

Lack of correlation between soluble CD14 and IL-6 in meningococcal septic shock

Arranz E, Blanco-Quirós A, Solís P, Garrote JA. Lack of correlation between soluble CD14 and IL-6 in meningococcal septic shock
Pediatr Allergy Immunol 1997; 8: 194–199. © Munksgaard 1997.

Meningococcal sepsis is a good model to study the dynamic response of cytokines and other soluble factors *in vivo* in the early stages of the disease. Levels of soluble CD14, interleukin-6 (IL-6), IL-6 receptor (IL-6R), and C-reactive protein (CRP) have been measured in plasma from 26 children with septic shock (nine of whom had disseminated intravascular coagulation) and from ten control children. All samples were collected at the onset, before treatment, and, when possible, 24 and 48 hours later. At admission, patients had significantly higher levels of IL-6 ($p<0.001$) and CRP ($p<0.001$), and lower levels of IL-6R ($p<0.005$) than normal controls. After 24 hours, there was a significant increase of sCD14 ($p<0.05$) and CRP ($p<0.001$). Although IL-6 showed a progressive decline since the onset, its levels were always higher than controls. There was an inverse correlation between IL-6 and both IL-6R ($p<0.001$) and CRP ($p<0.001$), probably due to the later increase of CRP. Nevertheless, sCD14 did not correlate with IL-6 levels. We have confirmed the finding of IL-6 as a sensitive and reliable inflammatory marker in septic shock. Moreover, the ratio IL-6/IL-6R may have a prognostic value, given a putative role of IL-6R in modulating the effects of IL-6 in meningococcal sepsis.

**E. Arranz, A. Blanco-Quirós,
P. Solís, J. A. Garrote**
Department of Pediatrics and Immunology,
University of Valladolid, Spain

Prof. Alfredo Blanco-Quirós, Dept. de Pediatría,
Facultad de Medicina, c. Ramón y Cajal 5, 47005-
Valladolid, Spain

Key words: septic shock; interleukin-6; soluble
CD14

Received June 6, 1997
Accepted December 5, 1997

Septic shock remains a major cause of mortality in children and adults. Meningococcal septic shock is a good model to assess the dynamic response *in vivo* of different factors in the early stages of the disease as its sudden onset inevitably leads to immediate admission of patients. Attention has been currently focused on the role of cytokines and LPS-protein binding complex (CD14) in inflammatory responses and cardiovascular changes. Increased levels of TNF- α , IL-1 β , and IL-6 have been reported in severe infectious disease and sepsis (1), even though this finding is not specific since it also occurs in other conditions (2, 3). TNF- α and IL-1 β are involved in response to sepsis by gram-negative bacteria, inducing similar effects to those caused by bacterial lipopolysaccharide (LPS): hypotension, fever and synthesis of acute phase proteins (4). Interleukin-6 acts via an IL-6 receptor with two subunits, a ligand-binding (gp80) and a signal transducer protein (gp130)(5). It has recently been reported that the soluble form of IL-6R was decrease in neonates (6) and adults with sepsis (7). CD14 is also a cellular receptor for LPS presented as a complex with LPS-

binding protein (8). We and others have previously reported an increase of the soluble form of CD14 in serum from patients with clinical septicemia (9, 10). Differences reported in several septic models may be explained by the short half-life of cytokines and the very rapid change in their levels, which fluctuate in a complex pleiotropism. The aim of the study was to assess the time-related profile of cytokines and other soluble factors in the early stages of septic shock due to meningococcal infection.

Materials and methods

Patients

The study includes 26 children with septic shock, age range: 6 months–8 years (mean: 4 years, 2 months). The diagnosis of meningococcal sepsis was based on epidemiological data and clinical symptoms, according to the American Consensus criteria (11) modified for children (12), which defines septic shock as a systolic hypotension <60 mm Hg persisted for at least one hour. Nine patients

(34%) had a more severe condition, with disseminated intravascular coagulation (DIC) and multiorgan dysfunction syndrome (MODS: severe respiratory, renal, cardiac, and neurologic failure). The diagnosis of DIC was reached in children who had clinical symptoms of thrombosis and bleeding, less than 150.000/ μ l platelets, prolonged coagulation times and increased levels of fibrin split products, as reported (13). *Neisseria meningitidis*-type B was identified in blood or CSF from 18 patients (no other bacteria was found in any case). Results were similar in positive and negative blood culture cases; therefore, all patients were included in the same group. Only one child in the study died. Ten patients admitted for routine assessment of various ailments, i.e. iron deficiency anemia, abdominal pain or minor surgical problems, were used as controls. The mean age was 4 years-8 months (range: 2 months-7 years); five were girls and five were boys. All laboratory studies were eventually found to be normal.

Whole blood samples were collected at the time of admission in EDTA-containing tubes. Sample collection was repeated 24 hours later in eight patients, with a stable condition, who had not received plasma or blood, and after 48 hours in three other cases. Plasma was separated within 2 hours of collection by centrifugation under mild conditions for 20 min at room temperature, and stored at -20°C until assessment.

Methods

Soluble CD14, IL-6 and IL-6R were measured by ELISA according to the test protocol and standards provided by the manufacturer: sCD14 (Immuno Biological Lab, Germany), IL-6 and IL-6R (R&D Systems, USA). The sensitivity of the tests were 1.1 $\mu\text{g/ml}$ for sCD14; 0.70 pg/ml for IL-6, and 0.14 ng/ml for IL-6R. The commercial kit does not rule out the possibility that both free and complexed IL-6R molecule are detected.

C-reactive protein was quantified by radial immunodiffusion (Behring, Germany) with a sensitivity of 20 $\mu\text{g/dl}$.

Statistical analysis

The difference between groups was assessed by the Mann-Whitney U-test. In order to evaluate the relationship between two different parameters, the Spearman's rank correlation coefficient was calculated. Statistical significance was defined as a p value <0.05 . Data showed a non-parametric distribution, for this reason they are presented as median and interquartile range. All statistical calculations were performed using the software package StatView® Student v1.0 (Abacus Inc. USA).

Table 1. Serum levels of IL-6, IL-6R, soluble CD14 and C-reactive protein in meningococcal sepsis (median value and interquartile range)

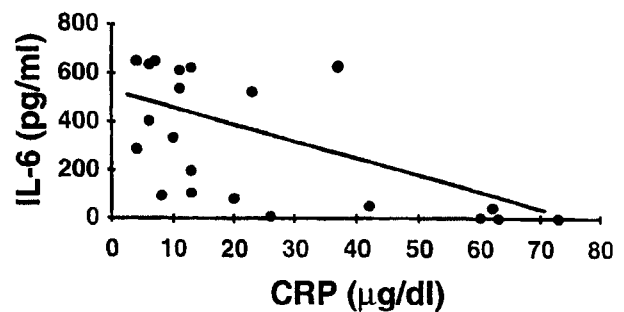
	Time (hours)	n	IL-6 (pg/ml)	IL-6R (ng/ml)	sCD14 ($\mu\text{g/ml}$)	CRP ($\mu\text{g/L}$)
Sepsis	0	26	448*** (161-624)	10.2** (4.6-13)	3.3 (2.6-4.0)	110*** (6.2-182)
	24	8	104*** (8-632)	14.5 (6.5-17.8)	4.0* (3.5-5.4)	370*** (237-622)
Controls		10	0.0	20.4 (17.6-21.6)	3.0 (2.2-3.6)	<20

*** $p<0.001$; ** $p<0.005$; * $p<0.02$ vs controls.

Results

Soluble CD14 was not significantly increased at the onset of the disease (3.3 $\mu\text{g/ml}$ in patients vs 3.0 in controls); however, 24 hours later, it was clearly higher (4.0 $\mu\text{g/ml}$, $p<0.05$) than controls (Table 1). At the time of admission, CRP also showed a significant increase (110 $\mu\text{g/L}$, $p<0.001$) which was even higher 24 hours later (370 $\mu\text{g/L}$, $p<0.001$). CRP levels were inversely correlated with IL-6 ($p<0.02$) (Fig. 1a).

CRP / IL-6 CORRELATION ($p:0.001$)



IL-6R / IL-6 CORRELATION ($p:0.001$)

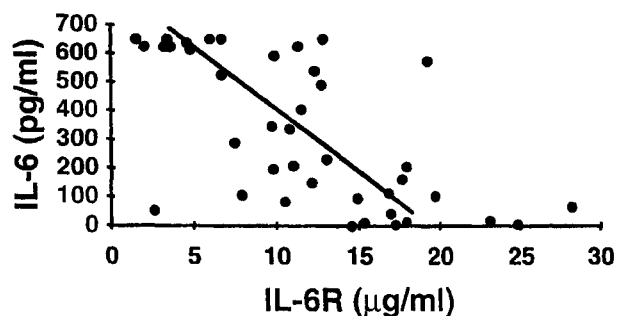


Fig. 1. Inverse correlation between serum levels of IL-6 and (a) C-reactive protein ($p:0.001$). (b) IL-6 receptor ($p:0.001$).

Table 2. Serum levels at 0 and 24 hours in patients with or without disseminated intravascular coagulation (DIC)

		IL-6 (pg/ml)	IL-6R (ng/ml)	sCD14 (µg/ml)	CRP (µg/L)
at 0 h	DIC+	287	7.5	3.4	130
	DIC-	539	10.5	3.1	105
		N.S.	N.S.	N.S.	N.S.
at 24 h	DIC+	650*	6.0**	3.8	370
	DIC-	38*	16.3**	4.5	430
		*p:0.01	**p:0.03	N.S.	N.S.

The finding of high IL-6 levels (448 pg/ml) was a constant feature in all patients with sepsis, and those levels were higher than controls at any time (Table 1). On the other hand, at the onset of the disease all 26 septic patients had IL-6R levels lower than controls (10.2 vs 20.4 ng/ml, $p < 0.005$). Serum IL-6R levels in patients were lower than controls at the time of admission, and there was an increase correlation between IL-6R and IL-6, particularly in those cases with low IL-6 levels ($r: 0.664$, $p < 0.001$) (Fig. 1b). Profiles of IL-6, IL-6R and sCD14 from eight patients were studied at the onset of the disease and after 24 hours. In three cases, the determinations were repeated 48 hours later and the results for IL-6 (149 pg/ml) and IL-6R (17.9 µg/ml) showed a trend similar to that observed after 24 hours, whereas sCD14 (3.0 µg/ml) returned to normal.

At the onset IL-6, IL-6R, sCD14 and CRP levels were similar in DIC-positive (DIC+) and DIC-negative (DIC-) groups. Nevertheless, after 24 hours IL-6 levels (650 vs 38 pg/ml, $p: 0.01$) were higher in DIC+ as compared to DIC- patients, and the IL-6R levels (6.0 vs 16.3 ng/ml, $p: 0.03$) were lower (Table 2).

The only patient who died during the study showed high levels of IL-6 (624 and 650 pg/ml) and very low of IL-6R (3.2 and 6.0 ng/ml) after 24 hours, whereas sCD14 levels were only slightly increased (3.67 and 3.81 µg/ml).

Discussion

We had previously found high levels of IL-6, TNF α and sCD14 in neonatal sepsis. sCD14 was particularly increased in patients with sepsis by gram-negative as compared to gram-positive bacteria (10), whereas IL-6, TNF α , CRP, and fibronectin had similar levels in both groups (10, 11). Soluble CD14 has also been found increased in adults with septic shock, and it is very likely that sCD14 will play a pathogenetic role in sepsis by LPS-induction of cytokine synthesis, which can be blocked by specific anti-CD14 antibodies (14). In the present study, we

highlight the finding of a peak sCD14 concentration 24 hours after the onset of meningococcal infection, whereas 48 hours later patients showed similar levels to those found at the onset.

In gram-negative bacterial sepsis, LPS induces macrophage synthesis of TNF and IL-6 (15). It was suggested that, in addition to a direct toxic effect, TNF might also mediate cytokine synthesis in septic shock, but recent reports have shown that endotox-

Variation of IL-6, IL6R and sCD14 levels in the first 24 hours

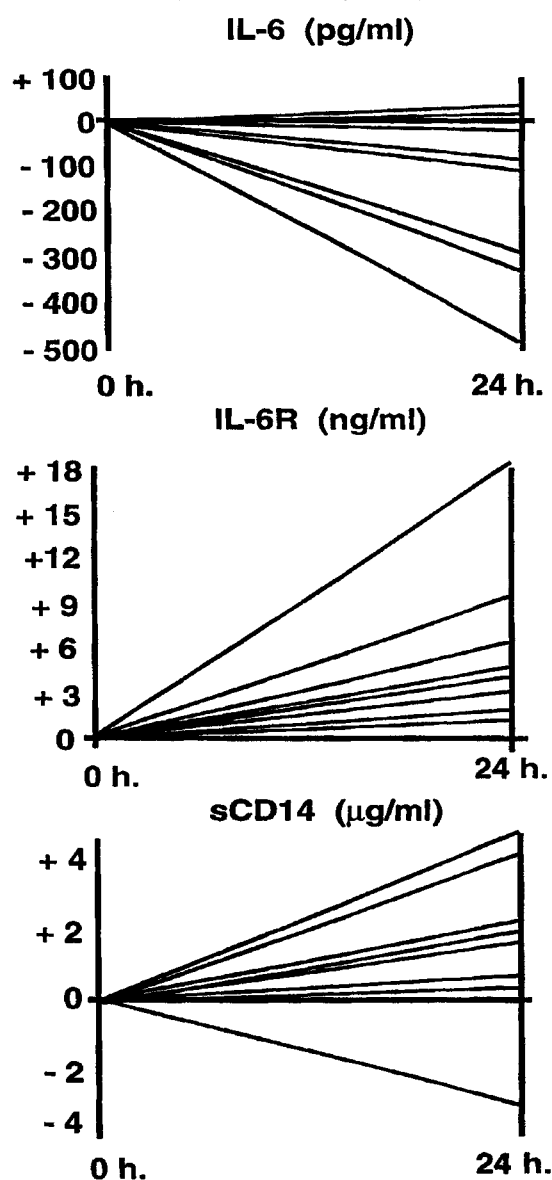


Fig. 2. Time-related changes of IL-6, IL-6R and soluble CD14. IL-6 decreases 24 hours later in most patients, and the increase of IL-6R began at the onset of the disease. However, the highest sCD14 levels are detected after the onset of meningococcal sepsis.

in-induced release in adult severe systemic infection with septic shock but not in mild infections (17), and, therefore, it may discriminate sepsis from other causes of fever. A correlation between increased IL-6 levels and the size of the lesion has been found in burned patients, particularly in cases with more than 40% of burned skin (18). Besides, the infection agent may also be relevant, and patients with bacterial meningitis have been shown to have significantly higher IL-6 levels in CSF samples than patients with aseptic meningitis (19).

Patients with disseminated intravascular coagulation (DIC+) showed always a more severe clinical picture and more intense hypotension. The only child who died had DIC+. The levels of all factors were studied here were similar in patients with DIC+ and DIC- at the onset of sepsis (time 0). Nevertheless after 24 hours IL-6 was significantly higher and IL-6R lower in group CID+ as compared to CID-, at 24 hours. By contrast sCD14 and CRP levels did not show differences between both groups. These data and other reports (20) suggest that IL-6 is a good marker of the sepsis.

In our study, the rise of IL-6 levels in children with meningococcal sepsis was the most common and early finding. Serum IL-6 has a very short half-life; however, septic patients showed higher IL-6 levels than controls at any time of the study, although its levels were declining. This cytokine plays a critical role in the induction of CRP synthesis in the liver, and this may explain the finding of a peak CRP level 24 hours later than the highest IL-6 titer at the onset. Other authors have also reported increased IL-6 levels, followed by CRP, in patients with hematological malignancies and sepsis, although IL-6 decreased rapidly during the antimicrobial treatment (21).

Compared to other reporters, and given the severity of the disease, it may be argued that our patients showed lower levels of some of the factors than should be expected. However, even though there were no other inclusion criteria but the diagnosis, it is possible that those patients severely ill died before entering the study, and therefore only cases with moderate and mild forms of the disease were included. Moreover, the lack of homogeneity between groups after the onset of the disease makes us think that some of the results should be interpreted with caution. This was due to technical problems during the sampling procedure at the early stages of the disease, as we were unable to collect samples from patients in need of critical care.

High levels of IL-6 have been associated with increased susceptibility to septic shock. Both TNF α and IL-6 determinations may have a prognostic value in newborns, children and adults with septic shock (22–24), and their levels are frequently higher

in non-survivor than survivor patients (25). In general, very high IL-6 levels in serum and CSF have been related to severe symptoms, poor prognosis and increased mortality, especially in meningococcus infection (26–28). In severe intra-abdominal sepsis, IL-6 levels predicted mortality in 86% of cases, although no correlation was found between levels of TNF α , IL-1 and LPS and the mortality rate (29). Only cancer patients with chemotherapy-induced granulocytopenia who died from sepsis showed increased levels of IL-6 before death (30), and, in patients with severe leukopenia due to hematological malignancies, these levels remained high only in those who died from septic shock, whereas the association between CRP and shock and mortality was weak (31).

At the onset of the disease, all patients with sepsis had low IL-6R levels, even lower than controls, and they started to increase 24 hours later. The IL-6 receptor is expressed at the cell surface some minutes after synthesis and it is rapidly turned over with a half-life of 2–3 hours (5). It has been observed that anti-inflammatory drugs, such as dexamethasone, increase mRNA for IL-6R (31). A possible explanation of the decrease in IL-6R levels during sepsis might be either protein consumption after blocking or reducing the biologic activity of IL-6 or the result of the internalisation of IL-6 linked to its membrane receptor (7).

Serum levels of IL-6R were inversely correlated with those of IL-6, and this probably reflects the existence of a modulatory effect between both the cytokine and its soluble receptor, since serum IL-6R has been shown to enhance IL-6 effects on murine and human cells (33). Treatment with IL-6 and IFN γ reduces the number of IL-6R molecules at the cell surface (34). IL-6 decreases IL-6R mRNA expression, indicating a pathway by which cells regulate their response to IL-6. Furthermore, LPS, TNF α , IL-1 β , and IFN α induced IL-6 production and at the same time suppressed IL-6R mRNA synthesis (32). However, the controversy on the effect of IL-6 over its receptor is still open, since there are also reports which show that neither LPS nor IL-6 down-regulates IL-6R (35).

The decrease of IL-6R which coincides with the rise in IL-6 levels may be due to an augmented complex formation with the ligand and its subsequent clearance or to the decreased generation of IL-6R in sepsis. In any case, low levels of IL-6 might play a modulating role in the effects of IL-6, as others have recently proposed (20).

The commercial kit did not rule out the possibility that the capture antibody reacts with free and complexed IL-6R, but this seems irrelevant in our study because both molecules are decreased. The assay does not exhibit detectable cross reactivity with

other cytokines tested, IL-6 among them, and the addition of recombinant IL-6 to samples did not influence the detection of IL-6R and vice versa (20).

In summary, the severity of septic shock may be related to the levels of IL-6 rather than sCD14. Levels of IL-6 increase in the first place and this is followed by changes in sCD14, CRP and IL-6R. IL-6 levels did not correlate with sCD14 levels, suggesting an independent activation in meningococcal sepsis. An increased ratio IL-6/ IL-6R may have a prognostic value in septic shock due to a putative protective effect of IL-6R.

Acknowledgements

This study was partially supported by a grant from the Consejería de Sanidad (Grant VA 41/95). Junta de Castilla y León, Spain.

References

- HACK CE, DE GROOT ER, FELT-BERSMA RJF, et al. Increased plasma levels of interleukin-6 in sepsis. *Blood* 1989; 74: 1704-10.
- WHICHER JT, EVANS SW. Cytokines in diseases. *Clin Chem* 1990; 36: 1269-81.
- HELMINEN M, VESIKARI T. Increased interleukin-1 (IL-1) production from LPS-stimulated peripheral blood monocytes in children with febrile convulsions. *Acta Paediatr Scand* 1990; 79: 810-16.
- VAN DEVENTER SJH, BULLER HR, CATE TJW, et al. Experimental endotoxemia in human analysis of cytokine release and coagulation. *Blood* 1990; 76: 2520-6.
- GERHARTZ C, DITTRICH E, STOYAN T, et al. Biosynthesis and half-life of the interleukin-6 receptor and its signal transducer gp130. *Eur J Biochem* 1994; 223: 265-74.
- LERNBECHER T, SCHROD L, KRAUS D, et al. Interleukin-6 and soluble interleukin-6 receptor in cord blood in the diagnosis of early onset sepsis in neonates. *Acta Paediatr* 1995; 84: 806-8.
- FRIELING JTM, VANDEUREN M, WIJDEJES J, et al. Circulating interleukin-6 receptor in patients with sepsis syndrome. *J Infect Dis* 1995; 171: 469-72.
- WRIGHT SD, RAMOS RA, TOBIAS PS, et al. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS-binding protein. *Science* 1990; 249: 1432-3.
- JABARA HH, VERCELLI D. Engagement of CD14 on monocytes inhibits the synthesis of human Igs, including IgE. *J Immunol* 1994; 153: 972-8.
- BLANCO-QUIRÓS A, SOLIS G, ARRANZ E, et al. Serum levels of CD14 in neonatal sepsis by gram-positive and gram-negative bacteria. *Acta Paediatr* 1996; 85: 728-32.
- American College Of Chest Physicians/Society of Critical Care Medicine Consensus Conference. Definition of sepsis and organ failure guidelines for the use of innovative therapies in sepsis. *Crit Care Med* 1992; 20: 864-74.
- SÁEZ-LLORENS X, MCCracken GH. Sepsis syndrome and septic shock in pediatrics: Current concepts of terminology, pathophysiology and management. *J Pediatr* 1993; 123: 497-508.
- BLANCO-QUIRÓS A, ALVAREZ-GUISASOLA FJ, SOLIS P, et al. Fibronectin in meningococcal sepsis. Correlation with antithrombin III and protein C. *Acta Paediatr* 1990; 79: 73-6.
- LANDMANN R, REBER AM, SANSANO S, ZIMMERLI W. Function of soluble CD14 in serum from patients with septic shock. *J Infect Dis* 1996; 173: 661-8.
- BATTAFARANO RJ, KIM SK, DAHLBERG PS, et al. Lymphocyte derived cytokines augment macrophage tumor necrosis factor-alpha and interleukin-6 secretion during experimental gram-negative bacterial sepsis. *J Surg Res* 1995; 58: 739-45.
- VASILESCU C, BERGER D, BUTTENSCHON K, et al. Endotoxin-induced release of interleukin 6 and interleukin 1 beta in human blood is independent of tumor necrosis factor alpha. *Eur Surg Res* 1996; 28: 55-62.
- GAETA G, DEVONDERWEID U, LUCHESI A, et al. Levels of interleukin 6 in neonatal sepsis. *Fundam Clin Immunol* 1994; 2: 169-73.
- KOWALVERN A, WALENGA JM, HOPPENSTEADT D, et al. Interleukin-2 and Interleukin-6 in relation to burn wound size in the acute phase of thermal injury. *J Am Coll Surg* 1994; 178: 357-62.
- MATSUZONO Y, NARITA M, AKUTSU Y, TOGASHI T. Interleukin-6 in cerebrospinal fluid of patients with central nervous system infections. *Acta Paediatr* 1995; 84: 879-83.
- OSTERMANN H, KRATZ-ALBERS K, MESTERS RM, KIEHL M, KIENAST J. Reciprocal changes in circulating interleukin-6 and its soluble receptor during evolving sepsis in leukocytopenic patients. *J Infect Dis* 1997; 176: 825-8.
- RINTALA E, PULKKI K, MERTSOLA J, et al. Endotoxin interleukin-6 and phospholipase-A2 as markers of sepsis in patients with hematological malignancies. *Scand J Infect Dis* 1995; 27: 39-43.
- GIRARDIN E, ROUX-LOMBARD P, GRAU GE, et al. Imbalance between tumor necrosis factor-alpha and soluble TNF receptor concentrations in severe meningococemia. The J5 study group. *Immunology* 1992; 76: 20-3.
- BUCK C, BUNDSCHU J, GALLATI H, et al. Interleukin-6: A sensitive parameter for the early diagnosis of neonatal bacterial infection. *Pediatrics* 1994; 93: 54-8.
- DE BONT ES, MARTENS A, VAN RAAN J, et al. Tumor necrosis factor, interleukin-1 beta and interleukin-6 plasma levels in neonatal sepsis. *Pediatr Res* 1993; 33: 280-3.
- YOSHIMOTO T, NAKANISHI K, HIROSE S, et al. High serum IL-6 level reflects susceptible status of the host to endotoxin and IL-1/tumor necrosis factor. *J Immunol* 1992; 148: 3596-603.
- 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.
- TIAO G, RAFFERTY J, OGLE C, et al. Detrimental effect of nitric oxide synthase inhibition during endotoxemia may be caused by high levels of tumor necrosis factor and interleukin-6. *Surgery* 1994; 116: 332-8.
- MUSTAFA MM, LEBEL MH, RAMILO O, et al. Correlation of interleukin-1 beta and cachectin concentrations in cerebrospinal fluid and outcome from bacterial meningitis. *J Pediatr* 1989; 115: 208-13.
- PATEL RT, DEEN KI, YOUNGS D, et al. Interleukin-6 is a prognostic indicator of outcome in severe intra-abdominal sepsis. *Br J Surg* 1994; 81: 1306-8.
- STEINMETZ HT, HERBERTZ A, BERTRAM M, DIEHL V. Increase in interleukin-6 serum level preceding fever in granulocytopenia and correlation with death from sepsis. *J Infect Dis* 1995; 171: 225-8.
- ANTONELLI M, RAPONI GM, MARTINO P, et al. High IL-6 serum levels are associated with septic shock and mortality in septic patients with severe leukopenia due to hematological malignancies. *Scand J Infect Dis* 1995; 27: 381-4.
- HOFFMANN R, HENNINGER HP, SCHULZE-SPECKING A,

- DECKER K. Regulation of interleukin-6 receptor expression in rat Kupfer cells: modulation by cytokines, dexamethasone and prostaglandin E₂. *J Hepatol* 1994; 21: 543–50.
33. DIAMANT M, HANSEN MB, RIENECK K, et al. Stimulation of the B9 hybridoma cell line by soluble interleukin-6 receptors. *J Immunol Methods* 1994; 173: 229–35.
34. LASFAR A, WIETZERBIN J, BILLARD C. Differential regulation of interleukin-6 receptors by interleukin-6 and interferons in multiple myeloma cell lines. *Eur J Immunol* 1994; 24: 124–30.
35. SCHOESTER M, HEINRICH PC, GRAEVE L. Regulation of interleukin-6 receptor expression by interleukin-6 in human monocytes – a reexamination. *FEBS-Lett* 1994; 345: 131–4.