ORIGINAL

Increased frequency of NK and gamma delta 1 T cells in a cohort of patients with sepsis[*]

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Abstract

Introduction: The mechanisms involved in the immunopathogenesis of sepsis are not well established. The clinical and therapeutic relevance of several soluble mediators has been described and the contribution of cellular components with immunoregulatory roles has begun to be elucidated.

Objective: To describe changes in the frequency and production of IFN-γ and IL-10 occurring in NK cells and γδ T lymphocytes in a cohort of patients with different manifestations of septic syndrome.

Materials and methods: This was a prospective cohort study. Patients with sepsis (n=26), and severe sepsis (n=83) from adult emergency rooms and intensive care units, as well as healthy volunteers (n=8), were included. For all participants, the frequency and phenotype of NK cells and γδ T lymphocytes and the percentage of IFN-γ and IL-10 positive NK and γδ T cells were evaluated by flow cytometry. The NK cells were phenotyped based on the expression of CD56 and CD16 and the γδ T cells on the expression of δ1 and δ2 chains.

Results: Patients with sepsis and severe sepsis exhibited an increase in the frequency of NK cells with changes in the proportion of the CD56^{bright}/CD16$^-$, CD56^{bright}/CD16^{dim} and CD56^{dim}CD16$^-$ subpopulations; these cells showed a proinflammatory cytokine profile. A decrease in the Vδ2 subset of γδ T lymphocytes was also observed.

Conclusions: Our results indicate a role for NK and γδT cells during sepsis, however, their exact contribution in the pathogenesis of sepsis syndrome requires further studies.

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Incremento en la frecuencia de células NK y células T gamma delta 1 en una cohorte de pacientes con sepsis

Resumen

Introducción: Los mecanimos involucrados en la inmunopatogénesis de las sepsis no han sido claramente establecidos. La importancia clínica y terapéutica de diferentes mediadores solubles ha sido descrita y la contribución de componentes celulares con propiedades inmunorreguladoras ha empezado a dilucidarse.

Objetivo: Describir los cambios en la frecuencia y en la producción de IFN-γ e IL-10 que se observa en células NK y linfocitos T γδ en una cohorte de pacientes con diferentes manifestaciones del síndrome séptico.

Materiales y métodos: Estudio de cohorte prospectiva, donde pacientes con sepsis (n = 26) y sepsis grave (n = 83) provenientes de las salas de emergencias y unidades de cuidado intensivo, y controles sanos (n = 8) fueron incluidos. Tanto la frecuencia y fenotipo de las células NK y T γδ como el porcentaje de células IFN-γ+ e IL-10+ fueron evaluados mediante citometría de flujo. Las células NK fueron fenotipificadas con base en la expresión de las moléculas CD56 y CD 16 y los T γδ con base en la expresión de las cadenas δ1 y δ2.

Resultados: En los pacientes con sepsis y sepsis grave se observó un incremento en la frecuencia de las células NK con cambios en las proporciones de las subpoblaciones CD56^{bright}/CD16$^-$, CD56^{bright}/CD16^{dim} y CD56^{dim}CD16$^-$; en estas células se observó un perfil de citocinas proinflamarias. Se observó una reducción en el porcentaje de células Vδ2.

Conclusiones: Los resultados sugieren un papel de las células NK y linfocitos T γδ durante la sepsis; sin embargo, su contribución en la patogénesis de este síndrome requiere estudios adicionales.

Introduction

Sepsis is considered the main cause of death in non-coronary intensive care units and one of the most common causes in the general population worldwide, with a mortality rate close to 30% and an annual increase in the incidence rate of 9%. This syndrome is complex and involves a continuum of pathological processes of varying severity, including different clinical stages such as sepsis, severe sepsis and septic shock.[1] The immune response to sepsis is characterized by an initial pro-inflammatory phase manifested by high levels of the tumor necrosis factor-α (TNF-α), interleukin (IL) 1ß, IL-6, IL-8, leukotrienes, adhesion molecules and reactive oxygen species.[2] The second phase is characterized by anti-inflammatory mediators produced for limiting the inflammatory process; however, patients who survive the initial phase of sepsis may develop a state of immunosuppression.[3]

Inmmunosupression during sepsis, also known as compensatory anti-inflammatory response syndrome (CARS),[4] is characterized by defects in phagocytosis, antigen processing and presentation, apoptosis of dendritic cells, neutrophils and T and B lymphocytes, functional alteration of monocytes induced by the low expression of class II histocompatibility molecules, and the increased production of anti-inflammatory cytokines like IL-4, IL-10, TGF-ß and the soluble receptor of TNF-α.[5,6] During the immunosuppressive stage, patients are prone to acquire secondary infections and therefore the probability of death increases.[7]

The balance between the pro- and anti-inflammatory events of the immune response is a critical event in the outcome of the patient and, although soluble anti-inflammatory mediators have been described as key components of the evolution of sepsis, little is known about the role of lymphocytes of the innate immunity that can mediate immune regulation. Diverse evidence supports the importance of natural killer (NK) cells and γδ T lymphocytes during sepsis.[8-11] However, the role of these cells during the pathogenesis of the disease is yet controversial, mainly because most of the available knowledge comes from animal models whose data cannot be easily transferred into the humans.[8-11] Considering that innate lymphocytes could play an important role during both, the pro-inflammatory and the immune suppressive stages of sepsis, the aim of the present study was to evaluate the frequency, phenotype and functional response of NK and γδ T cells in a cohort of patients with different manifestations of the septic syndrome.

Materials and methods

Patients and study design

This cross-sectional study with prospective data collection included 114 patients with sepsis admitted to the emergency department (ED) or to intensive care units (ICUs) at the "Hospital Universitario San Vicente de Paúl" (Medellín, Colombia). This is a 550-beds, fourth level University Hospital with an admission rate of approximately

1,800 patients per month through the ED, and it is a reference institution for a region of approximately 3 million habitants. Patients in this study belong to a larger cohort examined for the research "*Toward an operative diagnosis in sepsis: a latent class approach*".[12] All patients were >18 years old with clinical suspicion of infection, and were admitted in the ED or ICUs within 24 hours before recruitment to the study. Sepsis and severe sepsis were defined by clinical consensus of three experts with clinical practice in internal medicine, infectious diseases and intensive care. This consensus followed the criteria proposed by the last international conference of sepsis definitions.[13]

The general protocol for each patient after recruitment and within 24 hours was: collection of baseline clinical data; calculation of entrance Sepsis Organ Failure Assessment (SOFA) score; Acute Physiology and Chronic Health Evaluation (APACHE II) score, and blood sampling (one EDTA tube of 10 ml). During seven days, patients were monitored with daily recording of any relevant data registered in medical or nurse records, using a standardized case report form. The local scientific ethical committee ("*Comité de* ética *del Centro de Investigaciones Médicas de la Facultad de Medicina de la Universidad de Antioquia*") approved sample collection on the basis of informed consent. To provide a panel of values from healthy donors, we included peripheral blood samples from eight adults, four males, with median age of 46 years within the range of 39-49 years.

Cell phenotyping

Peripheral blood mononuclear cells (PBMC) from patients with diagnosis of sepsis or severe sepsis were isolated using density gradients (Ficoll Histopaque 1077, Sigma Aldich) and cryopreserved until their processing. Cells were thawed after 3-5 months of freezing and in all cases viability was higher than 90%. To determine the frequency and phenotype the following combination of monoclonal antibodies were used: anti-CD3 APC-Cy7, CD16 FITC, and CD56 PE for NK cells and anti-CD3 APC-Cy7, anti-γδTCR FITC, CD8 PE-Cy5 or anti-δ1FITC and anti-δ2 PE for γδT lymphocytes. Most of the antibodies were from eBioscien cie (San Diego, CA, USA), the anti-δ2 antibodies were from Becton Dickinson-Pharmingen (Franklin Lakes, NJ, USA) and the anti-δ1antibody was from Thermo Scientific Pierce Biotechnology (BD Biosciences, Rockford, IL, IL USA).

To evaluate the functional profile of these cells, we determined the production of IFN-γ and IL-10 by intracellular staining, in response to phytohaemagglutinin (PHA) plus anti-CD28 antibody. Briefly, 1×10^6 PBMCs were cultured in 24-well plates coated with anti-CD28 antibody at 1 μg/ml, and then PHA (Sigma-Aldrich, St Louis, MO, USA) was added at 6 μg/ml. The cells were incubated at 37 °C and 5% of CO_2 for 22 hours and 8 μg/ml of brefeldin A (Sigma-Aldrich, St Louis, MO, USA) were added; the culture was further incubated for 26 hours under the same conditions. Next, the cells were fixed/permeabilized (staining buffer kit, e-Bioscience) and stained intracellularly using the antibodies anti-IFN-γ APC (eBioscience) and anti-IL-10 APC (BD Biosciences).

In all cases, the samples were run on a FacsCanto II flow cytometer (Becton Dickinson), collecting data at least on 5×10^4 lymphocytes (gated by forward and side scatter parameters) and analyzed using the Cell Quest Pro soft-ware (Becton Dickinson). All appropriate isotype controls were run for each sample.

Analysis

Comparisons between groups were made with the Kruskal Wallis test and post-hoc comparisons were based on the Dunn test, considering significant differences with p values lower than 0.05. The frequency of cells subpopulations are depicted as the percentage of each cellular type among the total of peripheral lymphocytes. The results are presented as means and standard deviation or medians with minimal and maximal values, according to the type of data; in the Figures the data are represented as median and interquartile range (IQR). Functional analyses are presented as delta percentage (the percentage on stimulated cells minus and the percentage on non stimulated cells), and as ratios between the percentage of IFN-γ positive cells and the percentage of IL-10 positive cells as a way to reflect the net balance of TH1/ TH2 responses, or between the percentage of δ2 and δ1 positive cells to demonstrate the switch in the proportion of these subpopulations in peripheral blood. For the analysis the GradPad Prims 5 software was used.

Results

Study population

A total of 114 patients were included, most of them with severe sepsis (n= 88), according to the presence of at least one organ dysfunction. However, compared to the usual presentation in sepsis, the general findings indicated a particularly young and healthy population with a median age of 50 years (37-67 years) and 53% of them (60 patients) without any previous comorbidity. Also, the average values of the SOFA (3 ± 2) and APACHE II (12 ± 5) scores suggested a relatively low severity. A microbiological confirmation of infection was possible in 44% (50 patients), blood cultures were requested in 89 patients (78%) and 27 of them (24% among the total study population) yielded a positive result. The main demographic and clinical characteristics of the patients, according to the clinical stage, are shown in Table 1.

Increased frequency of NK cells during sepsis

As shown in Figure 1, the percentage of NK, assessed among the total PBMCs and defined as $CD3^-$ and $CD56^+CD16^+/$ $CD56^+CD16^-/CD56^-CD16^+$ cells was significantly increased in patients with sepsis and severe sepsis compared with healthy donors (8,26% (4-30.6) and 8.56% (0.47-61.95) vs 2.99% (1.87-10.89), p=<0.05 for both, respectively). However, no differences were observed in the percentage of these cells between groups of septic patients. In addition, no differences were found among the three groups studied regarding the frequency γδT lymphocytes (median control group: 4.025% (0.95-6.8), patients with sepsis: 3.75% (0.64-17,27) and patients with severe sepsis 3.76% (0.07-22.5)). No differences were found in the absolute number of these cell populations among groups (data not shown).

Table 1 Demographic and clinical characteristics.

	Sepsis (n=26)	Severe sepsis (n=88)	Healthy donors (n=8)
Female gender [n (%)]	10 (38)	41 (46)	4 (50)
Age [median (IQR)]	37 (25-58)	54 (35-68)	46 (39-49)
Nosocomial infection [n (%)]	2 (7.7)	33 (37.5)	
28-day mortality [n (%)]	2 (7.7)	18 (20.4)	
Source of infection [n (%)]			
Symptomatic urinary tract infection	3 (11.5%)	17 (19.3%)	
Other infections of the urinary tract	1 (3.8%)	-	
Superficial surgical site	-	4 (4.5%)	
Deep surgical site	1 (3.8%)	-	
Bloodstream	1 (3.8%)	4 (4.5%)	
Clinical sepsis[a]	2 (7.7%)	14 (15.9%)	
Meningitis or ventriculitis	-	2 (2.3%)	
Mediastinitis	1 (3.8%)	-	
Sinusitis	-	1 (1.1%)	
Gastroenteritis	-	1 (1.1%)	
Intra-abdominal	2 (7.7%)	4 (4.5%)	
Other lower respiratory tract infection	-	1 (1.1%)	
Skin infection	-	1 (1.1%)	
Soft tissue infection	7 (26.9%)	8 (9.1%)	
Burn	-	1 (1.1%)	
Community-acquired pneumonia	6 (23.1%)	15 (17%)	
Other	2 (7.7%)	15 (17%)	
Blood culture ordered [n (%)]	14 (53)	75 (85)	
Gram-positive	1 (7)	9 (12)	
Gram-negative	0	17 (23)	
APACHE II[b] score	5 (3-8)	12 (9-16)	
SOFA[c] score	1 (0-1)	4 (3-6)	

Data are presented as mean ± standard deviation, number (percentage) and median (IQR) as indicated.
[a] According to CDC definition.
[b] APACHE (Acute Physiologic and Chronic Health Evaluation).
[c] SOFA (Serial Organ Failure Assesment).

Sepsis induces changes in the proportion of NK and γδ T cells subpopulations

NK cells were divided in five subpopulations according the differential expression of CD56 and CD16 molecules. The CD56^{dim}CD16^{+} and CD56^{-}CD16^{+} subpopulations represent around 90% of NK peripheral cells, exhibit highly cytotoxic activity, are involved in the antibody-dependent cell-mediated cytotoxicity and produce low amounts of IFN-γ. The CD56^{bright}CD16^{-}, CD56^{bright}CD16^{dim} subpopulations (around 10% of peripheral NK cells) do not have high

cytotoxic activity but are good producers of cytokines. Although the CD56^{dim} CD16^{-} subpopulation is not well characterized, it has been postulated that have cytotoxic activity but also can produce cytokines.[14] In our study population, CD56^{bright}/CD16^{-} NK cells were significantly diminished in patients with sepsis and severe sepsis compared with healthy donors (medians: 1,24% (0.06-14.31) and (3.11% (0-20.29) vs 7.11% (2.61-15.32), p<0.01 and p<0.05, respectively). The CD56^{bright}/CD16^{dim} cells were also decreased in both groups of patients compared with healthy donors but only the group of patients with severe
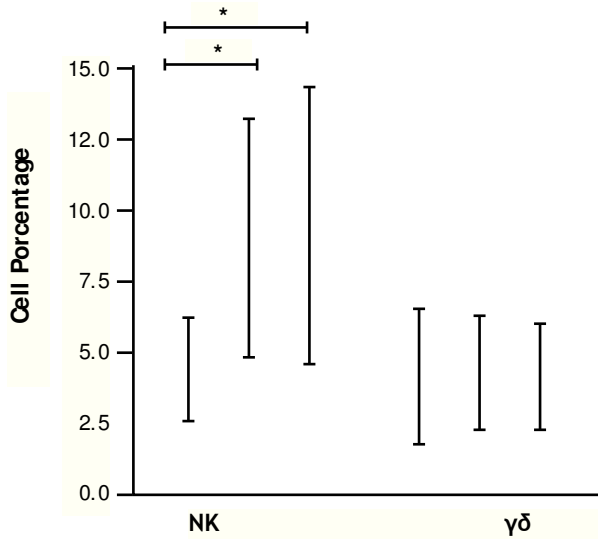
Figure 1 The frequency of NK and γδ T lymphocytes in PBMCs from patients with sepsis or severe sepsis and healthy donors, was determined by flow cytometry. NK cells were defined as CD3⁻ cells and the sum of CD56⁺CD16+, CD56⁺CD16⁻, CD56⁻CD16+. The γδ T lymphocytes were defined as positive cells for the γδ receptor. Median and interquartile range are represented. White bars represent controls, light grey bars represent patients with sepsis and dark grey bars represent patients with severe sepsis. The comparison among groups was performed with Kruskal Wallis test and the post-test comparissons with the Dun test. * p<0.05, ** p<0.01.

sepsis reached statistical significance (medians: 1.70% (0-18.53), vs. 4.42% (1,2-7), p<0.05). Conversely, the CD56^{dim}CD16⁻ subpopulation was increased in patients with severe sepsis compared with healthy donors (8.82%

(0.59-49.25) vs. 4.22% (1.75-7.5), respectively, p<0.05) and also compared with patients with sepsis (4.63% (1-23.78), p<0.05). No differences were found among groups in the frequency of CD56^{dim}/CD16⁺ and CD56⁻/CD16⁺ NK cells (Fig. 2A).

The frequency of Vδ2 and Vδ1 T lymphocytes showed a switch in the proportion of these subsets (Fig. 2B), with a reduction in circulating Vδ2T cells and a significant increase in Vδ1 observed in both groups of patients compared with healthy donors. The median percentages of Vδ2 T cells were: 2.67% (0.55-4.7) in healthy donors vs 0.71% (0.07-14.5) and 0.69% (0-13.9) in patients with sepsis and severe sepsis p>0.05 and p<0.05, respectively). The median percentages of Vδ1 T cells were: 0.95% (0,24-1.84) in healthy donors vs 2.37% (0.55-9.94) and 2,27% (0.07-14.18) p<0.01, for both patients with sepsis and patients with severe sepsis, respectively. When we evaluated the δ2/δ1 ratio, we found that for healthy donors it was 2.15 while for patients with sepsis and severe sepsis it was 0.31 and 0.32, respectively. Finally, we evaluated the expression of CD8 on γδ T lymphocytes, a subpopulation related with immunoregulatory activity, but we did not find differences among groups; the CD8 negative subpopulation of the γδ T lymphocytes was the most frequent in all groups (data not shown).

IFN-γ and IL-10 production by NK and γδ T cells

When the percentage of IFN-γ or IL-10 positive cells after stimulation with PHA/anti-CD28 was evaluated (Fig. 3), there were no significant differences in the production of IFN-γ by NK or γδ T cells among groups. However, both groups of patients have a significantly lower percentage of IL-10-positive NK cells compared with healthy donors
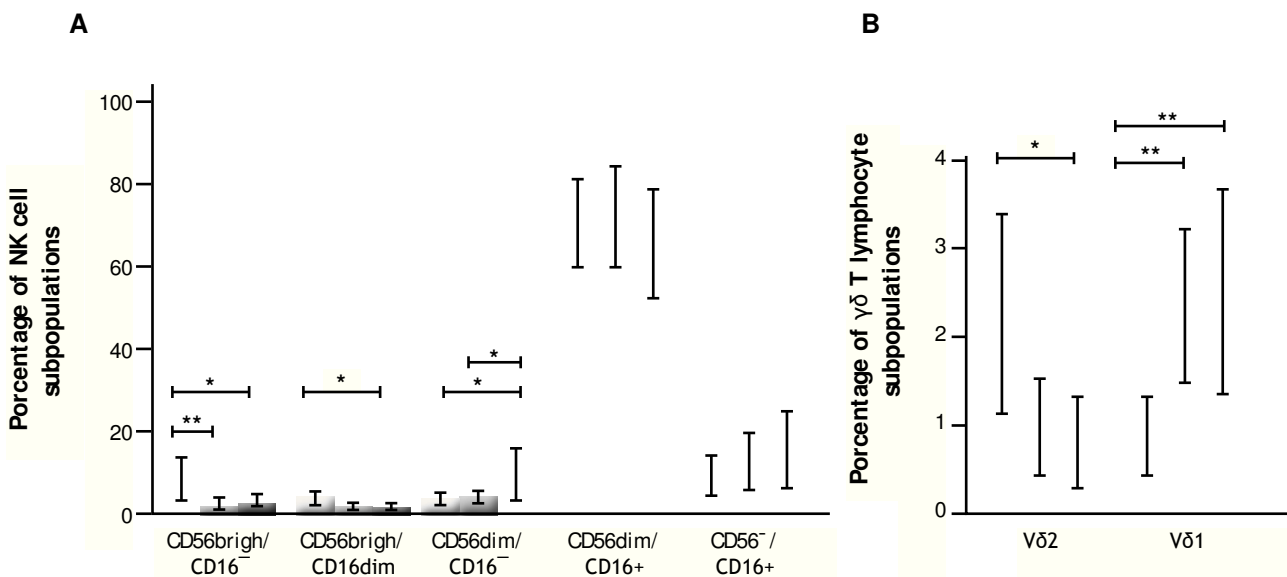
Figure 2 Innate lymphocyte subpopulations. The percentage of NK and γδ T lymphocyte subpopulations was assessed in patients with sepsis or severe sepsis and healthy donors. A) The percentage of each NK subpopulation was determined among total NK cells in PBMC by flow cytometry. The subpopulations were defined according to the differential expression of CD16 and CD56 molecules. B) γδ T lymphocyte subpopulations. The frequency of Vδ2 and Vδ1 cells were determined in PBMCs by flow cytometry. White bars represent controls, light grey bars represent patients with sepsis and dark grey bars represent patients with severe sepsis. Median and interquartile ranges are represented. The comparison among groups was performed with Kruskal Wallis test and the post-test comparisons with the Dunn's test. * p<0.05, ** p<0.01.
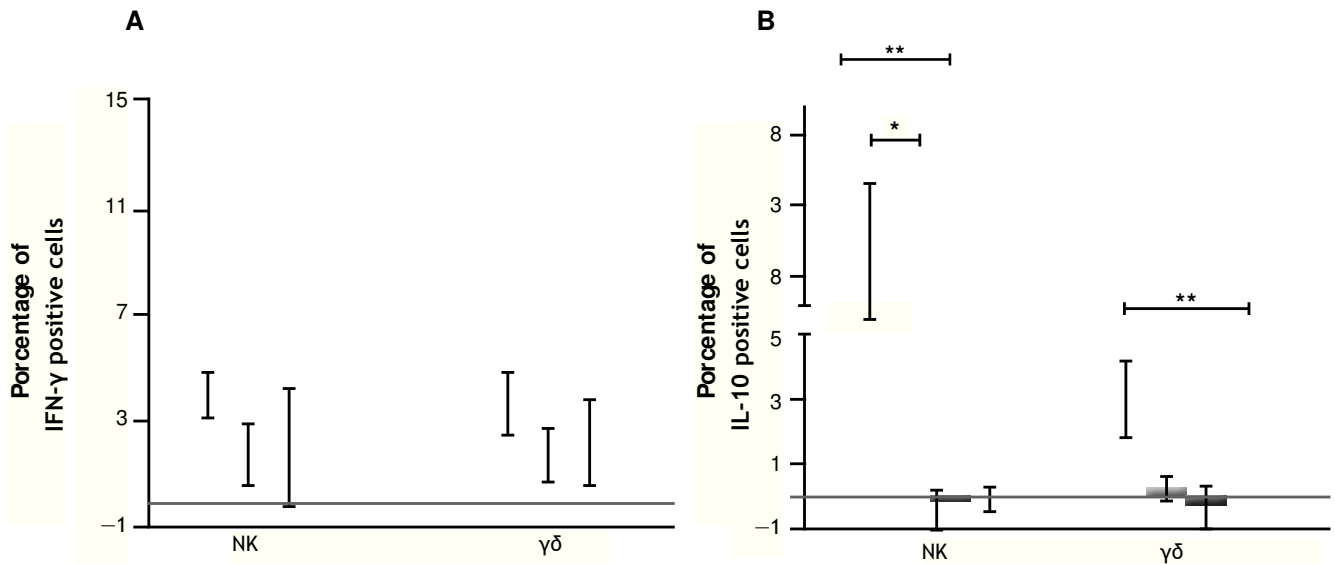
Figure 3 Cytokyne production by NK and γδ T lymphocytes. The production of IFN-γ (A) and IL-10 (B) by these cell subpopulations was evaluated after 48h PHA/anti-CD28 stimulation of PBMCs by intracellular flow cytometry. Since the CD56 molecule is down regulated after stimulation, we included anti-CD19 antibody (BD Biosciences) to better identify the NK population in the lymphocyte gate as cells CD3−CD19−. The percentage of IFN-γ or IL-10 positive cells and the median and interquartile range of delta values (percentage of cytokine positive cells after the stimulus minus the percentage of cytokine positive cells without stimulation) are represented. White bars represent controls, light grey bars represent patients with sepsis and dark grey bars represent patients with severe sepsis. The comparison among groups was performed with Kruskall Wallis test and the post-test comparissons with the Dunn's test. * p<0.05, ** p<0.01.

{0.07% (−3.1%-11.45%) for patients with sepsis (0.04% (−2.54%-1.6%) and for patients with severe sepsis vs 7.12% (4.92%-16.59%) for healthy donors p<0.05 and p<0.01; respectively}. Similarly, patients with severe sepsis and sepsis, have a lower percentage of IL-10 positive γδ T cells compared with healthy donors, although the difference was only significant with severe sepsis patients {3.73% (1.16%-4,25) for healthy donors vs −0.17% (−7.9%-1.94%) for those with severe sepsis, p<0.01 and 0.20% (−0.66%-3.88%), p>0.05 for sepsis group}.

Proinflammatory cytokine profile in NK cells during sepsis

As show in Table 2, in healthy donors the ratios between IFN-γ and IL-10 expressing NK and γδ T cells were close to 1, in both unstimulated and stimulated cells. Conversely, a preferentially proinflammatory profile was observed in patients since the percentage of IFN-γ positive cells was higher than the percentage of IL-10 positive cells in NK cells (p<0.05 for both unstimulated and stimulated cells)

Table 2 Th1 / Th2 cytokine profile.

	Ratio of IFNγ+/IL-10+ cells			
	Healthy donors	**Sepsis**	**Severe sepsis**	**P value***
NK				
UN	1.05	5.97	7.79	0.0283
PHA/ anti-CD28	0.94	9.2	12.18	0.0101
iNKT				
UN	1.41	0.89	0.65	0.3051
PHA/ anti-CD28	0.97	1.25	1.5	0.4596
γδ				
UN	1.33	1.25	1.31	0.774
PHA/ anti-CD28	1.19	3.67	3.12	0.3841

PHA: phytohemagglutinin; UN: cells cultured without stimulus.
  * The comparison among groups was performed with Kruskall Wallis test.

and in γδ T cells, although in this latter cell subpopulation the difference was not significant.

Discussion

The role of innate lymphocyte subpopulations during the pathogenesis of sepsis has been recently underlined based on their immunoregulatory properties and the potential influence in the outcome of the patient. However, the characterization of these cells in patients with sepsis has not been fully established.

The role of NK cells during the pathogenesis of sepsis is controversial.[15] Here we show that the percentage of peripheral NK cells is increased in patients with sepsis, finding that is in agreement with a previous report in neonates with sepsis[16] and in patients with severe Gram-negative sepsis.[17] In addition, it was also described that patients with severe sepsis who died have higher counts of NK cells in blood and early activation patterns in comparison with those patients who survived.[18,19]

Although we found that the percentage of IFN-γ-positive NK cells do not differ from that of controls, the overall pro-inflammatory response of NK cells could be higher in patients, since there was a significant decrease in the percentage of IL-10 NK positive cells when compare with controls. We speculate that the decreased percentage of IL-10 NK positive cells in patients might be related to the decrease in the CD56^{bright} NK subpopulations which is primarily involved in cytokine production.[20]

In the context of increased percentage of NK cells and lower production of anti-inflammatory cytokines, NK cells can promote the overactive immune response observed in the initial phases of sepsis. This would be in agreement with what has been postulated in experimental models of sepsis, where there is increasing evidence of the detrimental role of NK cells during the disease.[8,10,21]

Despite the low severity of our studied population we found an increase in the percentage of the subpopulation CD56^{dim}/CD16$^-$ in severe sepsis patients.

The origin and functional profile of CD56^{dim}/CD16$^-$ NK subpopulation has not yet been clarified. The increased percentage of these cells in patients with severe sepsis might suggest a possible association between a cytotoxic activity and the severity of sepsis; unfortunately, we could not perform cytotoxicity assays. In a previous study, an association between the activation of cytotoxic cells and higher mortality and severity of organ dysfunction in patients with sepsis was established.[22] However, it cannot be ensured that the increased frequency of the cytotoxic subpopulations represents an increase in cytotoxic activity, given that previous reports in other pathological contexts, such as HIV infection, have indicated that despite an increased frequency of these cells their functional activity is greatly altered.[23]

γδ T cells represent a small proportion (1-10%) of the total T lymphocytes in peripheral blood, where Vδ2 T cells are the most prevalent subset (>80%). A smaller subset has the receptor V δ1 involved in the recognition of stress-induced MHC-related antigens.[24] In contrast to previous studies, we did not find differences in the frequency of γδ T lymphocytes in PBMCs among the three groups studied. Matsushima

et al.[25] reported an early decrease in the frequency of γδ T lymphocytes in whole blood from patients with SIRS and patients with sepsis, and Venet et al.[26] reported a significant decrease in γδ T lymphocytes in septic shock patients. These differences could be explained, in addition to the particular characteristics of our study population, by the type of sample, since in the studies of Venet et al. the frequency was established in peripheral blood while in our study, the frequency was determined in PBMCs.

The expression of CD8 on γδ T lymphocytes was also evaluated since the CD8 positive subpopulation has been reported to play a regulatory function in pathological conditions.[27] No differences were found among the groups. Nevertheless, the role that γδ T lymphocytes play during the disease may be attributed to other phenotypic characteristics such as the presence of the variable segments Vδ1 and Vδ2 in their receptor. In fact, there was an interesting switch with a dramatic decrease in the percentage of Vδ2 T cells and the consequent increase of Vδ1 T cells in patients with sepsis. A similar change has been reported in HIV-infected individuals, particularly in patients co-infected with opportunistic pathogens.[28] The reduction of Vδ2 T cells could be related to the response to quimiotactic stimulus or to apoptosis susceptibility by sustained activation,[29,30] while the increase in peripheral Vδ1 T cells could be related to recirculation of these cells from mucosa tissues.[31]

Considering the role that Vδ2 T cells play in the initial control of infections and their marked reduction in patients with sepsis, we can speculate on the importance of this subset in preventing secondary infections, which can be enhanced by the lack of effector cells, and the presence of regulatory cells that favor the expansion or acquisition of infections. However, we found a lower percentage of IL-10-positive γδ T lymphocytes in response to stimulation, a finding that suggests the importance of others immunoregulatory populations during infection. In the setting of our experimental design, it was not possible to establish whether the reduction in the Vδ2 subset of γδ T cells is a predisposing factor to the development of sepsis or is a consequence of the infection itself.

Since these entire cell subpopulations are potent producers of IFN-γ in experimental models of sepsis, they are believed to play a significant role in the dysregulated septic response. In this study we observed that both NK and γδ T cells from patients with severe sepsis tend to produce more IFN-γ than IL-10. Considering the early role of IFN-γ in promoting the functional activity of macrophages and other antigen-presenting cells,[32] and the importance of IL-10 for the regulation of the immune response, the production of these cytokines may have differential implications in the pathogenesis of the septic syndrome, depending on its progression. However, it is important to note that the ability of these cell subpopulations to produce either pro-inflammatory or anti-inflammatory cytokines are conditioned by the type of stimuli and also by the functional maturation of other cell populations, that can amplify immune responses and could be associated with deleterious or beneficial outcomes in patients with sepsis.

Taken together, the results suggest that alterations in innate immune cell subpopulations during sepsis are more evident in severe cases. These patients exhibit an increase

in the frequency of NK, mainly the CD56^{dim} subpopulation with a proinflammatory cytokine profile and also a switch in the proportion of δ2 and δ1 subpopoulation. Since our study population exhibited low severity, the magnitude of the phenotypic changes in most severe cases should be much more evident. However, the clinical relevance of these findings remains to be established.

Conflicts of interest

All the authors confirm that there are no conflicts of interest associated with this publication.

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