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Short communication

Deficit of interleukin 7 in septic patients

Juan C. Andreu-Ballester [a,*], Carmen Cuellar [b,1], Carlos Garcia-Ballesteros [c,2], Jaime Pérez-Griera [d,3], Victoria Amigó [c,4], Ana Peiró-Gómez [e,4], Carlos Peñarroja-Otero [f,5], Ferran Ballester [g,6], José Mayans [h,4], Constantino Tormo-Calandín [i,7]

[a] Research Department, Arnau de Vilanova Hospital, Valencia, Spain
[b] Department of Parasitology, Faculty of Pharmacy, Complutense University, Madrid, Spain
[c] Hematology Department, Arnau de Vilanova Hospital, Valencia, Spain
[d] Biopathology Department, Clinic Universitary Hospital, Valencia, Spain
[e] Emergency Department, Arnau de Vilanova Hospital, Valencia, Spain
[f] Casa de la Salud Hospital, Valencia, Spain
[g] Faculty of Nursing, University of Valencia, Spain
[h] Hematology Department, Arnau de Vilanova Hospital, Valencia, Spain
[i] Catholic University School of Medicine, Valencia, Spain

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ABSTRACT

We recently demonstrated an overall decrease of all αβ and specially γδ T cell subsets in patients with sepsis compared with healthy subjects. IL-7 is a crucial factor for development of γδ T cells and survival in sepsis but its association with sepsis severity, evolution of organ failure and death still has not been investigated. Sera from 78 patients who met criteria for sepsis were analyzed vs control group. Septic patients showed the lowest levels of IL-7. Patients with severe sepsis reached levels of IL-7 higher than those observed in the groups of uncomplicated sepsis and septic shock. The frequency of γδ T cells at admission was lower in septic patients vs control group. At the time of admission, the frequency of γδ T cells in septic patients who subsequently died was lower than the observed in the group of patients that instead survived.

1. Introduction

Sepsis continues to have a high incidence and mortality in Spain and in other countries [1,2]. We recently demonstrated an overall decrease of all αβ and γδ T cell subsets in patients with sepsis compared with healthy subjects. Furthermore, the largest reduction was detected in γδ T cells. In addition, the reductions of T cells, specifically, CD56+ γδ T cells were significantly associated with mortality [3]. These results agree with the major theories for the pathogenesis of sepsis: The initial immune response in sepsis is considered as a hyper-inflammatory response resulting in the production of a "cytokine storm" with increased levels of TNF-α, IL-1β, and IL-6, but then an un-controlled anti-inflammatory response may be developed and patients enter a hypo-inflammatory phase considered as an "immune paralysis" and consequently they fail to mount adequate immune responses [4].

IL-7 is a pluripotent cytokine produced by stromal and epithelial cells in the bone marrow and thymus and by fibroblastic reticular cells in T-cell zones of secondary lymphoid organs [5]. IL-7 is a crucial factor for lymphocytes and it is absolutely required for development of γδ T cells [6].

Sepsis models and clinical trials showed that IL-7 promoted T cell functionality and improved survival in sepsis [7,8]. Therefore, this cytokine is being used, along with other immunotherapeutic agents, for restoring T cell function and therefore improving their evolution and prognosis [9].

The cytokine profile in plasma of patients with severe sepsis has been measured in several studies but association with sepsis severity,

* Corresponding author. Research Department, Arnau de Vilanova Hospital, San Clemente 12, 46015, Valencia, (Spain).
E-mail addresses: jcandreu@ono.com (J.C. Andreu-Ballester), cuellarh@farm.ucm.es (C. Cuellar), garcia_carbar@gva.es (C. Garcia-Ballesteros), perez.griera@gmail.com (J. Pérez-Griera), amigo_vic@gva.es (V. Amigó), ampeiro733y@hotmail.com (A. Peiró-Gómez), carlospenyarroja@medinserv.es (C. Peñarroja-Otero), ballester_fer@gva.es (F. Ballester), mayans_jos@gva.es (J. Mayans), tormo_con@gva.es (C. Tormo-Calandín).
[1] Tel.: +34 913941818.
[2] Research Department and Hematology Department. Arnau de Vilanova Hospital. Valencia (Spain). Tel.: +34 963868784; fax: +34 963868580.
[3] Tel.: +34 629791324.
[4] Tel.: +34 963868500; fax: +34 963868580.
[5] Tel.: +34 696473939.
[6] Spanish Consortium for Biomedical Research in Epidemiology and Public Health (CIBERESP), and Centre for Public Health Research (CSISP-FISABIO), Valencia (Spain). Tel.: +34 961 92 57 79; fax: +34 961 92 57 03.
[7] Professor of Catholic University (School of Medicine). Valencia (Spain). Tel.: +34 961622333; fax: +34 961622433.

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evolution of organ failure and death was not investigated. Venet F et al. showed that IL-7 pathway remains activable in septic patients, suggesting that IL-7 plasmatic levels may be increased in a homeostatic manner after septic shock as a consequence of marked lymphopenia [8]. White M et al. showed that patients with post-operative infection and sepsis had deficient IL-2 and IL-7 gene expressions in peripheral blood leukocytes, although IL-7 levels were similar to the control group [10]. Bozza et al. [11] compared cytokine concentrations among patients with severe sepsis and septic shock, and observed that concentrations of IL-7 were significantly increased in septic shock as compared with severe sepsis.

The aim of this study was to measure serum levels of IL-7 in septic patients on admission and their relation to frequency of γδ T cells, time evolution of sepsis and mortality.

2. Material and methods

2.1. Study population and design

In this case–control study, sera from 78 patients who met criteria for sepsis were analyzed [3]. All patients were admitted to the Emergency and Intensive Care Unit of Arnau de Vilanova of Valencia (Spain). Sepsis was defined according to internationally established criteria [12], as well as their different stages, sepsis without organic failure and with organic failure (severe sepsis) [13]. In addition, patients had to meet the following requirements: not suffering from immunodeficiency or autoimmune diseases, have not been vaccinated in the last six months, and have not been subjected to immunosuppressive therapy. The control group (78 subjects) was recruited from relatives of patients admitted to the hospital who were not relatives of septic patients. They should have the same characteristics of the patients in addition to not suffer acute infectious diseases. Both groups were matched by sex and age (±5 years). The Research and Ethics Committee of Arnau de Vilanova Hospital approved the study.

2.2. Flow cytometry and ELISA

Blood samples were taken from sepsis patients and controls at the time of admission and diagnosis in the Emergency Department or Intensive Care Unit of the hospital, and prior to any therapeutic action.

Peripheral blood samples obtained by venipuncture were collected in K3-EDTA anticoagulant and processed within 6 h of collection. Blood cell counts were performed using Coulter LH750 automated hematology analyzer (Beckman Coulter, Fullerton, CA). CYTO-STAT tetraCHROME (Beckman Coulter) CD45-FITC (clone: B3821F4A)/CD4-RD1 (clone: SFCI12T4D11)/CD8-ECD (clone: SFCI21Thy2D3)/CD3-PC5 (clone UCHT1) and CYTO-STAT tetraCHROME (Beckman Coulter) CD45-FITC/CD56-RD1 (clone: N901/NKH-1)/CD19-ECD (clone: J3-119)/CD3-PC5 monoclonal antibody reagents were used for the peripheral blood subpopulations, and CD4-PC7 (clone: SFCI12T4D11), CD8-FITC (clone:B9.11), CD56-PE and CD56-PC7 (clone:N901 (NKH-1)), CD3-PC5 (clone: UCHT1), TCRab-PE (clone:IP26A), TCRgd-FITC (clone: IMMU510) and TCRgd-PE (clone: IMMU510) are all purchased from Beckman Coulter.

Cytometry analysis was performed using a Beckman-Coulter multiparameter analyzer, Cytomics FC 500, and later analyzed with CXP Software (Beckman Coulter, Fullerton, CA). Minimums of 50.000 events were acquired. Absolute counts of circulating cell subsets were calculated using the percentages obtained by flow cytometry and the leukocyte count was obtained from the hematological analyzer using a dual-platform counting technology.

For the determination of serum IL-7 levels the human IL-7 Instant ELISA kit (eBioscience, San Diego, CA) was used according to the manufacturer's instructions. Serum samples were maintained at −80 °C until analytical determinations were done. According to the product information, the detection limit of the human IL-7 was determined to be 9.5 pg/ml, mean of six different assays. For the sample assay we used a standard curve with eight different point concentrations, from 0 to 1000 pg/ml.

2.3. Statistical analysis

The assumption of a normal distribution for continuous variables was verified using graphic tests and the Kolmogorov–Smirnov test with a Lilliefor's significance correction. When normality was assumed, the Student *t* test was used to compare the means of the quantitative variables. When the hypothesis of normality of the quantitative variable was not accepted, the nonparametric Mann–Whitney *U* test was used. To analyze the correlations between T cell subsets and IL-7, Spearman Rho test was used. The data were analyzed using the statistical software SPSS, version 19.0.

3. Results and discussion

3.1. Serum levels of IL-7 at admission were lower in septic patients

The characteristics of patients with sepsis are shown in Table 1. The SOFA (mean 4.8) and APACHE II (mean 15.5) scores are low for a sepsis cohort, but correspond to the case-mix of the study population, 78 patients of which only 19 (24.4%) had septic shock, while the rest of the population had uncomplicated sepsis in 19 cases (24.4%), and severe sepsis in 40 (51.2%).

Septic patients showed lower levels of IL-7 (24.6 ± 15.3 pg/ml) vs control subjects (39.9 ± 37.8 pg/ml), P = 0.045. IL-7 levels were lower in all stages of sepsis (uncomplicated sepsis, severe sepsis and septic shock) with respect to the control group. Patients with severe sepsis reached levels of IL-7 (28.0 ± 15.4 pg/ml) higher than those observed in the groups of uncomplicated sepsis and septic shock (20.5 ± 12.3 and 21.1 ± 11.4 pg/ml, respectively) although these levels were lower than in the control group (Fig. 1A).

IL-7 is a crucial factor for lymphocytes and it is absolutely required for development of γδ T cells. IL-7-deficient (IL-7(−/−)) mice have reduced numbers of B and TCR αβ cells, but lack mature TCR γδ cells [6]. For these reasons IL-7 should be undoubtedly implied in the

Table 1
Characteristics of patients with sepsis.
N = 78. *1 patient with each of the following diagnoses: Diverticulitis, appendicitis, pelvic inflammatory disease, gangrene, malaria, intestinal obstruction, acute pancreatitis, primary spontaneous peritonitis and septic arthritis. S.D.: Standard deviation.

	Mean ± S.D.		No. (%) of patients
Age − yr	68.9 ± 18.4	- Abscess	3 (3.8)
APACHE II score	15.5 ± 6.1	- Acute cholangitis	2 (2.6)
SOFA score	4.8 ± 3.6	- Undetermined	2 (2.6)
	No. (%) of patients	- Cellulitis	2 (2.6)
Sex		- Others*	9 (11.7)
Male	46 (59.0)	Organic failure	
Female	32 (41.0)	Acute respiratory failure	38 (48.7)
Emergency department	36 (46.2)	Acute renal failure	28 (35.9)
Intensive care unit	42 (53.8)	Neurologic	24 (30.8)
Stages of sepsis		Shock	19 (24.4)
Sepsis	19 (24.4)	Metabolic	19 (24.4)
Severe sepsis	40 (51.2)	Acute hepatic failure	17 (21.8)
Septic shock	19 (24.4)	Hematologic	14 (17.9)
Positive cultures	33 (42.3)	No. of organs with failure	
In-hospital death	20 (25.6)	0	19 (24.4)
Diagnosis		1	12 (15.4)
Pneumonia	43 (55.1)	2	18 (23.1)
Urinary tract infections	11 (14.1)	3	14 (17.9)
Acute cholecystitis	6 (7.7)	4 or more	15 (19.1)