Peer reviewed article

CD14 expression on monocytes and TNFα production in patients with septic shock, cardiogenic shock or bacterial pneumonia

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Summary

Objectives: In patients with septic shock, circulating monocytes become refractory to stimulation with microbial products. Whether this hyporesponsive state is induced by infection or is related to shock is unknown. To address this question, we measured TNFα production by monocytes or by whole blood obtained from healthy volunteers (controls), from patients with septic shock, from patients with severe infection (bacterial pneumonia) without shock, and from patients with cardiogenic shock without infection.

Measurements: The numbers of circulating monocytes, of CD14+ monocytes, and the expression of monocyte CD14 and the LPS receptor, were assessed by flow cytometry. Monocytes or whole blood were stimulated with lipopolysaccharide endotoxin (LPS), heat-killed *Escherichia coli* or *Staphylococcus aureus*, and TNFα production was measured by bioassay.

Results: The number of circulating monocytes, of CD14+ monocytes, and the monocyte CD14 expression were significantly lower in patients with septic shock than in controls, in patients with bacterial pneumonia or in those with cardiogenic shock ($p < 0.001$). Monocytes or whole blood of patients with septic shock exhibited a profound deficiency of TNFα production in response to all stimuli ($p < 0.05$ compared to controls). Whole blood of patients with cardiogenic shock also exhibited this defect ($p < 0.05$ compared to controls), although to a lesser extent, despite normal monocyte counts and normal CD14 expression.

Conclusions: Unlike patients with bacterial pneumonia, patients with septic or cardiogenic shock display profoundly defective TNFα production in response to a broad range of infectious stimuli. Thus, down-regulation of cytokine production appears to occur in patients with systemic, but not localised, albeit severe, infections and also in patients with non-infectious circulatory failure. Whilst depletion of monocytes and reduced monocyte CD14 expression are likely to be critical components of the hyporesponsiveness observed in patients with septic shock, other as yet unidentified factors are at work in this group and in patients with cardiogenic shock.

Keywords: cytokines; TNFα; septic shock; LPS; cardiogenic shock; pneumonia; CD14

Introduction

Septic shock is a syndrome in which pro-inflammatory and anti-inflammatory processes are dynamically interconnected and regulated. Central to these processes is the monocyte, which produces large quantities of pro-inflammatory cytokines when stimulated with bacterial products. During the course of sepsis, compensatory anti-inflammatory mechanisms may deactivate monocytes and lead to a state of relative "paralysis." Indeed, several studies have shown that monocytes of septic patients are hyporesponsive to potent infectious stimuli [1–2]. These observations support the hypothesis that anti-inflammatory response mechanisms may deactivate the monocytes and lead to a state of "immunoparalysis." However, it is unclear whether monocyte/macrophage hyporesponsiveness is the result of active down-regulation or simply reflects a state of "cellular exhaustion." To investigate whether down-regulation of TNFα production was related to shock or infection, we compared TNFα production of monocytes after stimulation with LPS, *E. coli* and *Staph. aureus*, using either whole blood or isolated peripheral blood mononuclear cells of normal vol-

unteers and of patients with septic shock, cardiogenic shock or bacterial pneumonia. To examine whether down-regulation of TNFα production was due to cell exhaustion or to a reduced expression of receptors for microbial products, we quantified the number of monocytes present in the blood as well as the expression of CD14, which is a receptor for lipopolysaccharide (LPS), and also for the peptidoglycan of Gram-positive organisms [4].

Methods

Patients

Three groups of patients observed in the Intensive Care Unit or in the Emergency Rooms of the Departments of Internal Medicine and Surgery were analysed. A control group of normal volunteers was included. The protocol of the study was approved by the Ethics Committee of the hospital.

Group 1: Patients with *septic shock* defined by leukocytosis, fever (>38°) or hypothermia (<35.6°C), tachycardia (>90 beats per minute), tachypnoea (respiratory rate >20 breaths per minute, or needing mechanical ventilation), and either hypotension (systolic blood pressure <90 mm Hg) or two of the following signs of systemic toxicity or peripheral hypoperfusion: unexplained metabolic acidosis (base excess = 5 mmol/l); arterial hypoxemia, ratio of the partial pressure of oxygen to the fraction of inspired oxygen <250 mm Hg; hyperlactacidemia; acute renal failure with urinary output <0.5 ml/kg/h; elevated prothrombin or partial thromboplastin time; reduction of platelet counts to less than half of the baseline value; sudden decrease in mental acuity; cardiac index of more than 4.l per minute per square metre of body surface area with systemic vascular resistance of less than 80 dynes × sec × cm^{-5}.

Group 2: Patients with *cardiogenic shock*, defined by hypotension (blood pressure <90 mm Hg or a decrease of >30 mm Hg in a hypertensive patient) or needing catacholamines to maintain blood pressure (dopamine or dobutamine >5 µg/kg/min), and by cardiac index <2 l/min/m^2 or systolic index <20 ml/m^2 and pulmonary capillary wedge pressure >16 mm Hg.

Group 3: Patients with severe community-acquired *bacterial pneumonia*. Community-acquired pneumonia was diagnosed according to the following criteria: a new infiltrate seen on chest X ray in the presence of two or more symptoms, signs or values such as fever of >38°C, cough, purulent sputum, leukocytosis of >10 000/mm^3. Pneumonia was considered to be microbiologically documented if isolates from sputum or tracheal aspirate cultures contained a predominant bacterium, more than 25 neutrophils and less than 10 epithelial cells per low power field (×100) on microscopy. Patients with nosocomial pneumonia were excluded.

Ten patients with septic shock (mean APACHE score: 22, ranging from 17 to 30), 10 patients with bacterial pneumonia (mean APACHE score:13, ranging from 4 to 23), 10 patients with cardiogenic shock (mean APACHE score:10, ranging from 6 to 20), and 10 controls were analysed. The clinical status of these patients has been previously described [5]. All patients with septic shock or pneumonia had positive cultures. Pathogens in patients with sepsis or pneumonia included *P. aeruginosa*, *E. coli*, *S. pneumoniae*, *S. pyogenes*, *Legionella* sepsis, and *P. mirabilis*. Two patients with sepsis had fungal (Candida) infections. Patients with cardiogenic shock had no signs of infection.

Blood sampling

Blood was collected in sterile heparin tubes containing. Samples were taken on day 1 or 2 in patients with septic or cardiogenic shock, and on day 1 in patients with bacterial pneumonia. Blood from normal volunteers was obtained from the Swiss Red Cross Transfusion Center.

Stimulation assay

200 µl heparinised whole blood was stimulated with 10 ng/ml of *E.coli* O111 LPS (Sigma, St. Louis, Missouri), 10^6 heat-killed *E. coli* O111 or *Staph. aureus*, for 4 hours at 37°C. TNFα was measured in the supernatant by bioassay, using WEHI clone 13 as target cells [6]. The lower limit of sensitivity of the bioassay is 25 pg/ml of bioactive TNFα.

An alternative protocol of stimulation was used in selected patients with septic shock. 10^6 peripheral blood mononuclear cells (PBMC), obtained by Ficoll centrifugation were mixed with 10% pooled plasma from normal volunteers and incubated with 10 ng/ml LPS for 4 hours at 37°C. TNFα was measured after 4 h incubation. In this short-term assay, monocytes are the major source of blood TNFα [6].

Endogenous TNFα measurement

Endogenous TNFα present in serum was measured by ELISA (Medgenix, Endotell, Basel, Switzerland).

Number of monocytes and CD14 expression

Total white blood cells and total monocyte counts were assessed in the haematology laboratory (Prof. M Schapira, CHUV). Peripheral blood mononuclear cells (PBMC) were obtained by Ficoll centrifugation. Using side-scatter analysis, the percentage of CD14+ monocytes was evaluated by reacting PBMC with an anti-CD14 mAb (Leu M3) labelled with FITC (Coulter clone, Instrumenten Gesellschaft, Zürich). CD14 expression was estimated by the mean fluorescence value observed on monocytes reacted with anti-CD14 labelled with FITC by FAC scan (FACS) analysis.

Statistical methods

Comparisons between groups were evaluated with the ANOVA ranks test.

Results

Production of TNFα in whole blood

Compared to controls, whole blood of patients with septic shock showed a profound reduction of TNFα release after stimulation with LPS, heat-killed *E. coli* or *Staph. aureus* (table 1). Production of TNFα was also markedly decreased in whole blood of patients with cardiogenic shock. In contrast, production of TNFα in whole blood of pa-

Table 1

TNFα production by whole blood of normal volunteers (controls), patients with septic shock, with cardiogenic shock or with severe bacterial pneumonia after stimulation with LPS, *E. coli* and *Staph. aureus.*

	TNFα (ng/ml) produced after stimulation with					
	LPS		*E. coli*		*Staph. aureus*	
		P		P		P
Controls	1.82 ± 1.69		6.01 ± 3.72		1.56 ± 1.94	
Septic shock	0.18 ± 0.44	0.003	0.36 ± 0.65	0.001	0.12 ± 0.26	0.003
Cardiogenic shock	0.23 ± 0.23	0.004	1.12 ± 0.78	0.002	0.31 ± 0.38	0.003
Pneumonia	1.20 ± 1.45	0.27	4.87 ± 4.66	0.87	1.32 ± 2.43	0.25

Whole blood was stimulated with 10 ng/ml LPS, 10^6 heat-killed *E. coli* or *Staph. aureus*, and TNFα measured in plasma after 4 h of stimulation. Data are expressed as mean ± SD of 10 samples for each group of patients. P values were calculated with the ANOVA rank test when comparing patients with controls. Values measured in the septic shock group versus those of the cardiogenic shock group, as well as values measured in the pneumonia group versus those of controls were not statistically different.

tients with bacterial pneumonia was similar to that of normal volunteers.

Role of monocytes versus plasma in TNFα down-regulation in septic shock patients challenged with LPS

The down-regulation of TNFα production observed in patients with septic shock could be mediated by inhibitory factors present in the plasma or due to hyporesponsive monocytes. To investigate this, whole blood of 6 patients with septic shock or their isolated PBMC in the presence of a pool of plasma obtained from normal volunteers were stimulated with LPS. TNFα production was 0.16 ± 0.22 ng/ml in whole blood and 0.17 ± 0.33 ng/ml in isolated PBMC, indicating that normal plasma did not restore TNFα production. Thus, in this group of patients with septic shock, defective TNFα production is not mediated by inhibitory factors present in plasma, but is probably due to quantitative and qualitative cellular defects.

Table 2

TNFα production and number of circulating monocytes, CD14+ monocytes, and CD14 expression in normal healthy volunteers (controls) and in patients with septic shock, cardiogenic shock or severe bacterial pneumonia.

	Median (range)	Mean ± SD
Controls		
TNFα (ng/ml)	1.35 (0.26–5.58)	1.82 ± 1.69
Total Mo $\times 10^{-3}$	17.5 (6–36)	20.9 ± 10.5
% CD14+ Mo	84 (52–92)	80.6 ± 12.1
CD14 (FU)	74 (50–90)	72.8 ± 16.4
Septic shock		
TNFα (ng/ml)	0 (0–1.4)	0.18 ± 0.44*
Total Mo $\times 10^{-3}$	<1 (<1–17)	6.3 ± 7.5*
% CD14+ Mo	35 (<1–95)	39.1 ± 38.6*
CD14 (FU)	15 (<1–70)	22.8 ± 24.4*
Cardiogenic shock		
TNFα (ng/ml)	0.13 (0.03–0.8)	0.23 ± 0.23*
Total Mo $\times 10^{-3}$	24 (11–44)	27.3 ± 12.8
% CD14+ Mo	85 (52–98)	85.4 ± 14.8
CD14 (FU)	72 (55–90)	71.3 ± 10.5
Bacterial pneumonia		
TNFα (ng/ml)	0.66 (0–4.4)	1.20 ± 1.45
Total Mo $\times 10^{-3}$	17 (<1–42)	19.8 ± 15.7
% CD14+ Mo	87 (<1–97)	70.5 ± 28.7
CD14 (FU)	55 (<1–92)	57.4 ± 25.9

Whole blood was stimulated with 10 ng/ml of LPS and TNFα content of supernatants was measured by bioassay. The total number of monocytes/ml of blood (Total Mo × 10^{-3}) was enumerated. Peripheral blood mononuclear cells (PBMC) were obtained by Ficoll centrifugation. The percentage of CD14+ monocytes was evaluated by reacting PBMC with a FITC-anti-CD14 mAb, and expression of CD14 was measured by FACS analysis (FU, fluorescence units). Results are expressed as median (range) and mean ± SD of the 10 individual samples/group. * p <0.001 when comparing controls with the other groups (ANOVA on ranks).

Correlation between monocytes, CD14 expression and TNFα production

We went on to investigate whether TNFα down-regulation was due to a reduction either in the total numbers of circulating monocytes or of CD14+ monocytes, or to a decreased expression of CD14 on monocytes. Indeed, LPS and other bacterial products induce cytokine release in blood via the monocyte receptor CD14 [7]. The total number of monocytes in the PBMC preparation, the percentage of monocytes expressing CD14 and the expression of CD14 evaluated by FACS analysis are shown in table 2. All but two patients with septic shock had decreased monocyte counts, a decreased number of CD14+ monocytes and a reduced expression of CD14 associated with low TNFα production. In contrast, patients with septic shock, patients with bacterial pneumonia (with the exception of 1 of 10) and those with cardiogenic shock had normal numbers of monocytes and CD14 expression similar to controls. Yet, despite normal monocyte counts and normal CD14 expression, TNFα production was markedly reduced in patients with cardiogenic shock.

Correlation between endogenous TNFα and TNFα produced by LPS stimulation in whole blood of septic shock patients

Initial serum TNFα concentrations in patients with septic shock were measured by ELISA, which measures both unbound TNFα and TNFα bound to soluble TNF receptors. Circulating TNFα levels ranged from 80 pg/ml to 520 pg/ml (Figure 1). However, this TNFα was not biologically active as it was undetectable by bioassay, which only detects unbound TNFα (data not shown), indicating that in these patients TNFα was associated with circulating TNF receptors. TNFα produced by stimulating whole blood of these patients with LPS was active by bioassay, and inversely correlated with serum TNFα initially present in the samples (fig. 1).

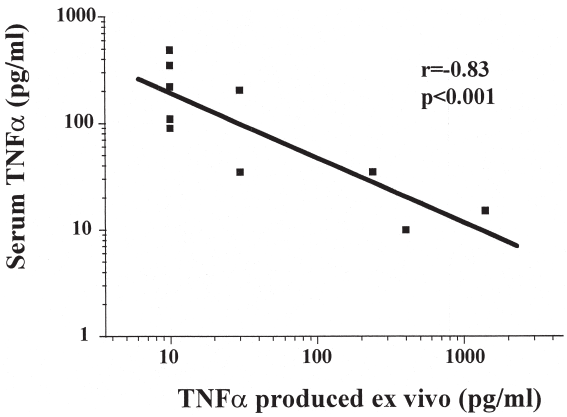

Figure 1

Correlation between serum concentrations of TNFα and TNFα produced ex vivo by LPS-stimulated blood of patients with septic shock. Serum TNFα was measured by ELISA. TNFα produced ex vivo by whole blood stimulated with LPS was measured by bioassay.

Discussion

A profound down-regulation of monocyte activation by bacterial products as measured by TNFα production was observed in patients with septic shock or with cardiogenic shock, but not in patients with bacterial pneumonia. Patients who did not produce ex vivo TNFα in response to LPS had the highest serum TNFα levels, suggesting that when monocytes have been activated in vivo, they were less likely to respond to a further stimulation in vitro.

It is well documented that monocytes presensitised with LPS have a markedly reduced capacity to produce pro-inflammatory cytokines (especially TNFα) in response to a second LPS stimulation [8]. This phenomenon also occurs in vivo and has been called tolerance, adaptation, or hyporesponsiveness. Several mechanisms have been proposed to explain how monocytes from healthy donors become tolerant to LPS. Among these are the down-regulation of LPS receptors, the induction of inhibitory molecules or the production of anti-inflammatory mediators, such as anti-inflammatory cytokines or glucocorticoid hormones [8]. Although similarities exist between LPS-tolerant monocytes and monocytes isolated from critically-ill or septic patients, there are important differences as well, as demonstrated in the present study. In septic shock patients, blood samples with the lowest numbers of monocytes and a reduced expression of CD14 produced less TNFα in response to LPS stimulation than blood samples containing normal monocyte levels. Reduced TNFα production in septic shock patients was clearly associated with a reduced number of monocytes and a decreased expression of CD14. The mechanisms of, and reasons for a profound down-regulation of CD14 receptors remain unknown. At low concentrations of LPS, CD14 is the receptor for LPS on cells of the myelomonocytic lineage [7], whereas CD18 might serve as an alternative receptor at high concentrations of LPS [9]. Expression of CD14 and CD18 on LPS-tolerant normal monocytes was found to be unchanged or even up-regulated [10–13], suggesting that down-regulation of receptors does not account for LPS tolerance in normal monocytes. In sharp contrast to what was seen in the tolerant state, monocyte CD14 expression has repeatedly been reported to be profoundly reduced in the severely injured or in septic patients [14–18]. The mechanisms responsible for decreased CD14 expression are far from understood. In vitro, bacterial products have been shown to increase CD14 expression and survival of monocytes [19], while anti-inflammatory cytokines usually down-regulate CD14 expression [20]. In fact, it has been documented that increased levels of circulating soluble CD14 are found in patients with septic shock [21]. Interestingly, down-regulation of CD14 expression has recently been shown to be sufficient to trigger monocyte apoptosis [20]. If similar mechanisms occur in septic patients, one could postulate that the anti-inflammatory response induces down-regulation of CD14 and therefore monocyte apoptosis.

Brandtzaeg and coworkers have investigated whether anti-inflammatory cytokines might be responsible for the profound down-regulation observed in the blood of septic patients. By mixing normal monocytes and plasma from septic patients (with or without shock), they observed that IL-10 present in the plasma of shock patients was responsible for the suppression of the response of normal monocytes to LPS [3]. While this situation may occur in patients with meningoccaemic septic shock with elevated IL-10 levels (up to 40 ng/ml), it is unlikely to contribute importantly in most cases of septic shock, in which serum levels do not exceed 1 ng/ml (a concentration which does not inhibit synthesis of pro-inflammatory molecules). In fact, the present observations rather suggest that the predominant cause of down-regulation is the monocyte itself, since monocytes of patients with septic shock did not respond to LPS stimulation even in the presence of plasma from healthy individuals. Yet, this does not rule out a contribution of anti-inflammatory mechanisms in the process of dysregulation.

Independently of CD14 expression, of tolerance states of monocytes or down-regulation due to anti-inflammatory mechanisms, a striking ob-

servation of the present study was the very low numbers of circulating monocytes found in septic shock patients, an observation that may *per se* account for the low levels of TNFα produced upon stimulation with microbial products. Since shock is known to trigger macrophage apoptosis [23], monocytes of septic patients may consist of a mixture of normal and dying monocytes. In contrast to patients with septic shock, patients with bacterial pneumonia or with cardiogenic shock had normal numbers of monocytes and normal expression of CD14. However, some of these patients failed to produce TNFα in response to LPS stimulation, which cannot be explained by CD14 down-regulation or the presence of apoptotic monocytes.

Although LPS, peptidoglycan and lipoteichoic acid, whole Gram-positive or Gram-negative bacteria may share a CD14-dependent signalling pathway [4], mechanisms other than CD14 down-regulation may also account for the profound dysregulation of monocyte function. Such mechanisms may explain why patients with cardiogenic shock exhibited normal monocyte counts and CD14 expression, and yet were hyporesponsive to LPS stimulation. The causes for down-regulation in cardiogenic shock patients are not known. The elevated levels of endogenous catecholamines in such patients together with the fact that these patients were treated with such amines could account for this down-regulation. Indeed, catecholamines are known to depress monocyte activation [24]. Similar mechanisms may also occur in patients with septic shock, since patients with septic shock usually also require catecholamines. To answer this question, a detailed analysis of the cytokine-inducing potential of whole blood of septic shock patients would require blood sampling before the administration of catecholamines. Yet, the more significant defect in cytokine production observed in samples of patients with septic shock compared to those with cardiogenic shock may be related to additional mechanisms involving monocyte function.

Conclusions

In summary, the present data indicate that numbers of monocytes in patients with septic shock are depleted and that sepsis is associated with a profound dysregulation of monocyte function more complex than explained by LPS tolerance alone. Such down-regulation was a hallmark of septic shock, and was not restricted to patients with Gram-negative infections. Down-regulation of monocytes was also found to occur in patients with cardiogenic shock, but not in patients with bacterial pneumonia. This suggests that the presence of an infectious focus alone does not necessarily lead to "immunoparalysis." The causes of "immunoparalysis" in sepsis remain to be determined.

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References

1 Munoz C, Carlet J, Fitting C, Misset B, Blériot JP, Cavaillon JM. Dysregulation of in vitro cytokine production by monocytes during sepsis. J Clin Invest 1991;88:1747-54.
2 Ertel W, Kremer JP, Kenney J, Steckholzer U, Jarrar D, Trentz O, et al. Downregulation of proinflammatory cytokine release in whole blood from septic shock patients. Blood 1995;85:1341-7.
3 Brandtzaeg P, Osnes L, Ovstebo R, Joo GB, Westwik AB, Kierulf P. Net inflammatory capacity of human septic shock plasma evaluated by a monocyte-based target cell assay: identification of interleukin-10 as a major functional deactivator of human monocytes. J Exp Med 1996;184:51-60.
4 Pugin J, Heumann D, Tomasz A, Kravchenko V, Akamatsu Y, Nishijima M, et al. CD14 is a pattern recognition receptor. Immunity 1994;1:509-16.
5 de Werra I, Jaccard C, Betz Corradin S, Chioléro R, Yersin B, Gallati H, et al. Cytokines, NO_3^-/NO_2^-, soluble TNF receptors and procalcitonin levels: comparisons in patients with septic shock, cardiogenic shock and bacterial pneumonia. Crit Care Med 1997;25:607-13.
6 Heumann D, Gallay P, Barras C, Zaech P, Ulevitch RJ, Tobias PS, et al. Control of LPS binding and LPS-induced TNF secretion in human peripheral blood monocytes. J Immunol 1992;148:3505-12.
7 Ulevitch RJ, Tobias PS. Recognition of endotoxin by cells leading to transmembrane signalling. Curr Biol 1994;6:125-30.
8 Ziegler-Heitbrock HWL. Molecular mechanism in tolerance to lipopolysacharide. J Inflamm 1995;45:13-26.
9 Ingalls RR, Arnaout MA, Golenbock DT. Outside-in signalling by lipopolysaccharide through a tail-less integrin. J Immunol 1997;159:433-8.
10 Marchant A, Duchow J, Delville JP, Goldman M. Lipopolysaccharide induces up-regulation of CD14 molecule on monocytes in human whole blood. Eur J Immunol 1992;22:1663-5.
11 Otterlei M, Sundan A, Ryan L, Espevik T. Effects of anti-CD18 and LPS on CD14 expression on human monocytes. Scand J Immunol 1995;41:583-92.
12 Haas JG, Bauerle PA, Riethmüller G, Ziegler-Heitbrock HWL. Molecular mechanisms in down-regulation of tumor necrosis factor expression. PNAS 1990;87:9563-7.
13 Takasuka N, Matsuura K, Yamamoto S, Akagawa KS. Suppression of TNF-α mRNA expression in LPS-primed macrophages occurs at the level of nuclear factor-kB activation, but not at the level of protein kinase C or CD14 expression. J Immunol 1995;154:4803-12.
14 Heinzelmann M, Mercer-Jones M, Cheadle WG, Polk HC. CD14 expression in injured patients correlates with outcome. Ann Surg 1996;224:91-6.
15 Ertel W, Krombach F, Kremer JP, Jarrar D, Thiele V, Eymann J, et al. Mechanisms of cytokine cascade activation in patients with sepsis: normal cytokine transcription despite reduced CD14 receptor expression. Surgery 1993;114:243-51.
16 Lin RY, Astiz ME, Saxon JC, Rackow EC. Altered leukocyte immunophenotypes in septic shock. Studies of HLA-DR, CD11b, CD14, and IL-2R expression. Chest 1993;104:847-53.
17 Lin RY, Astiz ME, Saxon JC, Saha DC, Rackow EC. Relationships between plasma cytokine concentrations and leukocyte functional antigen expression in patients with sepsis. Crit Care Med 1994;22:1595-602.
18 Fingerle G, Pforte A, Passlick B, Blumenstein M, Ströbel M, Ziegler-Heitbrock HWL. The novel subset of CD14+/CD16+ blood monocytes is expanded in sepsis patients. Blood 1993;82:3170-6.
19 Landmann R, Knopf HP, Link S, Sansano S, Schumann R, Zimmerli W. Human monocyte CD14 is upregulated by lipopolysaccharide. Infect Immun 1996;64:1762-9.

20 de Waal Malefyt R, Figdor CG, Huijbens R, Mohan-Peterson S, Bennett B, Culpepper J, et al. Effects of IL-13 on phenotype, cytokine production, and cytotoxic function of human monocytes. J Immunol 1993;151:6370-81.
21 Landmann R, Zimmerli W, Sansano S, Link S, Hahn A, Glauser MP, et al. Increased circulating soluble CD14 is associated with high mortality in Gram-negative septic shock. J Infect Dis 1995;171:639-44.
22 Heidenreich S, Schmidt M, August C, Cullen P, Rademaekers A, Pauels HG. Regulation of human monocyte apoptosis by the CD14 molecule. J Immunol 1997;159:3178-88.
23 Ayala A, Urbanich MA, Herdon CD, Chaudry IH. Is sepsis-induced apoptosis associated with macrophage dysfunction? J Trauma 1996;40:568-74.
24 Severn A, Rapson NT, Hunter CA, Liew FY. Regulation of tumor necrosis factor production by adrenaline and β-adrenergic agonists. J Immunol 1992;148:3441-5.

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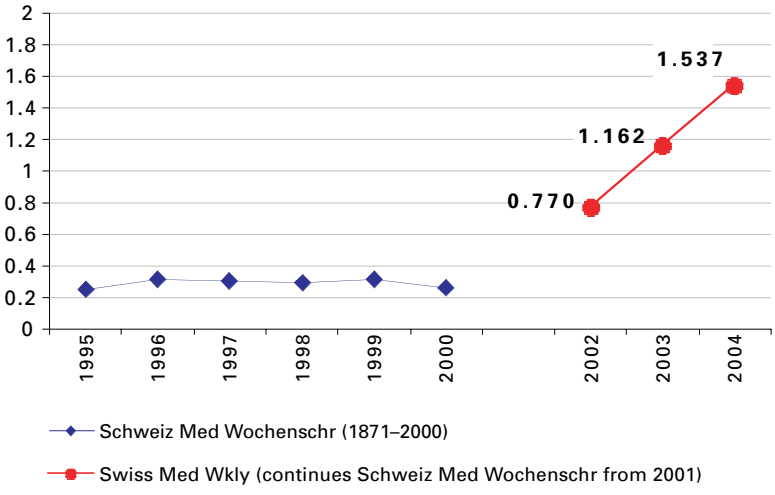

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