

THE systemic inflammatory response syndrome (SIRS) is an inflammatory process seen in association with a large number of clinical infective and non-infective conditions.

The aim of this study was to investigate the role of anti-inflammatory cytokines such as interleukin-4 (IL-4), interleukin-10 (IL-10), and transforming growth factor-beta (TGF-beta). Serum levels of IL-4, IL-10 and TGF- $\beta$  were determined in 45 patients with SIRS: 38 patients had SIRS of infectious origin, whereas seven patients had non-infectious SIRS. Twenty healthy subjects were used as controls.

Serum levels of IL-4, IL-10 and TGF- $\beta$  were determined by an immunoenzyme assay. A significant increase of IL-4 was observed in these patients at the time of diagnosis and 5 days later. In contrast, serum levels of IL-10 were not increased at the time of diagnosis, but a slight decrease was noted after 5 days. Serum levels of TGF- $\beta$  were not increased at time of diagnosis, and a slight increase was observed after 5 days. Serum levels of IL-4 were significantly higher in patients with infectious SIRS at the time of diagnosis, whereas no significant difference between infectious and non-infectious SIRS was noted for serum levels of IL-10 and TGF- $\beta$  at the time of diagnosis and 5 days later.

During SIRS, serum levels of IL-4 were significantly increased with a significant correlation between IL-4 and mortality, and only levels of IL-4 were significantly increased in the SIRS caused by infectious stimuli.

**Key words:** SIRS, IL-4, IL-10, TGF- $\beta$

## Anti-inflammatory response of IL-4, IL-10 and TGF- $\beta$ in patients with systemic inflammatory response syndrome

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## Introduction

The systemic inflammatory response syndrome (SIRS) is an inflammatory process seen in association with a number of clinical infective and non-infective conditions.<sup>1</sup> Besides the infectious insults caused by viruses, protozoa and bacteria that may cause SIRS, non-infectious causes may include pancreatitis, multiple trauma, ischaemia, haemorrhagic shock and immune-mediated organ injury.<sup>1</sup> In response to an infectious or non-infectious stimulus, the local environment produces cytokines which are primarily intended to evoke an inflammatory response, and successively small quantities of inflammatory cytokines are released into the circulation to enhance local response.<sup>2</sup> Several agents have been studied for their role as mediators of sepsis and septic shock, including bacterial toxins, cytokines and nitric oxide.<sup>3–5</sup>

Acute-phase response is initiated by tumour necrosis factor (TNF), IL-1, and the IL-8 family, which are grouped together and called proinflammatory cytokines.<sup>6</sup> Successively, acute-phase response is con-

trolled by a simultaneous decrease in proinflammatory mediators and release of endogenous antagonists. It is well known that IL-4, IL-10 and transforming growth factor  $\beta$  (TGF- $\beta$ ) are considered anti-inflammatory cytokines because, when administered to animals with infection or inflammation, they reduce the severity of disease and reduce the production of IL-1 and TNE<sup>7</sup>

In this study we determined serum levels of anti-inflammatory cytokines IL-4, IL-10 and TGF- $\beta$  in patients with infectious and non-infectious SIRS, to evaluate whether an anti-inflammatory cytokine profile is associated with an adverse outcome.

## Materials and methods

Subjects enrolled in this study were patients admitted to the Division of Infectious Diseases, General Hospital, Bergamo, and Divisions of Medicine, Critical Care Unit and Surgical Division of San Giovanni di Dio Hospital, Florence. We evaluated 45 patients (mean age: 53.9  $\pm$  21.0 years; 26 male and 19 female) who were negative for HIV-1 infection, and who met

**Table 1.** Serum levels of IL-4, IL-10 and TGF- $\beta$  in patients with SIRS at the time of diagnosis (set A), and five days later (set B)**Set A**

Time	IL-4 (pg/mL)	IL-10 (pg/mL)	TGF- $\beta$ (pg/mL)
Infectious and non-infectious SIRS	1170.4 $\pm$ 943.1*	19.3 $\pm$ 46.2	36.1 $\pm$ 82.7
Infectious SIRS	1243.2 $\pm$ 916.7+	21.6 $\pm$ 50.1	45.4 $\pm$ 90.6
Non-infectious SIRS	18.3 $\pm$ 2.0	16.1 $\pm$ 27.9	ND

\*  $p < 0.0001$  in comparison with the controls; +  $p = 0.001$  in comparison with infectious SIRS; ND = not detectable.

**Set B**

Time	IL-4 (pg/mL)	IL-10 (pg/mL)	TGF- $\beta$ (pg/mL)
Infectious and non-infectious SIRS	1050.3 $\pm$ 917.2 *	11.1 $\pm$ 28.4	47.9 $\pm$ 135.5
Infectious SIRS	1193.9 $\pm$ 876.5+	11.5 $\pm$ 30.6	55.1 $\pm$ 148.3
Non-infectious SIRS	18.4 $\pm$ 1.3	5.1 $\pm$ 11.5	23.0 $\pm$ 51.5

\*  $p < 0.0001$  in comparison with the controls; +  $p = 0.002$  in comparison with patients with non-infectious SIRS.

clinical criteria for SIRS.<sup>1</sup> Thirty-eight patients (mean age: 52.5  $\pm$  21.5 years) had infective SIRS, caused in seven patients by gram-positive infection, in six patients by gram-negative infection, in four by polymicrobial infection, in two patients by *Plasmodium falciparum* infection, in one patient by *Mycobacterium tuberculosis* infection, in one patient by *Mycoplasma pneumoniae* infection, in one patient by *Pneumocystis carinii* infection, and in 16 patients the aetiology was unknown. According to the clinical diagnosis, 10 patients had intra-abdominal infections, four had acute endocarditis, four patients suffered from urinary tract infection, four had pneumonia, three patients had acute enteritis, and 13 patients had other infections.

Blood samples were collected at the time of diagnosis of SIRS, and 5 days later. Serum was prepared by centrifugation at 1600  $\times g$  for 10 min, and stored at -75°C. The concentration of IL-4, IL-10 and TGF- $\beta$  was measured by using the ELISA technique (R and D Systems, Minneapolis, MN, USA). The sensitivity of ELISA kits to IL-4 and IL-10 was equal to or less than 5 pg/mL in all cases, whereas the sensitivity to TGF- $\beta$  was equal to or less than 7 pg/mL.

Data are expressed as mean and standard deviation,

and were compared using the non-parametric Mann-Whitney U test. Statistical analysis was performed with the significance level set at  $p < 0.05$ .

**Results**

Serum levels of IL-4, IL-10 and TGF- $\beta$  were determined in patients with infectious or non-infectious SIRS. In addition, serum levels of IL-4, IL-10 and TGF- $\beta$  were determined in 25 healthy control subjects (IL-4: 3.2  $\pm$  1.2 pg/mL; IL-10: 1.1  $\pm$  2.3 pg/mL; TGF- $\beta$ : 4.1  $\pm$  1.9 pg/mL). Serum levels of IL-4, IL-10 and TGF- $\beta$  were determined in patients with infectious and non-infectious SIRS at the time of diagnosis and 5 days later. A significant increase in serum levels of IL-4 was observed in patients with SIRS at the time of diagnosis, whereas IL-4 levels did not significantly change 5 days later (Table 1).

Furthermore, serum levels of IL-4 were significantly higher in patients with infectious SIRS than in those with non-infectious SIRS at the time of diagnosis (Table 1, set A), and 5 days later (Table 1, set B). In contrast, no significant difference between infectious and non-infectious SIRS was observed for serum levels of IL-10 at the time of diagnosis (Table 1, set A), and

**Table 2.** Serum levels of IL-4, IL-10 and TGF- $\beta$  in survived or not-survived patients with SIRS at the time of diagnosis and five days later

Patient groups with SIRS	IL-4 (pg/mL) 0 day 5 days	IL-10 (pg/mL) 0 day 5 days	TGF- $\beta$ (pg/mL) 0 day 5 days
Survivors (n 34)	935.6 $\pm$ 932.6 876.3 $\pm$ 897.0	23.8 $\pm$ 52.8 10.7 $\pm$ 30.9	43.2 $\pm$ 84.8 58.9 $\pm$ 152.8
Non-survivors (n 9)	1560.2 $\pm$ 872.6 1749.6 $\pm$ 613.2*	ND 10.1 $\pm$ 15.9	33.6 $\pm$ 100.9 ND

\*  $p = 0.041$  when compared with the survivors; ND = not detectable.

5 days later (Table 1, set B). In addition, serum levels of TGF- $\beta$  were not significantly different between patients with infectious SIRS and non-infectious SIRS either at the time of diagnosis or 5 days later (Table 1, set A and B).

Table 2 shows serum levels of IL-4, IL-10 and TGF- $\beta$  in survivor and non-survivor patients with SIRS at the time of diagnosis and 5 days later, respectively. As can be seen in Table 2, we did not find a significant correlation between serum levels of IL-4, IL-10 and TGF- $\beta$  and risk of death in patients with SIRS at the time of diagnosis. In contrast, IL-4 was significantly associated with a fatal outcome in patients with SIRS.

## Discussion

The results of this study show that response of anti-inflammatory cytokines IL-10 and TGF- $\beta$  was not significantly increased in early and late stages of SIRS, whereas high circulating levels of IL-4 were found in early and late stages of SIRS, with a significant correlation between IL-4 and mortality. In addition, only levels of IL-4 were significantly increased in SIRS caused by infectious stimuli. The pro-inflammatory state of the acute phase response to infectious or non-infectious stimuli also initiates anti-inflammatory activity involving mediators such as IL-4, IL-10, IL-11, TGF- $\beta$  and IL-1ra.<sup>8</sup> Eventually, they should stimulate a compensatory systemic anti-inflammatory response to downregulate the pro-inflammatory response.<sup>9</sup> In particular, IL-4 is able to downregulate human alveolar macrophages and peripheral blood monocytes, stimulated by bacterial endotoxin, to produce IL-1 $\alpha$  and IL-1 $\beta$  and TNF- $\alpha$ .<sup>10</sup> More recently, van Dissel *et al.*<sup>11</sup> have shown that febrile patients with early and advanced stages of SIRS had a fatal outcome with a high ratio of IL-10 to TNF- $\alpha$ . These findings shed new light on the debate concerning the efficacy and safety of pro-inflammatory cytokine inhibition in the management of sepsis.

By blocking the action of pro-inflammatory cytokines, the circulating cytokine profile may be forced into anti-inflammatory cytokine activation that results in exacerbation of systemic disease and adverse outcome in febrile patients. The mechanisms that

determine the amount and balance of various pro-inflammatory and anti-inflammatory cytokines are still largely unknown. The pro- and anti-inflammatory mechanisms are often dysregulated by several host and microbial factors, which determine the pattern and magnitude of the human cytokine response involved in infection.

In conclusion, compensatory systemic anti-inflammatory response which downregulates the pro-inflammatory response has not been fully clarified yet, and the possible therapeutic activity of anti-inflammatory cytokines is not supported by current experimental and clinical studies.

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