Intensive Care Med (1999) 25: 106–109

BRIEF REPORT

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T helper cell subset ratios in patients with severe sepsis

Received: 26 May 1998
Final revision received: 9 October 1998
Accepted: 16 October 1998

This work was supported financially by the Anaesthetic Research Society

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Abstract *Background:* T helper 1 (Th1) lymphocytes produce interferon γ (IFNγ), favouring cell mediated immunity; Th2 cells secrete interleukin-4 (IL-4), favouring humoral immunity. Cytokines produced in sepsis may effect Th subset predominance and subsequent immune responses.
Methods: We measured Th subsets in ten patients with severe sepsis, seven APACHE II score-matched non-septic critically ill control patients, and ten healthy subjects. Mononuclear leukocytes were isolated and Th subsets identified by flow cytometry. *Results:* The median (range) Th1/Th2 ratio was 0.46 (0.2–2.5) in patients with sepsis, which was significantly lower than both non-septic controls (median 2.5 (0.2–5.9), $p = 0.050$) and healthy subjects (median 3.9 (1.2–10.8), $p = 0.01$).
Conclusions: In patients with sepsis, Th2 antibody mediated (humoral) immune responses predominate. This type of response may lead to fibroblast activation and ultimately immunosuppression. Modulation of Th cell subset predominance may present a novel therapeutic option in the treatment of severe sepsis.

Key words Leukocytes · T cells · Sepsis · Cytokines · Interleukin-4 · Interferon γ

Introduction

T helper lymphocytes are important in both cell mediated and humoral immunity. The particular type of immune response is determined by the differentiation of precursor T helper (Th0) cells into Th1 or Th2 cells, which is dependent upon local cytokine concentration, antigen load and mode of antigen presentation. Each cell subset secretes a particular array of cytokines which further augment the differentiation into that subset [1]. Th1 cells produce interferon γ (IFNγ) and favour cell mediated immune responses, and Th2 cells secrete interleukin-4 (IL-4) and favour humoral immunity [2]. Because Th1 and Th2 subsets demonstrate differing patterns of cytokine production, the ratio of Th1 to Th2 cells may be important to the outcome of sepsis. In addition, the balance of cytokines produced in severe sepsis is likely to have a marked effect on Th subset predominance and hence the nature of the subsequent immune response.

The ratio of Th subsets has not previously been studied in patients with severe sepsis. We therefore measured Th1 and Th2 subsets in patients with sepsis syndrome compared to non-septic critically ill control patients. A group of healthy younger subjects was also included for reference.

Methods

Subjects were recruited following local Ethics Committee approval. Ten patients, (median age 60), with severe sepsis were studied. Patients were within 12 h of fulfilling the criteria for severe sepsis as recommended by the Consensus Meeting of the American Thoracic Society and the American Society of Critical Care Medicine: clinical suspicion of acute infection, hypothermia or hyperthermia (< 36.0 C or > 38.0 C), tachypnoea (> 20 breaths/minute or venti-

Table 1 Patient data

Severe sepsis		Non-septic	
Age	Diagnosis/source of sepsis	Age	Diagnosis
73	Cardiac failure/pneumonia	77	Road traffic accident
69	Femoral hernia/faecal peritonitis	23	Road traffic accident
56	Jejunostomy/mediastinitis	69	Post laparotomy
53	Thoracotomy for oesophageal tear/mediastinitis	65	Post coronary artery bypass surgery
68	Cardiac failure/pneumonia	77	Road traffic accident
39	Rhabdinomyolysis/pneumonia	73	Pancreatitis
77	Lobectomy/pneumonia	58	Emphysema, respiratory failure
36	Varicella/pneumonia		
56	Community acquired pneumonia		
64	Trauma/faecal peritonitis		

lated) and tachycardia (> 90 beats per minute) plus an elevated leukocyte count ($> 12 \times 10^9$/l), plus secondary organ dysfunction (either renal failure or hypotension). In addition, seven patients (median age 69), who were critically ill on the intensive care unit, following trauma or surgery, but without evidence of infection, were studied (Table 1). Ten healthy subjects, aged 25–45, recruited from laboratory and medical staff, were also studied.

Mononuclear leukocytes were isolated from heparinised blood samples using density gradient centrifugation (Ficoll-Hypaque, Pharmacia Biotech, St Alban's Herts, UK) and were cultured for 4 h at 37 °C in 5 % CO_2 /95 % air with 20 ng/ml phorbol myristate acetate (PMA, Sigma Chemical Co Ltd., Poole, Dorset, UK) and 1 μM ionomycin (Sigma). Monensin (2 μM, Boehringer Mannheim**,** Lewes, East Sussex, UK) prepared initially in dimethylsulphoxide (Sigma) was also included.

Washed cells (1×10^6) were incubated for 30 min with conjugated antibodies against the cell surface antigens CD3 and CD4 (Sigma) and then fixed with 4 % w/v paraformaldehyde (Sigma) and rendered permeable with 0.1 % w/v saponin (Sigma). The cells were pelleted and stained with conjugated antibodies targeted to the intracellular cytokines IFNγ and IL-4 (Pharmingen, Cambridge, UK) prior to analysis by flow cytometry. Conjugated isotype control antibodies were used to detect non-specific binding.

A FACScan flow cytometer with a 488 nm argon laser (Becton Dickinson, Cowley, Oxon) was used, with filter settings for fluorescein isothionate at 530 nm and for phycoerythrin at 585 nm. Ten thousand cells were analysed and analysis gates were set according to both forward and side scatter profiles. The results were calculated as the ratio of the percentage of IFNγ producing (Th1) cells to IL-4 producing (Th2) cells and are presented as medians (ranges). Statistical analysis was performed using Microsoft Excel 3.5 with Astute statistical add-in. The data were not normally distributed and were therefore analysed using Mann-Whitney U test with a p value of ≤ 0.05 or less being considered statistically significant.

Results

Admission APACHE II scores were similar in patients with severe sepsis and in the non-infected critically ill controls, with median (range) values of 18 (12–30) and 17 (11–30), respectively. The ratio of IFNγ-producing T cells (Th1) to IL-4-producing T cells (Th2) was 0.46 (0.2–2.5) in the patients with sepsis, which was significantly lower than in both the non-septic critically ill control patients; median 2.5 (0.2–5.9, $p = 0.050$), and the younger healthy subjects; median 3.9 (1.2–10.8, $p = 0.01$, Fig. 1).

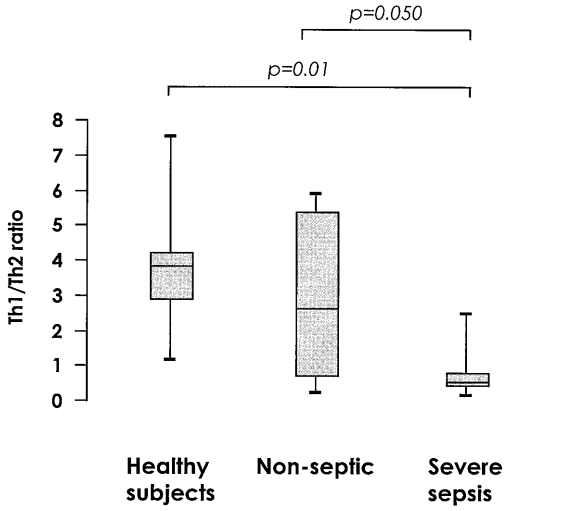

Fig. 1 T helper cell subset ratios in 10 patients with severe sepsis, 7 non-septic critically ill control patients and 10 young healthy subjects. Box and whisker plots show median, 25th and 75th percentiles and range as vertical lines. Data were compared using Mann Whitney U test

The number of IFNγ-positive Th1 cells, expressed as a percentage of total CD4 positive Th cells, was lower in the sepsis patients [9.9 (1.9–16.4) %] compared to non-septic control patients [16.0 (6.0–48.7) %], $p = 0.017$, Fig. 2. The number of IL-4 positive Th2 cells was higher in the sepsis patients [20.5 (3.5–43.1)%] compared to non-septic control patients [9.1 (3.6–26.2) %], $p = 0.047$, Fig. 2. The percentage of total Th cells within the CD3 positive T lymphocyte population was not different between sepsis and non-sepsis patients [30.4 (7.5–55.8) % and 32.2 (21.7–61.5) % respectively, $p = 0.34$].

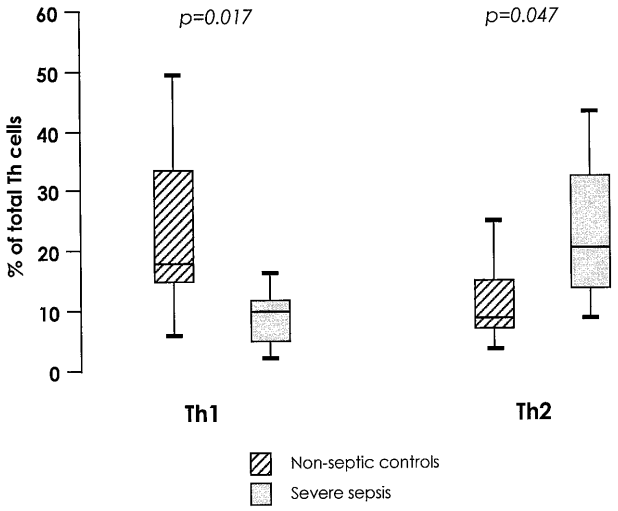

Fig. 2 Th1 and Th2 cells expressed as a percentage of total Th cells in 10 patients with severe sepsis and 7 non-septic critically ill control patients. Box and whisker plots show median, 25th and 75th percentiles and range as vertical lines. Data were compared using Mann Whitney U test

Discussion

We have shown that the predominating T helper cell subset in patients with sepsis is Th2, which is different from results found in critically ill but non-septic patients. This was due to relatively larger numbers of Th2 cells and smaller numbers of Th1 cells, without a change in the number of total Th cells.

Subpopulations within the classical CD4 positive T cell subsets differ markedly in the array of cytokines they produce [1]. On initial stimulation, naïve Th cells produce IL-2, but after re-stimulation and further differentiation these cells produce a broader range of cytokines specific to each cell subset. Th1 cells produce IFNγ and tumour necrosis factor β (TNFβ or lymphotoxin) and Th2 cells produce IL-4, IL-5, IL-6 and IL-13 [2]. We chose to use the measurement of intracellular IFNγ for Th1 cells and IL-4 for Th2 cells. Within the mononuclear cell population, IFNγ is produced by cytotoxic T cells (Tc) and natural killer cells in addition to Th1 cells, but the use of CD4, which selects T helper cells only, confers specificity. The use of TNFβ, also secreted by T cells, is less sensitive. For Th2 determination, we used intracellular IL-4, which is produced only by Th2 cells. IL-5, although also secreted only by Th2 cells, is produced in smaller quantities and therefore greater sensitivity is achieved with the choice of IL-4. Other cytokines produced by Th cells are less specific.

In the present study, there was an alteration in the balance of Th1 to Th2 cells, caused by relatively fewer IFNγ positive Th1 cells and more IL-4 positive Th2 cells within the CD4 positive T helper cell population, in patients with severe sepsis, but not in those critically ill patients without evidence of infection. The predominance of Th2 cells would lead to increased IgE synthesis and B cell activation. IL-13 and IL-4 have similar actions, with pronounced anti-inflammatory actions on macrophages. In addition, both Th1 and Th2 cells also produce IL-10, and IL-10 suppresses cytokine production by the Th1 cell subset [3], which is likely to result in a relative decrease in the Th1 cell population. Conversly, in animals, IFNγ suppresses IL-10 release, and IFNγ has been suggested as a restorer of immunocompetence in sepsis-associated immuno-suppression [4]. However, the effect of IFNγ administration on Th cell subsets is not known.

Markedly increased circulating concentrations of IL-10 have been reported in patients with sepsis [5], and these were highest in those patients who died [6, 7]. In a murine caecal ligation and puncture model of sepsis, exogenous IL-10 failed to improve morbidity or mortality [8]. We suggest that our findings may represent IL-10 mediated suppression of Th1 cells leading to Th2 predominance of antibody mediated (humoral) immune responses, leading to B cell proliferation, and preferential release of IL-4, IL-5, IL-10 and IL-13. This type of response may lead to fibroblast activation and, ultimately, immunosuppression. A study of polymicrobial sepsis in mice also showed that Th2 responses predominate [9]. Further study is required, both to identify the relationship between the circulating cytokine concentration and Th cell subset predominance, and also the effect on infection risk and morbidity. It has recently been suggested that although, in vitro, T cell clones commonly secrete distinct sets of cytokines in response to certain stimuli, cytokine regulation in Th cells may be more dissociated under physiological conditions [reviewed in 10]. However, there are circumstances where the modulation of certain immune effector mechanisms may be clinically useful. Indeed, IFNγ administration in patients with depressed monocyte function restores ex vivo TNF responses and HLA-DR expression [11]. It is possible that, in the future, modulation of Th cell subset predominance via early cytokine therapy may present a novel therapeutic option in the treatment of severe sepsis.

Acknowledgements This work was undertaken as an Anaesthetic Research Society Vacation Scholarship. We would also like to express our thanks to Dr Andrew Dick of the Department of Ophthalmology for his help with flow cytometry.

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