2.07	0.07	0.00	4.21	0.55	0.101
IL-15	0.00	24.9	0.00	13.2	91.7	30.2	**
IL-17	31.3	106.4	52.0	52.6	117.7	84.6	0.095
Eotaxin	11.4	67.0	30.1	0.00	22.3	9.1	**
G-CSF	149.1	757.6	362.5	82.5	256.3	125.5	**
GM-CSF	28.0	62.0	41.7	51.0	102.8	71.6	**
IFN-γ	67.2	126.5	103.8	41.2	101.5	57.1	**
IP-10	113.7	371.4	223.5	25.5	730.2	97.1	0.708
MCP-1	344.7	2125.1	848.6	693.2	11 495.0	3036.1	**
MIP-1α	14.5	40.4	27.3	26.7	103.0	49.9	**
MIP-1β	216.3	486.2	295.7	161.6	707.5	334.7	0.857
PDGFbb	647.8	2544.6	1507.9	35.6	91.0	50.3	**
RANTES	OOR>	OOR>	OOR>	102.0	1145.0	716.9	
TNF-α	9.8	42.8	24.7	36.7	101.5	50.8	**
VEGF	102.0	229.6	135.8	60.1	173.8	73.5	**

Note. **$p<0.01$ compared to the exudate. OOR (out-of-range), beyond the limits of calibration.

of intact neutrophils in wound tissue even 3 weeks after injury [1].

A positive correlation was found between activity of MPO (marker enzyme for polymorphonuclear leukocytes) and concentrations of MIP-1α (r=0.47), MIP-1β (r=0.49), TNF-α (r=0.47), and GM-CSF (r=0.55) in wound exudate ($p<0.05$). Infiltration of wound tissue with neutrophils and high content of IL-8 are associated with the risk of secondary necrosis in burn wounds [9]. The results of our study indicate that burns wounds are characterized by high concentration of MCP-1, MIP-1α, TNF-α, and GM-CSF (in addition to the elevated level of IL-8). These compounds can modulate the number and function of neutrophils.

The dynamics of MPO activity and cytokine concentration in the plasma and exudate from patient B. of group 2 is shown in Figs. 1 and 2 (GM-CSF and TNF-α, respectively).

We conclude that the systemic inflammatory response after severe burns is characterized by a significant increase in the concentrations of IL-6, IL-8, and MCP-1. The development of serious complica-

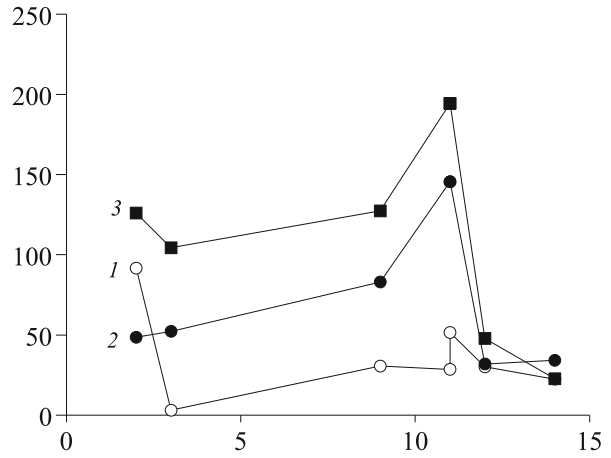

Fig. 1. GM-CSF concentration in the exudate and plasma (mg/g protein) and MPO activity in the exudate (U/g protein) of patient B. Total burn area, 35% of the body surface; deep burn area, 15% of the body surface. Tangential excision was performed on days 2 and 11 (extent of operation up to 15% of the body surface). The correlation coefficient between GM-CSF concentration and MPO activity in the exudate, r=0.85 (p<0.05). The correlation coefficient between GM-CSF concentrations in the exudate and plasma, r=0.28 (p>0.05). Plasma GM-CSF (*1*); exudate GM-CSF (*2*); exudate MPO (*3*). Here and in Fig. 2: abscissa, period after injury (days).

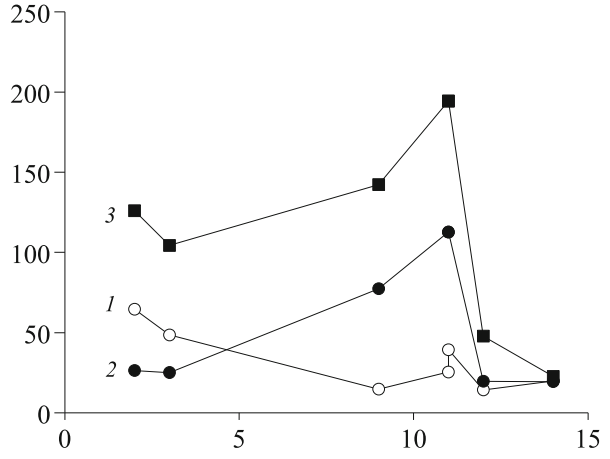

Fig. 2. TNF-α concentration in the exudate and plasma (mg/g protein) and MPO activity in the exudate (U/g protein) of patient B. Total burn area, 35% of the body surface; deep burn area, 15% of the body surface. Tangential excision was performed on days 2 and 11 (extent of operation up to 15% of the body surface). The correlation coefficient between TNF-α concentration and MPO activity in the exudate, r=0.71 (p<0.05). The correlation coefficient between TNF-α concentrations in the exudate and plasma, r=-0.17 (p>0.05). Plasma TNF-α (*1*); exudate TNF-α (*2*); exudate MPO (*3*).

tions is associated with an increase in the content of IL-ra, IL-6, IL-8, IL-10, TNF-α, IFN-γ, MCP-1, and G-CSF in blood plasma. The concentrations of IL-1β, IL-8, MCP-1, TNF-α, MIP-1α, and GM-CSF in burn wound of patients with systemic complications are much higher than in the plasma. These differences occur for at least 18 days after injury (period of active surgical treatment).

REFERENCES

1. E. V. Mikhal'chik, L. Yu. Pen'kov, L. Y. Budkevich, *et al*., *Det. Khir*., No. 3, 40-44 (2005).
2. E. V. Mikhal'chik, J. A. Piterskaya, V. A. Lipatova, *et al*., *Byull. Eksp. Biol. Med.*, **147**, No. 6, 696-699 (2009).
3. P. S. Andrews and N. J. Krinsky, *CRC Handbook of Methods for Oxygen Radical Research*, Ed. R. A. Greenwald, Florida (1985), pp. 297-302.
4. B. Carvalho, D. J. Clark, D. Yeomans, and M. S. Angst, *J. Vis. Exp*., **20**, 962 (2008).
5. C. C. Finnerty, D. N. Herndon, D. L. Chinkes, and M. G. Jeschke, *Shock*, **27**, No. 1, 4-9 (2007).
6. W. L. Garner, J. L. Rodriguez, C. G. Miller, *et al*., *Surgery*, **116**, No. 1, 42-48 (1994).
7. T. H. Han, S. Y. Lee, J. E. Kwon, *et al*., *Mediators Inflamm*., **13**, No. 4, 241-246 (2004).
8. S. Lu, J. Xiang, C. Quing, *et al*., *Chin. Med. J. (Engl.)*, **115**, No. 3, 323-325 (2002).
9. I. Ono, H. Gunji, J. Z. Zhang, *et al*., *Burns*, **21**, No. 5, 352-355 (1995).
10. D. A. Partrick, F. A. Moore, E. E. Moore, *et al*., *New Horiz*., **4**, No. 2, 194-210 (1996).
11. K. W. Shaheen, J. D. Punch, R. S. Rees, *et al*., *Surg. Forum*, **41**, 553-555 (1990).