



ORIGINAL ARTICLE

The impact of cystic fibrosis on the immunologic profile of pediatric patients[☆]

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KEYWORDS

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Abstract

Objective: To compare the immunologic state of 44 pediatric patients with cystic fibrosis (CF) with a control group consisting of 16 healthy individuals.

Methods: CF patients aged 3 to 12 years with moderate to good clinical score were selected for the study. Erythrocytic glutathione, production of reactive oxygen species, cytokines (TNF- α , IFN- γ , IL-8, IL-6, IL-10) in peripheral blood mononuclear cells cultures under spontaneous and BCG- or PHA-stimulated conditions, serum concentrations of TGF- β 2, IgA, IgG, IgM, IgE, and salivary IgA were evaluated.

Results: The spontaneous production of TNF- α , IL-6, and IL-10, the PHA-stimulated production of IL-6, and the serum TGF- β 2, IgA, and IgG were increased in samples from CF patients. Healthy subjects had a higher production of TNF- α in response to BCG.

Conclusion: Although CF patients appeared clinically stable, the results of their peripheral blood examinations demonstrated an impact on the immune system.

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PALAVRAS-CHAVE

Fibrose cística;
Espécies reativas de oxigênio;
Glutaciona;
Citocinas;
Imunoglobulinas

O impacto da fibrose cística no perfil imunológico de pacientes pediátricos**Resumo**

Objetivo: Comparar o estado imunológico de 44 pacientes pediátricos com fibrose cística (FC) a um grupo-controle formado por 16 indivíduos saudáveis.

Métodos: Foram selecionados para o estudo pacientes com FC com idade entre 3 e 12 anos, apresentando um escore clínico moderado e bom. Foram avaliados a glutaciona eritrocitária, a produção de espécies reativas de oxigênio, citocinas (TNF- α , IFN- γ , IL-8, IL-6, IL-10) em culturas de células mononucleares do sangue periférico em condições espontâneas e estimuladas por BCG ou PHA, a concentração sérica de TGF- β 2, IgA, IgG, IgM, IgE e IgA salivar.

Resultados: A produção espontânea de TNF- α , IL-6 e IL-10, a produção de IL-6 estimulada por PHA e TGF- β 2, IgA e IgG séricas aumentaram em amostras de pacientes com FC. Indivíduos saudáveis tiveram uma produção mais elevada de TNF- α em resposta a BCG.

Conclusão: Apesar de os pacientes com FC parecerem clinicamente estáveis, os resultados de seus exames de sangue periférico mostraram que houve um impacto sobre o sistema imunológico.

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Introduction

Cystic fibrosis (CF) is the most common severe Mendelian disease in infancy; it has a recessive autosomal character, and is more common in whites. The CF gene codifies a protein with 1,480 amino acid residues, identified as cystic fibrosis transmembrane conductance regulator (CFTR). This protein is present mainly in the epithelial cells of the airways, gastrointestinal tract, sweat glandules, and genitourinary system. CFTR functions as a chloride channel in the apical membrane of epithelial cells, and also has other important regulatory functions. Gene mutations may lead to the absence or malfunctioning of CFTR, contributing to the development of CF.^{1,2}

There is agreement that neutrophils and the existence of mutation in the CFTR gene play a role in CF inflammation. The increase in the reabsorption of sodium into the epithelial cells causes an elevated production of adenosine triphosphate (ATP) and, consequently, an increase of intracellular hydrogen peroxide and mitochondrial reactive oxygen species (ROS). Thus, it is possible that the production of ROS is responsible for the initiation and maintenance of the inflammatory process, when the excessive production of ROS activates the nuclear factor kappa-B (NF- κ B) pathway and increases the transcription of pro-inflammatory cytokines that may increase the production of ROS, perpetuating the cycle.^{3,4}

The defect in ROS neutralization may also be responsible for the oxidative stress in CF. CFTR is also involved in the transportation of glutathione (GSH) into the epithelial cells; thus, it is possible that the transportation of GSH is faulty in epithelial cells in patients with CF. GSH also prevents the degradation of NF- κ B-inhibitor (I- κ B) and therefore, low levels of intracellular GSH may promote the activation of NF- κ B, further increasing the concentration of pro-inflammatory cytokines, and consequently reducing the production of the anti-inflammatory cytokines.^{3,4}

A large number of studies has shown that inflammation has both local and systemic effects, and the improved clinical status of the patients mainly results from a better understanding of the natural course of disease, in particular inflammation. In this context, the aim of this study was to evaluate the inflammatory response and the immunological profile of patients with CF aged 3 to 12 years with a clinical score between moderate and good.

Methods

This cross-sectional study was conducted between January, 2009 and March, 2010. The inclusion criteria for CF patients were: diagnosis of CF confirmed by two tests showing elevated sodium and chloride concentrations and/or identification of two mutated alleles in the CFTR gene, patients between 3 and 12 years old, vaccinated with the BCG vaccine, and Shwachman score between moderate and good.

Regarding the healthy control group, the inclusion criteria were: age between 3 and 12 years, absence of clinical manifestations of disease, no family history of chronic disease, and vaccinated with the BCG vaccine. Exclusion criteria for the control group were: use of anti-inflammatory drugs, and report of fever or any injury a week before blood collection.

Parents or legal guardians of the children were informed about the objectives and methods employed in research, and being aware of the procedures and discomforts to which children would be submitted, with a capacity of free will and without coercion, were requested to sign the informed consent. The study protocol was approved by the Research Ethical Committee of the Medical School of Universidade Estadual de Campinas (UNICAMP), under No. 538/2008.

The survey was conducted with patients from the CF pediatrics outpatient unit, UNICAMP's Clinic Hospital, Campinas, São Paulo, Brazil. Fifty-two patients met the

inclusion criteria, and 44 agreed to participate. The group of healthy children was also recruited in Campinas, São Paulo. Twenty-one children were invited to enroll in the research, but only 16 met the established inclusion criteria.

Blood and saliva collection

Sample collections were performed by the department of clinical pathology of UNICAMP's Clinic Hospital. Eighteen milliliters (18 mL) of peripheral venous blood were collected only once, through standardized techniques in appropriate tubes for each procedure, after at least four hours of fasting. One milliliter (1 mL) of saliva was collected with plastic, sterile Pasteur pipette. The patients were advised to perform oral hygiene by washing with pure water. After collection, aliquots of saliva were centrifuged at 1 g for 7 min and stored at -80 °C until analysis.

Complete blood count

The overall and differential count of blood cells was accomplished through the Cobas-Argos 5 Diff (Roche Diagnostic), in the department of clinical pathology of UNICAMP's Clinic Hospital.

Erythrocyte reduced glutathione

The assay was performed using a minor modification in the method described by Beutler.⁵ Two hundred microliters (200 µL) of peripheral blood EDTA were lysed with 1.8 mL of distilled water. Then, 2 mL of 1.67% metaphosphoric acid solution were added, and the mixture was filtered. Four milliliters (4 mL) of 0.3M Na₂HPO₄ solution were added to 1 mL of the clear filtrate and read at 412 nm on a Beckman spectrophotometer. A second optical density reading was taken after the addition of 100 µL of dithiobis-nitrobenzoic acid (DTNB) solution to the filtrates. Results were expressed as mg/dL.

Production of reactive oxygen species by granulocytes

Measurements were performed by the modified technique of Emmendorffer et al.⁶ and Richardson et al.⁷ Dihydrorhodamine 123 (DHR 123) was used to quantify phorbol-12-myristate-13-acetate (PMA)-stimulated ROS production by granulocytes from peripheral blood. After lysing the erythrocytes, the events were recorded in an Epics XL-MCL flow cytometer (Beckman-Coulter - USA). Forward and side scatters were used to gate granulocytes. Basal ROS production was determined as the percentage of fluorescent events without PMA stimulus. PMA-stimulated ROS production was measured through the ratio of median fluorescence from PMA-stimulated granulocytes and median fluorescence from non-stimulated granulocytes.

Cytokine concentration in culture supernatant

The analysis of cytokine production was performed by the culture of peripheral blood mononuclear cells (PBMC),

modified from the protocol of Gaines et al.⁸ Lyophilized BCG (Moreau Rio de Janeiro vaccine vials) were freshly reconstituted with the culture medium RPMI 1640 (Sigma - USA) and used at 5×10⁵ UFC/mL. Phytohemagglutinin (PHA, Sigma - USA) was used as a positive control at 7.5 µg/mL. PBMC was diluted to 2×10⁶ cells/mL in supplemented RPMI, and incubated for 48 h (37 °C) in round-bottomed 96-well tissue culture plates with BCG, or PHA, or medium alone. The cultures' supernatants were collected and stored at -80 °C for enzyme linked immunosorbent assay (ELISA). Commercial kits (DuoSet, RD Systems - USA) were used to measure interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and interleukins (IL-10, IL-8 and IL-6) concentrations, as recommended by the manufacturer. All samples and points of the standardized curves were measured in duplicate. The results were expressed in µg/mL.

Concentration of transforming growth factor-β2 in blood serum

The transforming growth factor-β2 (TGF-β2) dosage was performed by ELISA (DuoSet, RD Systems - USA). The assays were performed following the manufacturer's protocols. The results were expressed in µg/mL.

Serum immunoglobulins (IgA, IgG, IgM e IgE) and salivary IgA

The determination of serum concentration of immunoglobulins (IgA, IgG, IgM, IgE) and salivary IgA were performed by nephelometry.

Statistical analyses

All the results were analyzed using the Statistical Package for Social Sciences (SPSS) for Windows, version 7.5.1 - USA. Data analysis was performed with Student's *t*-test, Mann-Whitney's test, and Spearman's test. Significance was defined as p-value < 0.05. GraphPad Prism (version 4.0, GraphPad Software - USA) was used to plot GSH, ROS production, cytokines, and immunoglobulins graphical data.

Results

In the present study 44 children with CF and 16 healthy subjects were included, paired by age. Table 1 shows the characteristics of the CF patients and healthy subjects.

As illustrated in Table 1, patients with CF and healthy subjects were paired by age. Regarding the colonization of the airways of patients, there was a high prevalence of colonization by *Staphylococcus aureus*, a relatively high presence of *Pseudomonas aeruginosa* and *Pseudomonas aeruginosa* mucoid, and a low incidence of *Burkholderia cepacia*.

The median values of leukocyte and erythrocyte cell numbers for the CF patients and healthy control group are shown in Table 2.

The number of total leukocytes was higher for the group of patients with CF. Mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) were both lower for CF children.

Table 1 Characterization of cystic fibrosis patients' group and healthy control group.

Study variables	CF patients (n = 44)	Healthy subjects (n =16)
<i>Characteristics</i>		
Age (months)	101.18±38.4 ^a	99.63±31.6 ^a
Gender (female/male)	22/22	12/4
Shwachman score	75 (40-85) ^b	-
<i>Associated disease</i>		
Pancreatic insufficiency	44	-
Hepatic disease	17	-
Diabetes	3	-
<i>Genetic study (CFTR mutation)</i>		
ΔF508 homozygote	14	-
ΔF508 heterozygote/R1162X	2	-
	1	-
ΔF508 heterozygote/N1303K	1	-
ΔF508 heterozygote/G542X	23	-
	4	-
ΔF508 heterozygote/Unknown	34	-
<i>Colonization</i>		
Staphylococcus aureus	11	-
Pseudomonas aeruginosa		
Pseudomonas aeruginosa mucoid	3	
Burkholderia cepacia		

CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator.

^aParametric data: mean ± standard deviation.

^bNon parametric data: median (minimum-maximum).

Table 2 White blood cells and red blood cells in patients with cystic fibrosis and in healthy subjects.

Variables	Patients with CF	Healthy subjects	p*
WBC (x10 ³ /mm ³)	9.91 (5.45-22.38)	6.39 (1.79-8.66)	< 0.001
LINFO (x10 ³)	3.89 (2.27-6.67)	2.79 (1.92-4.99)	0.006
MONO (x10 ³)	0.65 (0.11-3.44)	0.42 (0.24-0.91)	0.003
NEU (x10 ³)	4.82 (2.18-11.28)	3.13 (1.44-5.06)	0.001
RBC (x10 ⁶ /mm ³)	4.91 (4.18-5.74)	4.82 (4.17-5.24)	0.087
HCT (%)	39.5 (34.1-45.3)	40.55 (34.1-45)	0.366
HGB (g/dL)	13.05 (11.4-15.2)	13.35 (11.1-14.8)	0.503
MCH (pg)	27.1 (20.8-29.9)	28.4 (24.2-30.7)	0.011
MCHC (g/dL)	33.4 (29.7-35.3)	33 (30.5-34.5)	0.375
MCV (fL)	80.75 (69-88.1)	85.1 (79.2-89.7)	<0.001

Data expressed as median (minimum - maximum).

CF, cystic fibrosis; HCT, hematocrit; HGB, hemoglobin concentration; LINFO, lymphocytes; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MONO, monocytes; NEU, neutrophils; RBC, red blood cells; WBC, white blood cells.

*Mann-Whitney's test.

Glutathione in the erythrocytes, basal, and PMA-stimulated ROS production are shown in Figure 1. Erythrocytic GSH, basal, and PMA-stimulated production of ROS did not differ between healthy and CF groups.

Figure 2 shows the cytokines (TNF- α , IFN- γ , IL-8, IL-6, and IL-10) concentration in PBMC culture supernatants, serum TGF- β 2, and immunoglobulins (IgA, IgG, IgM, and IgE) from patients with CF compared to healthy children.

Patients with CF had significantly higher spontaneous production of IL-6, TNF- α , IFN- γ , and IL-10 than the healthy

subjects. PHA-stimulated production of IL-6 was also higher in CF patients. Healthy children showed higher production of TNF- α in BCG-stimulated PBMC when compared to CF patients. No difference between the two studied groups was observed for the other culture conditions. The concentration of TGF- β 2 in the blood serum of CF patients was higher than in healthy children.

IgA and IgM concentrations were higher for CF patients, compared to healthy subjects. No significant differences were observed between groups for IgG and IgE.

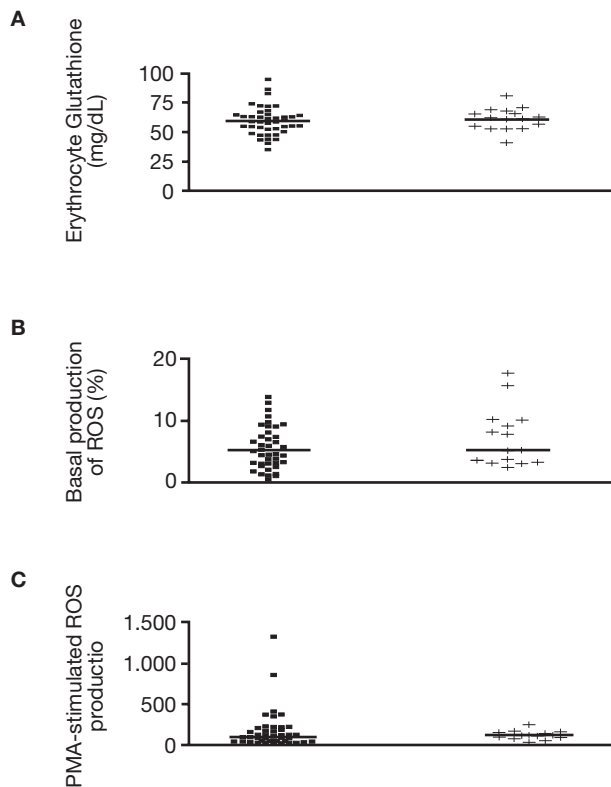


Figure 1 GSH in the erythrocytes, basal, and PMA-stimulated production of ROS of patients with CF (■) and healthy children (+). (A) Concentration of GSH in the erythrocytes (mg/dL), for CF patients (■) $n = 42$ and normal (+) $n = 16$; (B) percentage (%) of basal production of ROS, for CF (■) $n = 44$ and normal (+) $n = 16$ (C); PMA-stimulated ROS production, for CF (■) $n = 44$ and normal (+) $n = 16$. For all analyses, $p > 0.05$. CF, cystic fibrosis; GSH, glutathione; PMA, phorbol-12-miristate-13-acetate; ROS, reactive oxygen species.

Salivary IgA did not differ between the two groups, with median 7 mg/dL (from 1.3 to 52.5 mg/dL) for CF patients and 7.8 mg/dL (from 1.8 to 14.8 mg/dL) for healthy children.

There was a positive correlation between the Shwachman score and production of IL-6 in BCG-stimulated PBMC ($p = 0.04$), production of IFN- γ in BCG-stimulated PBMC ($p = 0.03$), and salivary IGA concentrations ($p = 0.02$). There was a negative correlation between the Shwachman score and IgM concentration in serum ($p = 0.04$), and IgG concentration in serum ($p = 0.004$).

Discussion

Analyzing the ROS production by peripheral granulocytes, it was verified that patients with CF did not show differences in basal or stimulated production in relation to the healthy children group. Differently from these data, ROS levels in two human CF lung epithelial cell lines were elevated compared with two non-CF controls.⁹ The increase of oxidation in the respiratory epithelium results directly from the absence

of CFTR function, or indirectly from the infiltration of inflammatory cells that produce a high quantity of hydrogen peroxide, as well as from a defective antioxidant system incapable of controlling the production of ROS.³

In the present study, it was observed that the glutathione concentration in peripheral erythrocytes from CF patients was not different from the group of healthy subjects. Other authors reported similar data, along with an inverse relation between erythrocytic GSH and pulmonary function in adult CF patients.¹⁰ The same correlation was identified between lymphocytic GSH and pulmonary function in children with CF.¹¹ In apical respiratory epithelium, a reduction of GSH was observed, and CFTR was responsible for the abnormal transportation of this tripeptide, which may indicate that CFTR is involved in maintaining the redox state of the airways.¹²

This oxidative/antioxidative agent imbalance associated with impaired metabolism of intracellular Ca^{2+} and pro-inflammatory cytokines activate the toll-like receptors (TLR) in the epithelial cells. The consequence is the activation of NF- κB and AP-1, resulting in the transcription of pro-inflammatory cytokines leading to the herding of neutrophils and macrophages to the region, increasing the production of ROS.³

As shown in this study, BCG-stimulated production of TNF- α in PBMC cultures was lower in CF subjects. However, the spontaneous cell production of this cytokine was higher in these patients. These results may indicate low BCG cellular memory in CF patients. Conversely, serum TNF- α concentration in adults and adolescents with CF was higher than in normal subjects.¹³

TNF- α concentration in the bronchoalveolar lavage fluid and in the sputum of children and adults with CF was elevated compared to healthy subjects.¹⁴⁻¹⁷ Children with CF chronically infected with *P. aeruginosa* had higher levels of TNF- α in their sputum.¹⁸

IFN- γ is closely involved in the inflammatory process and pulmonary function of CF patients. The results shown here reveal that there is a difference in the spontaneous production of IFN- γ among normal subjects and patients with CF, and that there was a positive correlation between the Shwachman score and the production of IFN- γ in BCG-stimulated PBMC. In a study by Moser et al.,¹⁹ low quantities of IFN- γ and high quantities of IL-4 were observed in PBMC of patients with CF chronically infected by *P. aeruginosa*, when compared to non-infected adult patients. In bronchial biopsy of patients with CF, a higher concentration of IFN- γ was associated with less aggressive disease, while low expression was associated with the presence of acute lung disease with frequent exacerbations.²⁰ Therefore, CF patients with high concentration of IFN- γ and predominant response of type $\text{T}_\text{H}1$ cells have better lung function.

In the present study, no difference in IL-8 production from PBMC cultures between patients with CF and healthy subjects could be demonstrated. A high concentration of IL-8 was identified in the bronchoalveolar lavage fluid of CF patients, even when infection was absent.¹⁴ However, other studies identified significantly elevated concentrations only in patients that showed low pulmonary function.²¹ The concentration of IL-8 in the sputum was also increased, as shown in a series of studies.¹⁶⁻¹⁸

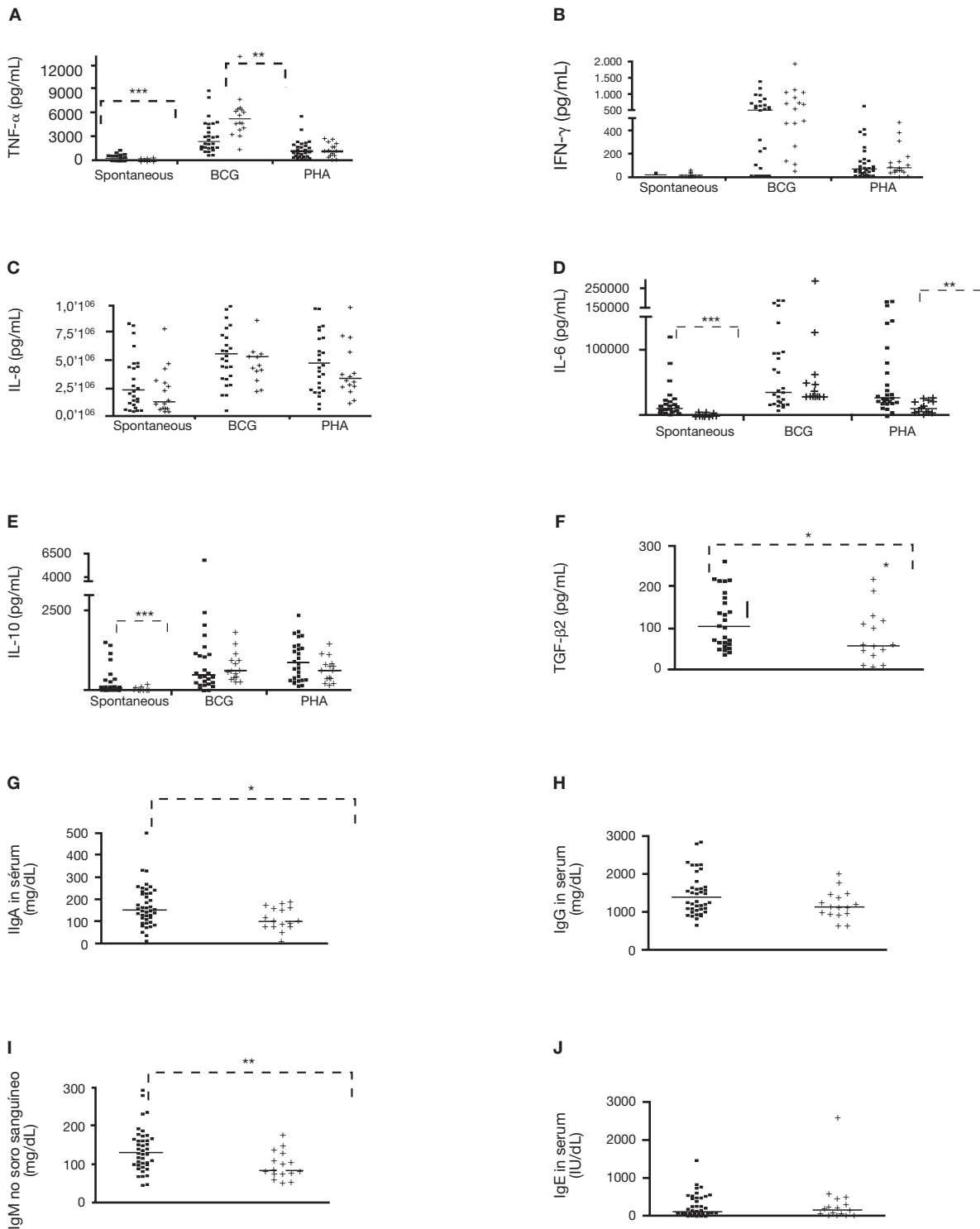


Figure 2 Cytokine concentration in the supernatant of PBMC culture (TNF- α , IFN- γ , IL-8, IL-6 and IL-10), and serum TGF- β 2 and immunoglobulin concentration (IgA, IgG, IgM and IgE) from patients with CF (■) and healthy children (+). The cytokine production in the supernatant of the PBMC culture was performed in non-stimulated cultures (spontaneous), stimulated by BCG or PHA, for 48 hours. (A) TNF- α production (pg/mL); (B) IFN- γ production (pg/mL); (C) IL-8 production (pg/mL); (D) IL-6 production (pg/mL); (E) IL-10 production (pg/mL); (F) concentration of TGF- β 2 (pg/mL) in the blood serum; (G) IgA serum concentration (mg/dL); (H) IgG serum concentration (mg/dL); (I) IgM serum concentration (mg/dL); (J) IgE serum concentration (IU/dL). The statistical test used was Mann-Whitney's, at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. IFN- γ , interferon- γ ; IgA, IgG, IgM, and IgE, immunoglobulins; IL-10, IL-8, and IL-6, interleukins; PHA, phytohemagglutinin; TGF- β 2, transforming growth factor- β 2; TNF- α , tumor necrosis factor- α .

The present findings revealed a higher spontaneous production of IL-6 in PBMC cultures of patients with CF, as well as in response to PHA; a positive correlation between the Shwachman score and the production of IL-6 in BCG-stimulated PBMC was also observed. During exacerbation and the clinical stability period, serum concentration of IL-6 in adult patients with CF was increased, and only after treatment with antibiotics did the serum levels of IL-6 returned to normal.²² The concentration of IL-6 in the bronchoalveolar lavage fluid was increased in CF patients infected with *P. aeruginosa*, similar to normal subjects in non-infected patients.¹⁵ In the sputum, the levels of IL-6 were significantly lower in CF patients in relation to healthy subjects.¹⁶ IL-6 exerts both inflammatory and anti-inflammatory effects.²³

In the present study, a high production of IL-10 was observed under spontaneous and PHA-stimulated conditions in PBMC cultures of CF patients. Lower levels of IL-10 were also observed in PBMC cultures from subjects with CF, in the presence of the tetanus toxoid, compared to healthy subjects.²⁴ Bonfield et al., in 1995,¹⁵ were the first to show that the levels of IL-10 in the bronchoalveolar lavage fluid of patients with CF were significantly lower than in healthy subjects.

In this study, blood serum levels of TGF- β 2 of CF patients were higher than in healthy subjects. In bronchial biopsy of patients with CF, higher expressions of TGF- β 1 were also found for patients with mild pulmonary disease, while in patients with frequent exacerbation the concentration was relatively lower.²⁰ Regarding the plasma concentration of TGF- β 1, normal levels were found in patients with normal hepatic function, and low levels were observed in patients with hepatic disease.²⁵

The higher spontaneous cellular production of IL-10 and the higher concentration of TGF- β 2 in the serum of the patients with CF, compared with healthy children, could be an adaptive response to the inflammatory status that occurs in this disease, since these two cytokines are considered important modulators of inflammation. Regarding these results, the normal values for ROS production by granulocytes and glutathione concentration in erythrocytes from CF patients are justified.

The present findings revealed that the levels of salivary IgA in patients with CF did not differ from those of healthy patients, and a positive correlation between the Shwachman score and salivary IGA concentrations was observed. Some researchers have reported high levels of serum IgA, with normal levels of salivary IgA,²⁶ while others have documented higher saliva and serum IgA in patients with CF, when compared to healthy subjects.²⁷

Most patients with CF evaluated in the present study showed serum levels of IgA, IgG, and IgM within the parameters of normality; however, when compared with healthy subjects, the concentration in the serum IgA and IgM was higher for CF patients, and a negative correlation between the Shwachman score and the IgM and IgG concentration in serum was observed. Some authors showed higher concentrations of serum IgA, IgG, and IgM in patients with CF than in normal and asthmatic subjects; additionally, the levels of IgG and its subclasses showed correlation to the levels of IgA.²⁸

In a study with 419 CF patients, it was observed that patients under 10 years of age had decreased IgG; however, older subjects showed normal or higher levels. The authors concluded that, with the progression of the pulmonary disease, these patients become more susceptible to develop hypergammaglobulinemia.²⁹ Similar results were obtained in a five-year prospective study, in which hypogammaglobulinemia-G was associated to the best weight for the age, fewer hospitalizations for pulmonary exacerbations, less colonization by *P. aeruginosa*, and consequently lower decline in pulmonary function over the time.³⁰

Patients with CF suffer from airway colonization and infection by a variety of organisms, whose host immune responses lead to chronic inflammation and respiratory failure. *Staphylococcus Aureus*, *Haemophilus influenza*, *P. aeruginosa*,³¹ and nontuberculous mycobacteria (NTM) are among such opportunistic pathogens.³² The CF patients in this study showed a high prevalence of colonization by *S. aureus* (77%) and *P. aeruginosa* (36%), and many of them had already been colonized by mucoid. Infection with *P. aeruginosa* affects the longevity of CF patients.³³ Initially, the infection occurs in different periods and is non-mucoid. Over time, *P. aeruginosa* infection becomes mucoid and can lead to biofilm formation, with high resistance to the action of antibiotics and innate immune responses.³⁴

The present results demonstrated that patients with CF have a variety of small but significant alterations compared to healthy children, such as a higher number of total leukocytes, higher spontaneous production of proinflammatory interleukins IL-6, TNF- α , and IFN- γ , as well as anti-inflammatory mediators IL-10 and TGF- β 2. These mediators are important in preventing the pathogen from entering the bloodstream and spreading to organs. The higher IgA and IgM serum concentrations among the CF patients could be due to this adaptive immune response, which fails to adequately control lung infection and the progression of chronic lung disease. Furthermore, the exact risk factors for infection with nontuberculous mycobacteria remain largely unknown. The ability to control intracellular *Mycobacterium* infection relies on cellular immunity and generation of a strong T-cell helper 1 (T_H1) response TNF- α or IL-1 β .³⁵ The lower TNF- α production from BCG-stimulated PBMC culture observed in the CF patients could be associated with higher risk for nontuberculous mycobacteria infection. Understanding these aspects will be essential for the development of improved therapies for CF.³⁶ Further research is needed to elucidate the potential mechanisms involved in the inflammation in CF and its impact on clinical outcomes.

Conclusion

Higher spontaneous production of TNF- α , IL-6, IL-10 and PHA-stimulated concentration of IL-6 were verified in PBMC cultures from CF patients, as well as higher TGF- β 2, IgA, and IgM concentrations in their serum. Conversely, healthy subjects presented increased BCG-stimulated production of TNF- α . Therefore, it can be concluded that even in clinically stable CF patients a higher stimulation of the immune system (humoral and cellular immunity) occurs when compared to healthy subjects.

Conflicts of interest

The authors have no conflicts of interest to declare.

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