$CD25^+CD8^+$, CLA^+CD4^+, $CD11a^+$ $CD4^+$ and $CD11a^+$ $CD8^+$ T Cell Counts Are Elevated in the Blood of Brazilian Patients With Chronic Plaque Psoriasis

Los linfocitos T $CD25^+CD8^+$ $CD4^+CLA^+$ $CD11a^+CD4^+$ y $CD11a^+$ $CD8^+$ están elevados en la sangre de los pacientes brasileños con psoriasis en placas crónica

To the Editor:

Psoriasis is a recurrent, chronic inflammatory disorder that mainly affects the skin and joints.[1] In view of the scarcity of studies on peripheral lymphocyte subpopulations in Brazilian psoriasis patients, we performed cytofluorometric analysis of peripheral blood mononuclear cells (PBMC) in psoriasis patients and healthy controls in order to characterize the lymphocyte subpopulations and certain molecules involved in cell activation and migration.

The study was approved by the Ethics Committee of Escola de Medicina e Cirurgia (UNIRIO, MEC, Brazil) and all patients signed an informed consent.

Twenty-five individuals were recruited from the Dermatology Department of HUGG/ UNIRIO/ MEC-Brazil. Seventeen had chronic plaque psoriasis and there were 8 healthy controls. None of the psoriasis patients was receiving systemic treatment. The physician's global assessment score[2-4] was 4 in 6 cases, 5 in 6 cases, and 6 in 5 cases.

A 20-mL blood sample was drawn from each patient and PBMCs were separated using the Ficoll-Hypaque gradient (Sigma Chemical Co, St. Louis, USA). Five microliters of each monoclonal antibody—anti-CD3, anti-CD4, anti-CD8, anti-CD11a (Immunotech, Beckman Coulter, France), anti-CD25, anti-CD69, and anti-CLA (BD Biosciences, California, USA)—were added according to the combinations listed in the Table 1. Laboratory procedures were performed according to the manufacturer's instructions.[5] The nonparametric Mann-Whitney and Kruskal-Wallis tests were used for the statistical analysis, which was performed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, California, USA).

Analysis of the lymphocyte subpopulations ($CD3^+$ or total T cells, $CD4^+$ or helper T cells, and $CD8^+$ or cytotoxic T cells) revealed no significant differences between patients with psoriasis and healthy controls. The relative percentages of each lymphocyte subpopulation in patients and controls are shown in Figure 1. Lymphocyte activation was determined through analysis of CD25 and CD69 expression (Figure 2, A and B). Higher percentages of activated ($CD25^+$ and $CD69^+$) cells were detected in the 2 lymphocyte subpopulations ($CD4^+$ and $CD8^+$) in psoriasis patients; the difference compared to controls was significant for the percentage of $CD25^+$ cells in the $CD8^+$ T-cell subpopulation (mean [SD], 39.2%[26.9]; median, 35.1%; $P<.05$), and there was a trend to elevation of the $CD25^+$ $CD4^+$ subpopulation. There was also a trend to elevation in the percentage of $CD69^+$ cells in both T-cell subpopulations ($CD4^+$: mean, 16.0% [19.6]; median, 7.8%; $CD8^+$, mean, 2.0% [1.47]; median, 2.23%) in psoriasis patients when compared to controls (Figure 2B). Migration of circulating T lymphocytes to the skin was studied through an analysis of CLA and CD11a expression. Compared to control subjects, patients with psoriasis presented an increase in the percentage of $CD4^+$ T cells expressing CLA (control group, mean, 19.30%[13.13]; median, 14.67%; psoriasis group, mean, 38.86% [20.67]; median, 39.76%; $P<.05$). However, this was not observed in the $CD8^+$ T cells, although increased CLA expression was detected in 4 of the 10 patients with high levels of $CD8^+$ lymphocytes (Figure 3A). A significant increase in the percentage of cells with CD11a expression was observed in psoriasis patients compared to controls in both $CD4^+$ (mean, 82.17%

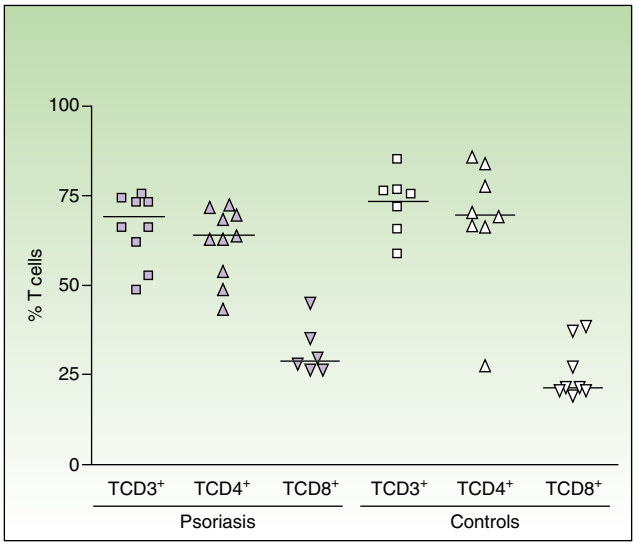

Figure 1 Quantification of T lymphocyte subpopulations ($CD4^+$ and $CD8^+$ T cells) in the peripheral blood of patients with chronic plaque psoriasis. The results are expressed as percentage of positive cells. The horizontal bars represent the median values of the results. Each point represents an individual.

Table 1 Set of monoclonal markers

Monoclonal markers	Analysis			Source
CD3-PC5/ CD4-PE/ CD8-FITC	CD3 in total lymphocytes	CD4 into CD3	CD8 into CD3	Immunotech, Beckman Coulter, France
CD4-PC5/ CD8-FITC/ CD25-PE	CD25 in total lymphocytes	CD25 into CD4	CD25 into CD8	BD Biosciences, CA, USA
CD4-PC5/ CD8-PE/ CD69-FITC	CD69 in total lymphocytes	CD69 into CD4	CD69 into CD8	BD Biosciences, CA, USA
CD4-PC5/ CD8-PE/ CLA-FITC	CLA in total lymphocytes	CLA into CD4	CLA into CD8	BD Biosciences, CA, USA
CD4-PC5/ CD8-PE/ CD11a-FITC	CD11a in total lymphocytes	CD11a into CD4	CD11a into CD8	Immunotech, Beckman Coulter, France

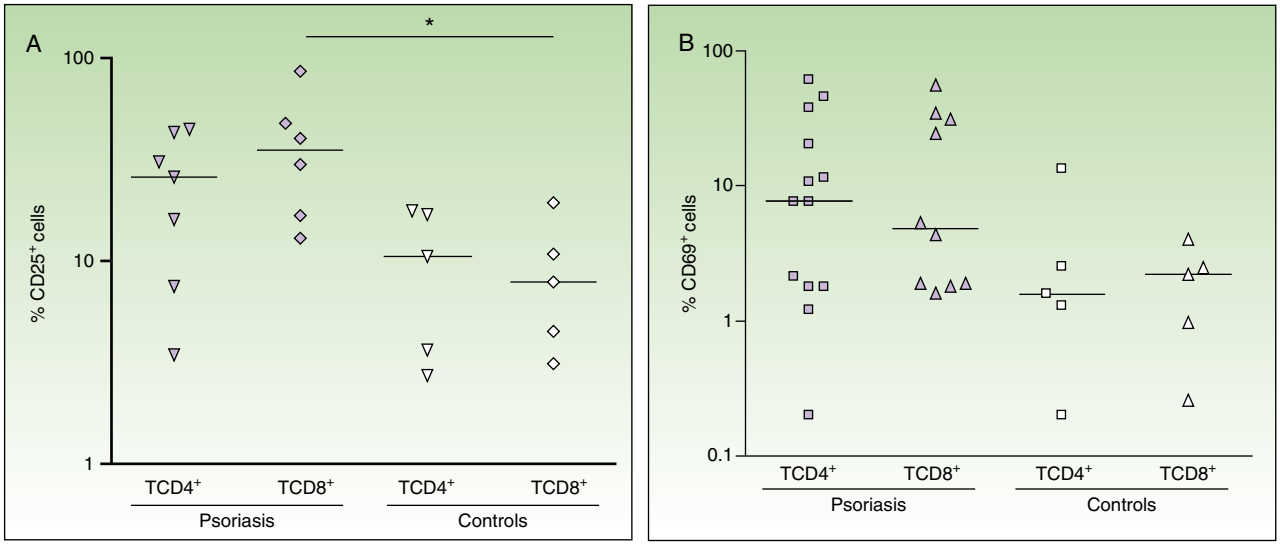

Figure 2 A and B, Assessment of the degree of activation of $CD4^+$ and $CD8^+$ T cells in psoriasis patients and controls. Each point represents an individual. The horizontal bar represent the median.

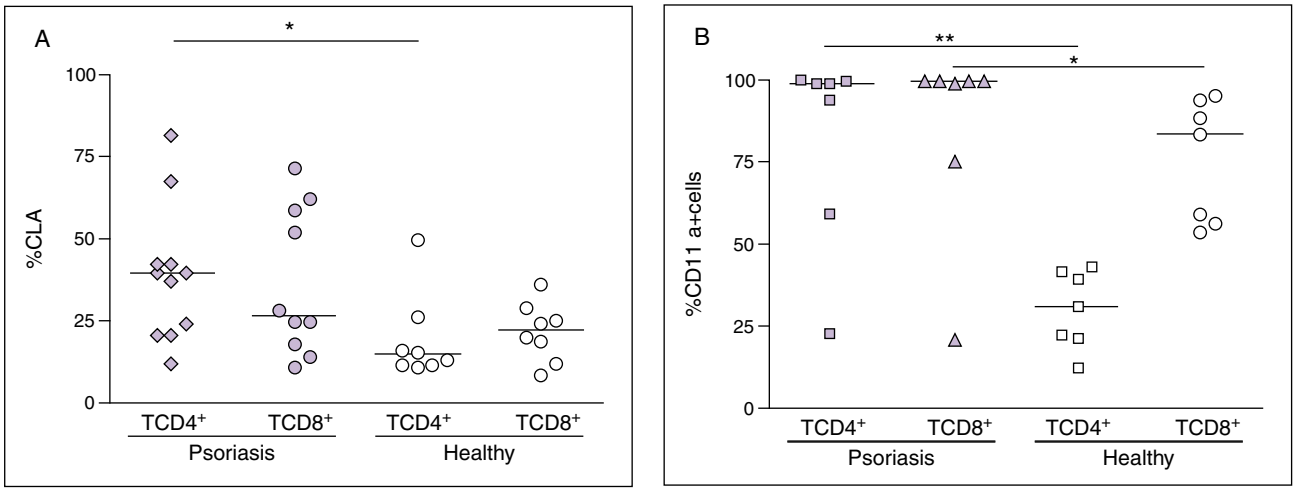

Figure 3 A, Elevated expression of molecules related to cutaneous cell migration (cutaneous lymphocyte associated antigen [CLA]) B. Endothelial tissue inflammation (CD11a) in $CD4^+$ and $CD8^+$ T cells in psoriasis patients and controls. Each point represents an individual. The horizontal bars represent the median values.

[29.91]; median, 99.45%; $P<.01$) and $CD8^+$ T cells (mean, 84.96 [29.77]; median, 99.75; $P<.05$) (Figure 3B).

Although our study is somewhat limited by the small sample size, the results showed that there was no change in the proportion of $CD4^+$ and $CD8^+$ T cells in the PBMC population in psoriasis patients when compared with general population, as has been reported in previous studies.[6] However, we observed qualitative differences between the 2 groups in the expression of activation molecules CD25 and CD69. Although the results were significant only for CD25 expression in $CD8^+$ T cells, there was a trend to increased expression of CD25 and CD69 in both $CD4^+$ and $CD8^+$ T cells, findings not reported by other authors.[7] The presence of activation molecules has been detected in the initial stages of psoriasis, even prior to the onset of clinically apparent lesions; these molecules are therefore presumably involved in lymphocyte recruitment and migration.[8,9]

Our data indicate that, although there is no significant increase or variation in the relative percentages of circulating mononuclear cells in psoriasis patients, these cells are qualitatively different because they express activation molecules that are involved in the initiation and progression of psoriasis lesions.

Acknowledgments

We are grateful to Ana Cristina de Almeida for her valuable suggestions, to Gustavo Estef Lino da Silveira for the linguistic revision, and to the medical staff of Serviço de Dermatologia-HUGG/ UNIRIO, Rio de Janeiro, Brazil. C Porto-Ferreira is a graduate student at Programa de Pós-Graduação em Ciências Médicas, FCM-UERJ, Rio de Janeiro, Brazil. AM Da-Cruz is a research fellow from FAPERJ, Rio de Janeiro, Brazil.

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Rhabdomyolysis During Isotretinoin Therapy

Rabdomiólisis durante el tratamiento con isotretinoína

To the Editor:

Isotretinoin therapy is used in nodular-cystic acne and acne vulgaris when they do not respond to other treatments. Muscle toxicity is a little-known complication and its true incidence and management remain to be determined.

A 16-year old male started therapy with 30 mg per day of isotretinoin (0.4 mg/kg/d) for papulopustular acne that was resistant to other treatments. In routine laboratory tests prior to the start of therapy with retinoids all parameters were normal. After 2 months the patient presented with severe cheilitis, so the dose was reduced to 20 mg per day (0.3 mg/kg/d). No adverse events were detected during follow-up with bimonthly physical examination and laboratory tests (complete blood count, basic biochemistry, and lipid profile), and the acne improved significantly. Because of the dose reduction the therapy was continued longer than usual. In a routine examination conducted after 11 months and close to the end of the therapy, the patient reported moderate fatigue. Blood tests revealed elevated creatine phosphokinase (CK) and myoglobin plasma levels, with values of 801 IU/l (normal range, 5-110 IU/L) and 504 ng/mL (normal range, 0-75 ng/mL), respectively. A few days before the sample was collected the patient had done weight-lifting exercises, which was not a usual activity for him. We discontinued the isotretinoin therapy and recommended abundant fluid intake and avoidance of strenuous exercise. In the results of laboratory tests after 3 weeks all parameters had returned to normal.

Rhabdomyolysis is caused by necrosis of striated muscle cells and subsequent release of toxic intracellular material into the blood stream.[1] It has been defined as an elevation of CK greater than 5 times the upper limit of normal.[2] It can be caused by toxins (substance abuse, alcohol, and drugs), trauma, overexertion, and muscle metabolic defects.[2] Drugs are one of the most common causes of rhabdomyolysis, although in most patients various causes are found simultaneously. The drugs involved are usually the antipsychotics and statins.[2] The typical clinical presentation of weakness, muscle pain, and reddish urine occurs in less than half of patients[1] and high levels of CK can be found in the absence of symptoms.[3]

Isotretinoin is a vitamin A derivative that is widely used in dermatology and is usually well tolerated. Shortly after its introduction several cases of elevated CK in patients receiving this therapy were reported. In recent years there have been almost no publications on the subject, probably because of the tendency to reduce the frequency and thoroughness of monitoring.[4] In some of the cases described the patients had severe muscle pain and weakness of acute onset.[5-7] In others rhabdomyolysis was detected in asymptomatic patients through the finding of elevated CK in routine