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Cytokine and nitric oxide production in an adult patient with staphylococcal scalded skin syndrome.

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Key words: Staphylococcal scalded-skin syndrome, cytokines, nitric oxide.

Abstract. Staphylococcal scalded-skin syndrome (SSSS) is an exfoliative dermatitis resultant from the infection with exfoliative-toxin-producing (ET) *Staphylococcus aureus*. This syndrome usually occurs in children, while adult cases are generally linked to renal-deficiency or immunossupresion. A case of a 74 years old woman presenting SSSS after hospital admission due to cardiovascular disorders is presented and discussed. Cytokines (TNF-α, IFN-γ, IL-6, IL-13 and IL-10) and nitric oxide (NO) production *in vitro* by whole blood leukocytes (WBL) were investigated. Leukocytes stimulated by lipopolysaccharide or phytohemagglutinin produced increased IFN-γ, TNF-α, IL-13 and NO levels after treatment. Based on these results, immunological aspects of the disease are discussed.

Producción de citocinas y óxido nítrico en uno paciente adulto con síndrome de la piel escaldada estafilocócica.

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Palabras clave: Síndrome de la piel escaldada, citocinas, óxido nítrico.

Resumen. El síndrome de la piel escaldada estafilocócica (SSSS) es una dermatitis exfoliante resultante de la infección por *Staphylococcus aureus* productores de toxinas exfoliantes. Este síndrome ocurre generalmente en niños, mientras que su manifestación en adultos está generalmente relacionada con deficiencia renal o inmunosupresión. Se presenta y discute el caso de una mujer de 74 años de edad con SSSS luego de su admisión en el hospital por complicaciones cardiovasculares. Fue evaluada la producción *in vitro* de cito-

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cinas (TNF-α, IFN-γ, IL-6, IL-13, IL-10) y óxido nítrico (NO) por células de sangre total (WBL). Los leucocitos estimulados por lipopolisacarideos y fitohemaglutinina produjeron elevados niveles de IFN-γ, TNF-α, IL-13 y NO en el final del tratamiento. Basados en estos resultados, se discuten los aspectos inmunológicos de la enfermedad.

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INTRODUCTION

Staphylococcal scalded-skin syndrome (SSSS) is an exfoliative dermatitis, resultant from an infection with exfoliative-toxin-producing (ET) *Staphylococcus aureus*. It is mainly characterized by the formation of large bullae without inflammatory cell infiltrate and the separation of extended areas of the epidermis at the stratum granulosum. No alteration in keratinocytes occurs. This disease is characterized by a positive Nikolsky sign, a dislodgement of intact superficial epidermis by a shearing force, indicating a plane of cleavage in the skin (1, 2). It is hypothesized that staphylococcal toxins could act directly at the desmosomes, cleaving desmoglein 1 (3). It has been shown that ETs have structural similarities to serine protease enzymes (4, 5) and esterase activity (6, 7), but protease activity still requires more studies. Moreover, ETs stimulate cytokine release with subsequent edema, which physically forces layers of skin apart at the desmosomes (8), probably acting as superantigens (9, 10). Although edema and reddening of skin occurs in SSSS, there are no studies about cytokine and NO production in patients during disease progression.

Clinical observations show that SSSS is usually seen in children, but there is a link between SSSS in adults and renal-deficiency. It was hypothesized that immune system maturation could protect adults from the action of toxins (11). By injecting recombinant ETA in both neonatal and adult mice, it was demonstrated that maturation of the adaptive immune response was not important for protection, while efficient toxin clearance from serum was associated with protection (11). On the other hand, the occurrence of SSSS in a patient suspected of having an acquired immunodeficiency syndrome-related complex but presenting normal renal clearance indicates that impaired immune response may be also important in *S. aureus* infection establishment and disease outcome (12). Also, a reported case of an adult SSSS, in which a patient presented normal opsonic activity and phagocytic function but marked defects in neutrophil chemotaxis and T-lymphocyte function (13), reinforces the importance of immune response in disease pathogenesis.

Little is known about the effective role played by the patient's immune response during SSSS and the occurrence of staphylococcal scalded skin syndrome in adults is limited to few reports on literature. Besides that, the increasing number of immunosuppressive events such as HIV infection and steroid therapy and the occurrence of intrahospital SSSS outbreaks (14) prompted us to provide some data on cytokine and NO production during SSSS.

CASE REPORT AND DISCUSSION

A case of a 74 year old woman presenting SSSS after hospital admission due to cardiovascular disorders is reported. During hospitalization, a sacral pressure ulcer developed and a few days later the patient presented oral candidiasis followed by classical

SSSS manifestations in the skin of her upper thorax and left shoulder. Clinical diagnosis was confirmed 4 days after skin manifestations by a *S. aureus* positive skin culture. At this time, before treatment (BT), venous blood was collected for biochemical and immunological studies and the patient was medicated (Phenergan 1 pill 8/8h oraly; Cefepime 1g + 50mL Saline 0.9%, 30min, 8/8h i.v.; Fluconazole 200mg/day i.v.). Blood samples were collected again, after treatment (AT), at day 14th for biochemical and immunological analyses when the patient recovered from the disease and returned home. After that, the patient did not return to the hospital and no further complications were observed. Blood analysis showed normal levels of many parameters, including those for renal function such as urea (31.0 and 33.0mg/dL, before and after treatment, respectively) and creatinine (1.0 and 1.0mg/dL). However, low values of total proteins (4.5 and 6.0g/dL), albumin (1.9 and 2.7g/dL), serum iron (19.0 and 29.0∝g/dL) and transferrin (108.0 and 158.0mg/dL), with elevated levels of alpha-1-glycoprotein (2.1 and 1.8g/L) and C-reactive protein (155.7 and 56.4mg/L), confirmed an acute phase response (15). Glucose measurements presented variable levels (145.0 and 67.0mg/dL) and the patient was HIV negative.

Cytokine measurement was performed on supernatants of whole blood leukocytes (WBL) cultures by a sandwich-ELISA assay using monoclonal antibodies matched pairs for TNF-α, IFN-γ, IL-6, IL-13 and IL-10 detection, following the manufacturer's instructions (BD OptEIA™ ELISA Set-BD Pharmingen). Supernatants were collected from WBL culture (1:4 diluted in saline 0,9%) stimulated with PHA (5μg/mL) or LPS (5μg/mL) at 37°C, 5% CO_2 atmosphere after 4 or 24h time periods. Nitric oxide (NO) production was measured in serum and WBL supernatants (50 μL), with 50 μL nitrate reduction solution (5mg/mL NADPH, 0,5M KH_2PO_4, 1U/100∝L nitrate reductase enzyme) (SIGMA) during 18h at 37°C. After this step, 100 μL of Griess reagent was added (0, 1% NEED, 1% sulphanilamide, 5% H_3PO_4) (Sigma) and the nitrite concentration was obtained against a $NaNO_3$ standard curve.

Seven control age-matched untreated adult individuals, also having pressure sores, had their samples collected twice within a 10 day interval following the same culture stimulation and protocol for cytokine and NO detection previously described for SSSS patient. This study was approved by the Research Ethical Committee of Universidade Federal do Triângulo Mineiro (UFTM/CEP protocol #404) and an informed consent was obtained from all individuals enrolled in it.

Pro and anti-inflammatory cytokine production by WBL increased after treatment (Fig. 1), except for IL-6 by LPS and PHA stimulated cells, that remained elevated (Fig. 1c). A cytokine release by PHA stimulated WBL may represent a T-cell response, while LPS stimulation activates innate immune response mainly *via* CD14/Toll-like receptors (TLR). It has been previously reported that IL-6 is highly produced after LPS-mediated TLR4 activation (16, 17), and that this cytokine seems to be essential for wound repair (18). Thus, the elevated IL-6 production by LPS stimulated WBL, observed in this study, suggests that the innate immune system of the patient is not suppressed. On the other hand, the elevated basal levels of IL-6 lead us to raise the question whether alterations on TLR signaling or polymorphisms, in these genes, are involved in SSSS physiopathology.

It has been previously demonstrated that drugs administered to our patient, such as phenergan (promethazine), cefepime, ranitidine and fluconazole, are unable to alter the cytokine pattern *in vitro*

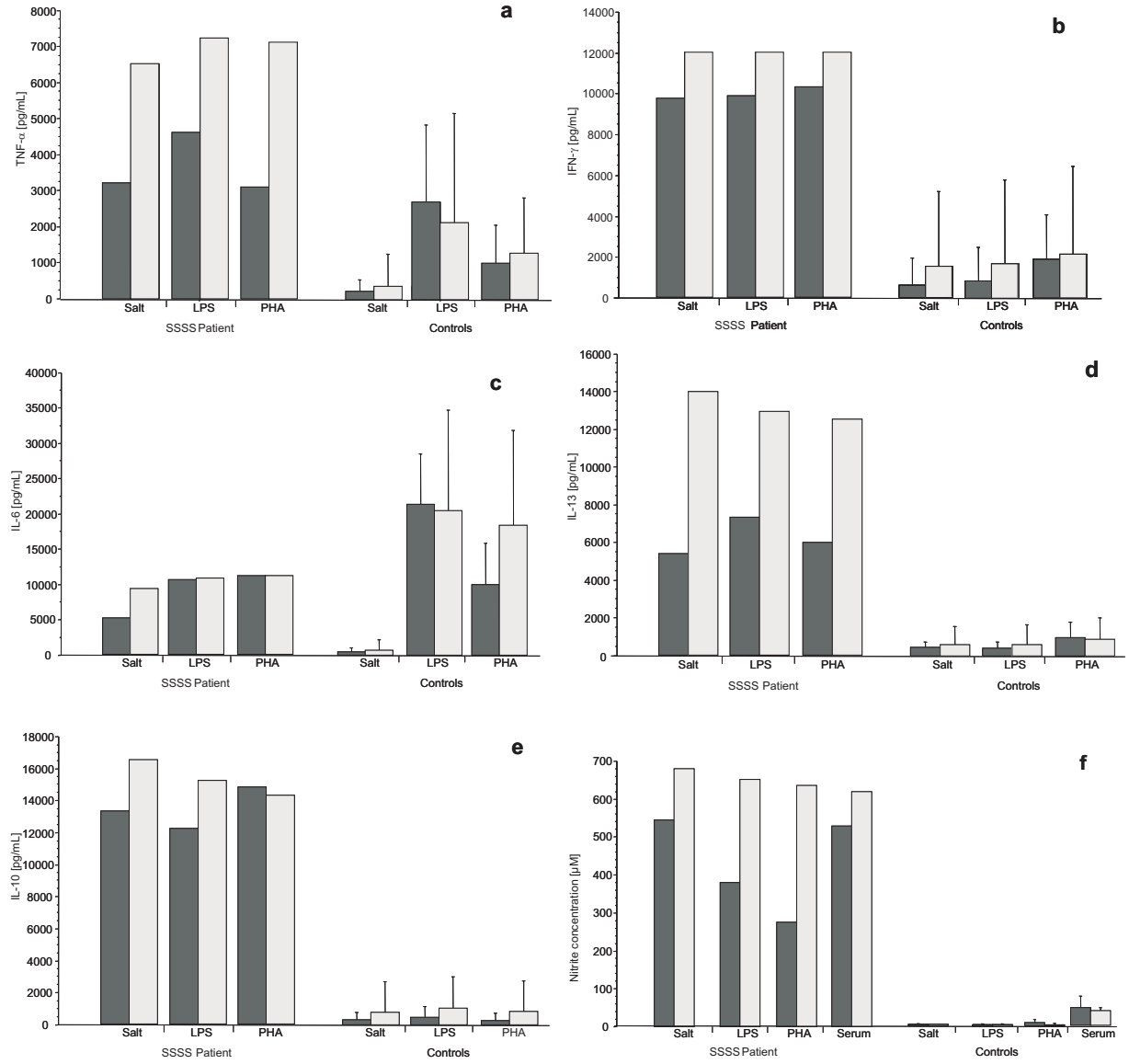

Fig. 1. Pro- and anti-inflammatory cytokines and nitric oxide during scalded-skin syndrome evolution. TNF-α (a), IFN-γ (b), IL-6 (c), IL-13 (d), IL-10 (e) and nitric oxide (f) produced *in vitro* by LPS and PHA stimulated whole blood leukocytes were measured by ELISA, on day 1 (before treatment - dark gray bars) and day 10 (after treatment-light gray bars) in SSSS patient's samples and uninfected control subjects.

or *in vivo* (19-22). Interestingly, an increase in the cytokine production, after SSSS treatment (Fig. 1), was associated with an improvement in the patient's general condition and elimination of microorganisms and toxins.

One relevant aspect is that immunoregulatory mechanisms seem to be working properly in this patient, since TNF-α and IFN-γ levels increased along with NO production after treatment. Interestingly, at the same time, PHA or LPS stimuli were unable to increase IL-10 production when compared to unstimulated leukocytes. Besides that, the patient's leukocytes produced more NO after treatment and

consequently, seemed to be able to kill intracellular pathogens. Increased NO production *in vitro* by WBL, correlated with augmented nitrite levels in serum, indicates that the phenomena observed *in vitro* could also occur systemically *in vivo*. Alternatively, IL-10 production in SSSS displays different patterns, depending on the type of stimuli used (PHA or LPS), suggesting an essential role of this cytokine in controlling inflammation and tissue healing.

Increased IL-6 levels were observed in control samples possibly due to its correlation with acute phase response (Cordeiro *et al*, unpublished data). However, all other cytokines were detected at higher levels in patient`s samples than in controls, which may reinforce the hypothesis that staphylococcal ETs could act as superantigens by stimulating a polyclonal lymphocyte response, although this issue needs to be more investigated.

This first work on cytokine and NO production in adult staphylococcal scalded skin syndrome could be useful in further studies concerning immunological mechanisms involved in this disease or alternative therapy implementation.

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