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Glucocorticoid Sensitivity and Proinflammatory Cytokines Pattern in Pemphigus

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Abstract Glucocorticoids (GC) represent the main treatment for pemphigus; however, some patients show GC resistance. GC sensitivity was evaluated in 19 pemphigus patients and 41 controls by the number of binding sites [B_{max} (fmol/mg protein)] and the affinity of GC receptor [Kd (nM)] to dexamethasone (DEX) as well as by the pattern of cytokine by DEX-mediated inhibition of concanavalin-A (Con-A)-stimulated PBMC proliferation. The Kd (15.7±2.8 *vs.*8.1±1.3) and Bmax (6.5±0.9 *vs.* 3.9±0.3) were higher in pemphigus than controls (p=0.002). Considering the values above the 95th percentile of normal group as a cut-off (K_d>24.9 nM and B_{max}>8.1 fmol/mg protein), elevated K_d and B_{max} were observed in 9.8% and 2.4% of controls and 15.8% and 36.8% of patients (p=0.02). PBMC proliferation was stimulated by Con-A and inhibited by DEX (p<0.001) in both pemphigus and control groups. IL-6 and TNFα (pg/mL) basal production were higher in patients than controls. There was an increment of these cytokines after Con-A stimulation, and they were inhibited by DEX (p=0.002) in controls and remained elevated in pemphigus (p<0.02). Patients and controls showed no difference in basal and stimulated production of IL-8 and IL-10. There is an alteration on GC sensitivity in pemphigus patients and a higher production of proinflammatory cytokines. Therefore, in pemphigus patients, proinflammatory cytokines might be involved in the mechanism of GC resistance and/or in its maintenance.

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Introduction

Pemphigus is an autoimmune blistering disease of the skin, which is characterized both histologically by cell/cell detachment of epidermal cells (acantholysis), and immunologically, by binding of autoantibodies to the adhesion molecules of keratinocytes, such as to desmogleins (Dsg) of epidermal cells besides other molecules [1]. Two major forms of the disease are recognized, as defined by clinical and histological findings. Pemphigus vulgaris (PV) affects the skin and mucous membrane and causes histologically acantholysis in the deep epidermis, primarily suprajacent to the basal cells. Pemphigus foliaceus (PF) usually affects the skin and generates acantholysis at the level of granular cells, forming blisters in the superficial epidermis [2]. The target antigens for the pemphigus autoantibodies have been shown to be localized at desmosomal junctions of epidermis which are transmembrane proteins from the cadherins family by immunoelectron microscopy. Biochemical and molecular genetic studies defined Dsg-3 (130 kDa) as the main autoantibody for PV [3, 4] and Dsg1 (160 kDa) for PF and PV [5, 6]. The stages of the evolution of pemphigus, its treatment, and the definition of therapy failure were recently defined by an international consensus [7].

Brazil is considered an endemic region for PF and the epidemiological factors might differ from European PF [8]. The pathophisiological mechanisms of acantholysis in endemic PF remain unclear despite of several studies [9–11]. While pemphigus is an antibody mediated disease, the role of T cells in its pathogenesis is being increasingly recognized [12]. T cells from pemphigus patients recognize desmoglein antigen and can influence the role of the cytokines on the clinical

course of the disease. Indeed, a high production of cytokines involved in the inflammatory response, such as interleukin 1 (IL-1) and tumor necrosis factor α (TNF-α), has been demonstrated in blood cells from PF and PV patients, while increased serum IL-6 production has been correlated to the disease activity in PV [13, 14]. In addition, previous studies observed that CD56+ CD3– natural killer cells (NK) were increased in patients with active PV, suggesting that NK cells contribute to Th2-based immune responses through IL-12Rb2 signaling impairment and up-regulation of IL-5 and Il-10 [15]. It has been also suggested that cellular and molecular interaction of the combination of NK, CD4+ T cells and Dsg1 and 3 influences the microenvironment of the skin and mucosal tissues during the preclinical and subsequent clinical stages [16]. Therefore, current information has been associated NK cells in the pathogenesis of blistering diseases, including pemphigus vulgaris patients [17].

The prognosis of PF is usually more favorable in PV than in PF; in both types it has been improved considerably since the introduction of corticosteroids [18]. Glucocorticoids (GCs) affect a variety of important functions throughout the body, such as glucose and fat metabolism, mediate stress response, influence the immune and central nervous system activity and have numerous effects on development and cell differentiation. Regulation of serum GC concentrations is under the influence of the hypothalamus-pituitary-adrenal (HPA) axis and circulating GCs themselves exert a negative feedback on both hypothalamic and the pituitary levels. GCs act via the cytoplasmic glucocorticoid receptor (GR), which is a member of the nuclear receptor family [19]. GR precursor mRNA is processed into at least two alternative splice products, hGRα and a nonligand-binding isoform, hGRβ, which acts as a dominant negative inhibitor of hGRα on glucocorticoid-responsive promoters [20, 21]. GC act by binding to a cytoplasmic GR, which then translocates to the nucleus to act as a transcription factor [19].

Current treatment regimens for pemphigus use high-dose systemic GC; unfortunately, the high doses and prolonged administration of corticosteroids that are often needed to control the disease result in numerous side effects, many of which are serious or even life-threatening [18]. However, some patients do not respond to GC regimen [22]. In an attempt to identify predictive factors of the response to GC, the pattern of dexamethasone (DEX) mediated inhibition of Concanavalin-A (Con-A) stimulated peripheral blood mononuclear cells (PBMC) proliferation has been used in different inflammatory and immune disease as asthma [23], rheumatoid arthritis [24], idiopatic nephrotic syndrome [25], patients awaiting renal transplantation [26]. These studies have demonstrated an increased lymphocyte resistance to the effects of GC in these disorders. In a recent study, we observed similar finding also in healthy subjects [27]. In addition, previous studies have demonstrated diminished GC receptor number and affinity in PBMC from rheumatoid arthritis [28] and asthma steroid-resistant patients [29]. It is important to point out that some GC alterations, such as an increased number of GR per cell, can be reversible in culture medium alone but sustained with the co-incubation of IL-2 and IL-4, suggesting that inflammation and cytokine secretion may also contribute to the acquired GC receptor defect found in asthma steroid resistant. Indeed, a disturbed immune regulation contributes to the corticosteroid resistance in some diseases [23, 24, 30].

There are no studies on GC sensitivity in pemphigus patients. In this study, we hypothesized that cytokines could differentially regulate the sensitivity of PBMC to GC in pemphigus patients. Taking advantages of different bioassays performed on the same subject, we evaluated the number and affinity of GC receptor, the DEX mediated inhibition of Con-A stimulated PBMC proliferation and cytokine profile in patients with pemphigus. We observed an alteration on GC sensitivity in untreated pemphigus patients. In addition, PBMC from pemphigus patients showed a higher production of proinflammatory cytokines, which were only partially inhibited even when higher GC doses were used. All these evidences suggest that proinflammatory cytokines could play an important role in the immunopathogenesis of pemphigus and might be involved in the mechanism of GC resistance or in the maintenance of this phenomenon in pemphigus patients.

Material and Methods

Subjects

This prospective study was approved by the institutional review board for human research of School of Medicine of Ribeirão Preto - University of São Paulo and informed consent in written form was obtained from all subjects and their parents.

We studied 19 patients with pemphigus followed at Dermatology Clinic of the University Hospital, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Brazil. The diagnosis of PF and PV were confirmed by the histopathological findings and by direct immunofluorescence. Patients with predominant erosions and crusts on the head and upper thorax were considered to have localized PF. Patients with PV presented erosions in oral and or genital mucosal regions and flaccid bullous spread throughout the tegument. At the time of the study, patients with pemphigus had never been treated for or were off corticosteroid therapy for at least consecutive 30 days interval. Clinical findings, localization of lesions, classification of pemphigus, previous treatment, and diagnostic confirmation by direct immunofluorescence can be seen in the Table 1.

CG sensitivity evaluated by the number of binding sites (Bmax) and the affinity of glucocorticoid receptor (Kd) by hormone binding assay and also the pattern of dexamethasone-mediated inhibition of concanavalin-A (Con-A)-stimulated

Table 1 Clinical findings in patients with pemphigus foliaceus and pemphigus vulgaris

Patients	Sex	Age (y)	Type of pemphigus	Clinical compromising	Time of onset of the disease	Previous systemic t reatment
1	F	38	Foliaceus	Localized form (seborheic areas)	14 y	Prednisone plus dapsone
2	M	16	Foliaceus	Generalized form	5 y	No treatment
3	F	51	Foliaceus	Localized form (seborheic areas)	6 y	Prednisone plus azathioprine
4	F	30	Foliaceus	Localized form (seborheic areas)	2 y	Prednisone plus azathioprine
5	M	33	Foliaceus	Localized form (seborheic areas)	3 y	No treatment
6	F	28	Foliaceus	Generalized form	1 y	Prednisone
7	M	19	Foliaceus	Generalized form	4 y	Prednisone plus chloroquine
8	F	45	Foliaceus	Generalized form	5 y	Prednisone
9	M	34	Foliaceus	Generalized form	1 y	No treatment
10	F	44	Foliaceus	Generalized form	12 y	Prednisone
11	M	16	Foliaceus	Generalized form	2 mo	No treatment
12	F	41	Foliaceus	Generalized form	10 mo	No treatment
13	F	36	Vulgaris	Vegetant variety with oral compromising	1 y	No treatment
14	F	27	Vulgaris	Generalized lesions with oral compromising	2 mo	No treatment
15	M	44	Vulgaris	Lesions on trunk with oral compromising	1 y	Dapsone
16	F	16	Vulgaris	Generalized lesions with oral compromising	1 mo	No treatment
17	M	50	Vulgaris	Generalized lesions with oral compromising	2 mo	No treatment
18	M	50	Vulgaris	Lesions on trunk with oral compromising	2 mo	No treatment
19	F	63	Vulgaris	Lesions on trunk with oral and genital compromising	1 y	No treatment

F female; *M* male; *y* years; *mo* months

peripheral blood mononuclear cells (PBMC) proliferation using different doses of DEX had been previously evaluated in 41control subjects (21 F, 20 M; 22–62 years old) [27]. In the present study, using both methods, we performed CG sensitivity analysis in 19 pemphigus patients (11 F, 8 M; 16–63 years old; PF= 12 and PV=7). In addition, in all these patients as well as in six healthy controls (4 F, 2 M; 32–63 years old), we also evaluated the IL-6, IL-8, IL-10 and TNF-α secretion in medium culture of PBMC in basal condition and after treatment with Con-A alone or associate with different doses (10^{-10} to 10^{-4} M) of DEX.

Cell Preparation

PBMC were isolated by density gradient centrifugation using Ficoll-Hypaque (Histopaque-Sigma Chemical Co, St Louis, MO), washed three times in Hanks buffered saline solution and resuspended in RPMI 1640 (Life Technologies, Gaithersburg, MD) containing 2 mM HEPES buffer (Sigma Chemical Co, St Louis, MO), 10% fetal calf serum, 100 IU ml^{-1} penicillin, 100 gml^{-1} streptomycin and 10 mg ml^{-1} gentamicin.

Binding Assay

PBMC DEX-binding assay was performed as previously described [25–27]. Cells were suspended in RPMI media and adjusted to 2×10^6 cells per tube in duplicate and incubated with six concentrations (1.56–50 nM) of DEX ([1,2,4,6,7-^{3}H] Dexamethasone, Amersham Life Science, England) at 37°C in the presence or absence of a 1.000-fold molar excess of unlabeled DEX (Dexamethasone Sigma, St Louis, MO, USA) for 1 h. After incubation, the cells were washed three times to separate bound from free steroid with 1.5 ml cold PBS and centrifuged at 400 × *g* for 10 min. After the third wash, the pellets were suspended in 100 μl of RPMI, transferred to vials and the radioactivity was counted in a β-counter. Specific binding was calculated by subtracting non-specific binding from total binding. Receptor assay data were analyzed by the method of Scatchard using computerized linear regression analysis. The binding capacity (B_{max}) was expressed as fmol of dexamethasone bound per mg of protein, and the dissociation constant (K_d), inversely proportional to ligand affinity, was expressed in nM.

Proliferation and in vitro corticosteroid sensitivity assay

To perform the in vitro steroid sensitivity assay we measured the inhibitory effect of DEX on Con-A-stimulated PBMC proliferation. PBMC (2×10^{-6} cells per well) were plated onto 96-well flat-bottomed plates (Nunc, Denmark) in triplicate and cultured at 37°C in the presence of 5% CO_2. Con-A at the dose of 50 μg ml^{-1} was used to stimulate the cells in the presence or absence of different doses (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M) of DEX. After 48 h of culture, the cells were pulsed with 1 μCi/well tritiated thymidine (^{3}H-

thymidine, Amersham, Pharmacia Biotech, UK) for 18 h prior to collecting the supernatants for cytokine quantification. The cells were harvested with a multiple automated sample harvester and radioactivity was counted in a liquid scintillation β-counter (Beckman, Fullerton, CA). Percent inhibition of proliferation by steroid was calculated using the following formula: $\{1-(x-n/y-n)\}100$, where: x = counts in Dex and Con-A; n = counts in RPMI alone, y = counts in Con-A alone. The IC_{50} was defined as the concentration of DEX that caused a 50% inhibition of cell proliferation.

Cytokine Quantification

The supernatants collected during cell culture were stored at −70°C for measurement of IL-6, IL-8, IL-10, and TNF-α levels by ELISA (BD OptEIA, BD Biosciences Pharmigen, San Diego, CA). The minimum detection limits for IL-6, IL-8, IL-10 and TNF-α were 4.7, 3.1, 7.8 and 7.8 pg/ml, respectively. Undetectable cytokine levels were considered as the minimum detection limit of each assay.

Statistical Analysis

All results are expressed as the Mean ± SE. Data were compared using One-Way ANOVA followed by Newman-Keuls Multiple Comparison Test. Mann–Whitney test was used when appropriate. Level of significance was set at $p<0.05$.

Results

Binding Assay

The linearity of the Scatchard plots indicates a single class of binding site affinity. The Kd (nM) and Bmax (fmol/mg protein) values were 8.1±1.3 and 3.9±0.3 in control and 15.7±2.8 and 6.5±0.9 in pemphigus patients. Patients with pemphigus had K_d and Bmax values significantly higher than healthy subjects ($p=0.002$ and $p=0.0002$, respectively). Considering the values above the 95th percentile of the normal group (K_d >24.9 nM and B_{max}>8.1 fmol/mg of protein), 4 out of 41 controls (9.8%) and 3 out of 20 (15.8%) pemphigus patients showed an elevated K_d ($p=0.02$) while one out of 41 controls (2.4%) and 7 out of 20 (36.8%) pemphigus patients showed an elevated B_{max} ($p=0.01$). Figure 1 shows a representative saturation curve and Scatchard plot of one individual with normal K_d and one individual with an elevated K_d.

Proliferation Assays

Basal lymphocyte proliferation was stimulated by Con-A in the control group (761±60.7 vs. 36866±2647 cpm; $p<0.001$) and pemphigus patients (780±92 vs. 43089±3360 cpm; $p<0.001$). There was no difference in lymphocyte proliferation on basal condition between the control and pemphigus groups. Different doses of DEX (10^{-10}, 10^{-8}, 10^{-6}, 10^{-4} M) inhibited lymphocyte proliferation in a dose-dependent manner in both control and pemphigus groups (Fig. 2). There was no difference in the IC_{50} between controls and pemphigus patients.

Cytokines

Cytokine levels were measured in the supernatant collected from fresh cultured PBMC in the basal condition and after stimulation with Con-A alone or Con-A plus different doses of Dex (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M) in control subjects and pemphigus patients. PBMC from pemphigus patients showed IL-6 basal production higher than controls (934.5±66.8 vs. 501.7±87.9 pg/mL; $p=0.005$). There was an increment of IL-6 secretion after Con-A stimulation in both controls (1982±370.8 pg/mL; $p=0.009$) and pemphigus patients (2469±101 pg/mL; $p=0.0001$), with no difference between groups. IL-6 secretion was significantly inhibited by DEX dose of 10^{-7} M in controls and pemphigus patients ($p=0.01$). However, in pemphigus patients the levels of IL-6 remained elevated compared to controls (p=0.01) even after a high doses of Dex (10^{-4} M). These data are shown in Fig. 3a.

PBMC from pemphigus patients showed TNF-α basal production higher than controls (77.7±12.1 vs. 7.8±2.1 pg/mL; $p=0.002$). There was an increment of TNF-α secretion after Con-A stimulation in controls (560.5±146.3 pg/mL; $p=0.002$) and pemphigus patients (2031±218 pg/mL; $p=0.0001$), however in the pemphigus group TNF-α secretion was higher than controls ($p=0.001$). TNF-α secretion was significantly inhibited by DEX dose of 10^{-8} M in controls ($p=0.002$) and pemphigus patients ($p=0.0004$). However, in pemphigus patients the levels of TNF-α secretion remained elevated compared to controls after all doses of DEX (p<0.02). These data are shown in Fig. 3b.

IL-8 basal production of PBMC from pemphigus patients (5713±309 pg/mL) showed no difference from controls (5748±441 pg/mL). IL-10 basal production of PBMC from pemphigus patients (90.5±19.3 pg/mL) also showed no difference from controls (47.8±10.2 pg/mL). In pemphigus patients, Con-A stimulated PBMC increased significantly IL-8 (6857±187.4 pg/mL; $p<0.0001$) and IL-10 (1103±78.7 pg/mL; $p<0.0001$) secretion compared to the basal production. The same was observed in controls: IL-8 (6741±284.6 pg/mL; $p=0.01$) and IL-10 (1032±118.6 pg/mL; $p=0.002$). IL-8 and IL-10 secretion were significantly inhibited by DEX dose of 10^{-7} M in pemphigus patients ($p=0.0004$) and in controls ($p=0.002$). Data on IL-8 and IL-10 secretion are shown in Fig. 3c and d, respectively.

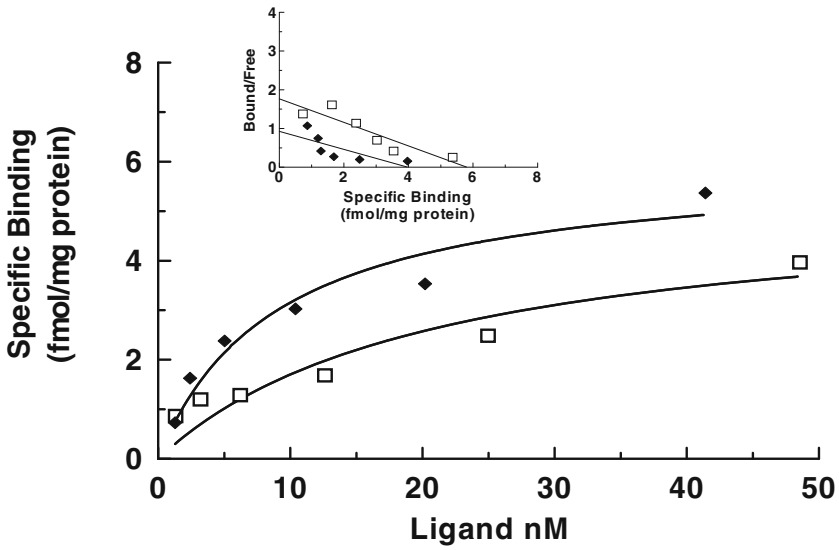

Fig. 1 Representative saturation curves of [3H] dexamethasone binding to peripheral blood mononuclear cell (PBMC) and the Scatchard plot (insert) of glucocorticoid receptor (GR) binding studies of one individual with normal Kd (□) and with an elevated Kd (♦)

Discussion

In the present study, taking advantages of two bioassays, the binding assay and DEX mediated inhibition of concanavalin-A (Con-A) stimulated PBMC proliferation, we observed that pemphigus patients showed an increased number and a decreased affinity of GR to DEX compared to healthy subjects, suggesting GC resistance in untreated pemphigus. In addition, we demonstrated that the GC resistance observed in these patients was associated with a proinflammatory cytokine pattern.

Some recent reports have shown an increasing incidence of pemphigus in UK and Brazil [31, 32]. The treatment regimen is individualized based on the subtype of pemphigus, age of the patient, severity and extent of the disease, and rate of disease progression. Local applications of steroids, including creams, gels, and swishes, can be used for lesions limited to one area of the body [33]. The only common guideline is to initiate treatment of PV or PF, with corticosteroids until the disease is controlled. Once a clinical response is observed, the dose of systemic corticosteroids can be tapered and eventually discontinued [34, 35]. However, there are patients, in whom the disease becomes exacerbated as the dose of prednisone is tapered or do not respond to GC treatment, suggesting GC resistance. The advances in the understanding of the pathogenesis of the disease and the development of new therapies decreased the mortality rate from 60% to 90% to about 10% [22, 35, 36]. B cell-directed therapy using anti-CD20 antibody rituximab directly interferes with pathogenic autoantibodies and induces rapid clinical remission and long-term control in

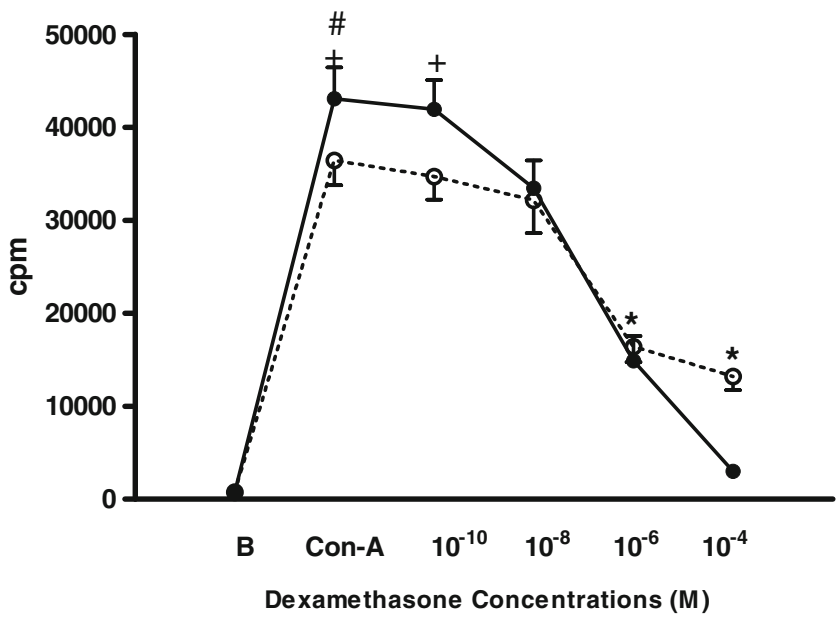

Fig. 2 Lymphocyte proliferation after incubation in pure culture medium (b; basal), or culture medium with concanavalin - A (Con-A), or culture medium with Con-A plus different doses of dexamethasone (10^{-10}, 10^{-8}, 10^{-6}, and 10^{-4} M), in control individuals (○) and in pemphigus patients (●). # Basal vs Con-A; * Con-A vs Dex10^{-6} M and Con-A vs Dex 10^{-4} M; + Control vs. Pemphigus

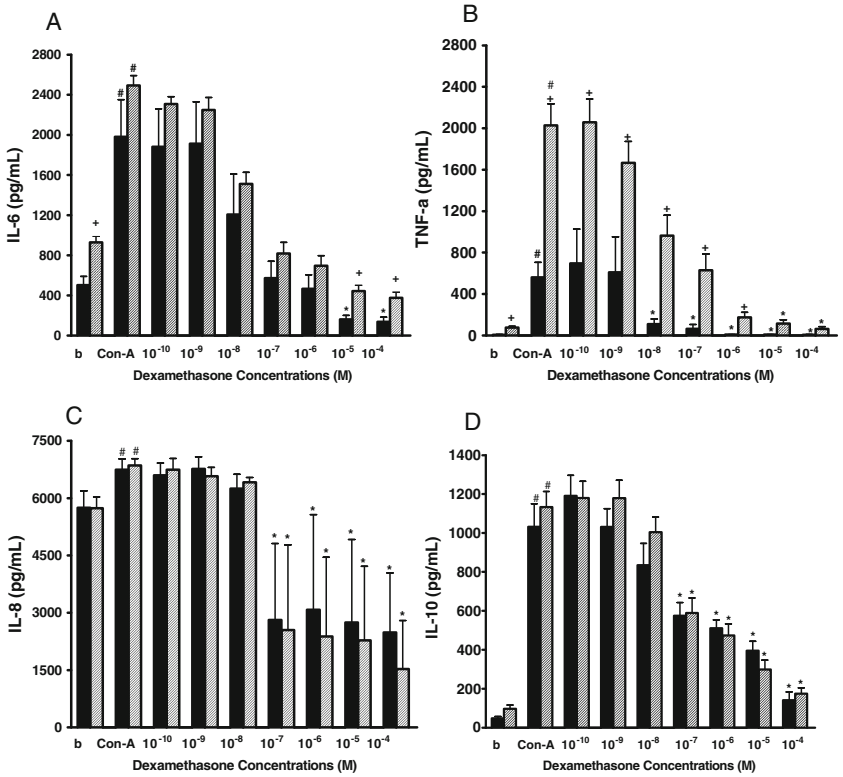

Fig. 3 Interleukin-6 (**a**), Tumor necrosis factor-α (**b**), Interleukin-8 (**c**) and Interleukin-10 (**d**) concentrations (pg/mL) in supernatants collected during peripheral blood mononuclear cell culture from controls (black bars) and pemphigus patients (hachured bars) after incubation in pure culture medium (b; basal), or culture medium with concanavalin - A (Con-A), or culture medium with Con-A plus different concentrations of dexamethasone (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M). # Basal vs Con-A; *Con-A vs DEX concentration; + Control vs. Pemphigus patients

difficult to treat pemphigus. Recent studies have demonstrated that low dose of rituximab is effective and safe although relapses may occur, mostly at the end of the second year of treatment [37]. However, cost-effectiveness studies with a long follow-up are required to determine the proper dosage of this expensive drug in pemphigus [38].

Studies on GC sensitivity in pemphigus patients are lacking; in the present study, we observed no difference in lymphocyte proliferation in basal condition and also in the dose that caused a 50% inhibition of cell proliferation (IC_{50}) between controls and pemphigus patients. Since the binding assay is more straightforward than the GC-mediated inhibition of mitogen stimulated PBMC proliferation, we also used this method for the prediction of GC resistance in patients with pemphigus. These patients showed a higher K_d compared to normal subjects, which denotes diminished GR affinity, indicating GC resistance. We also evaluated the number of binding sites (Bmax), which values were also significantly higher than controls. These results would suggest a compensatory increase in the number of GC binding sites in order to overcome the GR resistance in pemphigus. Since binding assays evaluate only the GRα isoform, which is the classic ligand-binding protein for GC, we can not rule out an overexpression of GRβ inducing steroid insensitivity in pemphigus, as well established in GC resistant patients with asthma, rheumatoid arthritis and ulcerative colitis [29, 30, 39].

Studies have suggested that the overexpression of GRβ in inflammatory diseases would be a consequence of the inflammation more than a constitutional characteristic contributing to the development of the disease [40, 41]. Interestingly, many other GC alterations can be reversible in culture but sustained with cytokine co-incubation, suggesting that cytokine secretion may contribute to the acquired GR defect found in asthma steroid resistant [23]. In addition, while pemphigus is an antibody mediated disease, the role of T cells in its pathogenesis is being increasingly recognized [14, 42]. Therefore, increased production of cytokines can be associated not only with pemphigus pathogenesis but also can represent another possible mechanism of GC resistance in pemphigus. Therefore, in the present study, cytokine profile was also studied in order to better understand the mechanisms underlying the GC resistance in patients with pemphigus.

PBMC from pemphigus patients showed a higher production of TNF-α—a proinflammatory cytokine—than controls, in basal and after Con-A stimulation. In addition, TNF-α secretion was significantly inhibited by low dose of DEX in controls and remained elevated in pemphigus patients even after high doses of DEX. Similar result was observed with another proinflammatory cytokine—IL-6 -, which levels remained elevated in the medium culture from PBMC of pemphigus patients even after high doses of DEX. On the other hand, no differences were observed in IL-8 and IL-10 production neither in basal nor in stimulated conditions in pemphigus patients. Thus, proinflammatory cytokines were only partially inhibited in patients with pemphigus. These findings might reflect a predominance of Th1 cells and suggest

that proinflammatory cytokines might play a role in the mechanism of GC resistance in pemphigus patients.

Few studies have been undertaken to measure serum cytokine levels in patients with pemphigus [14, 42, 43]. In these studies, while IL-6 and TNF-α levels were increased, low to undetectable levels of IL-8 and IFN-γ were found in serum of pemphigus patients. Our data, using a different approach, such as the basal, mitogen-stimulated proliferation and the DEX mediated inhibition of Con-A stimulated PBMC cytokine secretion in culture medium, reinforce those previous studies. In addition, increased expression of IL-6 and TNF-α around the blistering process of pemphigus has also been reported and could play a mediator role in pemphigus lesions by increasing epithelial damage [44]. More recently, the presence of TNF-α, IFN-γ, and IL-1 in the inflammatory exudates of lesions and an expression of proinflammatory cytokines, such as TNF-α and IL-6, around the blister were demonstrated in pemphigus patients by in situ hybridization. These inflammatory mediators might contribute to tissue injury by their ability to directly or indirectly induce apoptosis [8, 45]. Overall, as expected, the cytokine levels decrease in the course of treatment with conventional therapy, which causes general immunosuppression and elimination of proinflammatory cytokine-producing cells [46]. Altogether, these evidences suggest that proinflammatory cytokines not only can be involved in the immunopathogenesis of pemphigus but also in GC resistance observed in a subset of pemphigus patients.

In conclusion, we described for the first time, an alteration on GC sensitivity in untreated pemphigus patients. In addition, PBMC from pemphigus patients showed a higher production of proinflammatory cytokines, suggesting that they could play a role in the immunopathogenesis of pemphigus and also might be involved in the mechanism of GC resistance and/or in the maintenance of this phenomenon in pemphigus patients. The possible impact of GC resistance in a less favorable outcome regarding frequent partial remission, treatment failure, relapse, the need for high-dose corticosteroids, and treatment dependence remains to be established.

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