CELL AND HUMORAL IMMUNITY IN ENDEMIC PEMPHIGUS FOLIACEUS

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SUMMARY

A study was conducted on 16 patients with pemphigus foliaceus, ten of them with the localized form (group G₁) and six with the disseminated form (group G₂). These patients were submitted to full blood counts, quantitation of mononuclear cell subpopulations by monoclonal antibodies, study of blastic lymphocyte transformation, and quantitation of circulating antibodies by the indirect immunofluorescence test, in order to correlate their clinical signs and symptoms and laboratory data with their immunological profile, and to determine the relationship between circulating autoantibody titers and lesion intensity and course of lesions under treatment.

Leucocytosis was observed especially in group G₂. All patients showed decreased relative CD₄⁺ and CD₈⁺ values and a tendency to decreased relative values of the CD₄⁺ subpopulation. Blastic lymphocyte transformation indices in the presence of phytohemagglutinin were higher in patients (group G₁+G₂) than in controls. The indirect immunofluorescence test was positive in 100% of G₂ patients and in 80% of G₁ patients. The median value for the titers was higher in group G₁ than in group G₂.

Analysis of the results as a whole permits us to conclude that cell immunity was preserved and that there was a relationship between antibody titers detected by the direct immunofluorescence test and extent of skin lesions.

KEYWORDS: Endemic pemphigus foliaceus; Cell immunity; Humoral immunity; Fogo Selvagem.

INTRODUCTION

The etiology of pemphigus foliaceus is still unknown. Nowadays, this condition is believed to be an autoimmune disease with circulating autoantibodies (AB) directed against the desmossomal protein, desmoglein 1, 22, 23, 24. The presence of these antibodies, usually of the IgG class, can be demonstrated by the direct immunofluorescence test in the lesions 22, 27 or by indirect immunofluorescence in the serum of patients 1, 18.

Different investigators have used different solutions and substrates for the indirect immunofluorescence test. The results have not been uniform, leading to controversy about the correlation between circulating antibody titers and severity of the disease 1, 5, 6, 11, 14, 18, 25. Studies on cell immunity 4, 13, 21 have shown discrepant results, with doubts still existing about the behavior of cell immunity in the pemphigus foliaceus.

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*In memory.
The objectives of the present study were to define the most sensitive indirect immunofluorescence technique for the identification of antiepidermis antibodies, and to correlate clinical signs and symptoms and laboratory data with the immunological profile of patients with pemphigus foliaceus, i.e., to determine the relationship between initial antiepidermis antibody titers and lesion intensity, and the relationship between the evolution of these antibody titers and the course of the lesions under treatment. The cell immunity behavior of the patients was also studied.

MATERIAL AND METHODS

Material

The study was conducted on 16 patients with endemic pemphigus foliaceus diagnosed on the basis of clinical signs and symptoms, and histopathologic examination, and direct immunofluorescence of the lesions.

The clinical classification of the patients utilized for group characterization was that proposed by the "Cooperative Pemphigus Group" and systematized in the literature (DIAZ et al. apud FRIEDMAN et al. 11), which recognizes two active forms of the disease, i.e., a localized form and a generalized form. Thus, the patients were divided into two groups, G1 and G2, respectively with the clinical characteristics described below.

Group G1: small quantities of localized lesions on the scalp, face and anterior and posterior trunk. This group consisted of ten previously untreated individuals, seven females and three males aged 15 to 68 years.

Group G2: lesions of generalized distribution intensely involving the scalp, face, anterior and posterior trunk, and limbs (pre-erythrodermal or erythrodermal phase). This group consisted of six previously untreated individuals, three females and three males aged 12 to 68 years.

In addition to the patients, a third group, G0, was used as control. This group consisted of six normal individuals who were nonconsanguineous relatives of six patients in G1 and G2, and of eight normal volunteers from Botucatu (SP), five males and nine females aged 15 to 65 years.

METHODOLOGY

Cell immunity

Cell immunity was studied in G1, G2, and G0 subjects by quantitation of the subpopulation of mononuclear cells and by the determination of blastic lymphocyte transformation.

For the quantitation of the mononuclear cell subpopulation on the basis of monoclonal antibodies in venous blood, we used indirect immunofluorescence (avidin-biotin system described by GATASS et al. 13) and the following monoclonal antibodies: OKT3, OKT4, OKT8, and OKB1 (Ortho Diagnostics System, Raritan, NJ, USA).

The production and characterization of the function of these monoclonal antibodies have been described in the literature 21, 22. OKT3 reacts with T lymphocytes of the CD8 group, OKT4 with helper/inducer T cells (CD3), OKT8 with cytotoxic/suppressor T cells (CD8), and OKB1 with B cells (CD20).

Blastic lymphocyte transformation was evaluated by the method of MOTA et al. 20.

The results are reported as blastic transformation index (BTI), calculated by the following ratio:

\[
BTI = \frac{\text{counts per minute of stimulated cells}}{\text{counts per minute of control cells}}
\]

DETERMINATION AND QUANTITATION OF CIRCULATING ANTIBODIES

Circulating antibodies were determined by the indirect immunofluorescence (IF) test according to COONS and KAPLAN 7 in the sera of all patients of groups G1 and G2 and in six individuals of group G0, nonconsanguineous relatives of patients. Blood samples were collected from G1 and G2 patients before corticotherapy, during the first, second, third and fourth week after the beginning of treatment, on the occasion of monthly return visits, and during the reactivation of the clinical signs and symptoms in patients in whom this occurred. Reactivation of clinical signs and symptoms was defined as the appearance of new lesions and greater facility in obtaining a positive Nikolsky sign.

Blood samples were collected only once from healthy individuals.
Blood was transferred to dry polyethylene tubes, centrifuged and separated from serum, which was stored frozen at -20°C until the time for use.

Anti-human IgG fluoresceinated conjugates (Laboratórios Hoechst do Brasil) were used at 1:40 dilution in a solution containing nine parts Tween 80 to one part Evans Blue and a substrate consisting of normal human foreskin obtained after postectomy. The skin fragments were placed in saline solution, immediately wrapped with aluminum paper and frozen in liquid nitrogen at -160°C for 24 hours. After freezing, they were stored in a freezer at -20°C until the time for slide preparation.

The immunofluorescence test was performed using Tris-acetate (TAS - calcium) as buffer solution, pH 7.5, prepared with 12.11g of 0.01 M Trizma base, 85g sodium chloride, 5.55g calcium chloride, and 2g sodium azide diluted in 1000ml distilled water. This solution was stored in a refrigerator until the time for use, when it was again diluted 1:10 in distilled water. The buffer solution was used for serum dilution and for slide washing.

The reactions were read up to 24 hours after preparation using a Jenaval (Carl Zeiss, Jena) fluorescence microscope at 40 to 100x magnification, plus immersion oil and a type 510 filter. The reactions analysed were compared to reactions performed with negative control serum and positive control serum. Each slide was examined twice at different times by two persons.

The reactions were classified by intensity as follows:

0 - negative reaction
+ - positive reaction of weak intensity
++ - positive reaction of moderate intensity
+++ - positive reaction of strong intensity
++++ - positive reaction of very strong intensity.

**STATISTICAL METHODS**

To evaluate the variables related to cell immunity, groups G₁, G₂, and G₃ were compared by analysis of variance for a randomized experiment, calculating the F value and the p level of significance as recommended by SNEDECOR & COCHRAN. Contrasts between group means were performed by the method of Scheffé, considering alpha = 0.05 and comparing group pairs and well as the patient group (G₁ + G₂) with the control group (G₃).

**RESULTS**

The values obtained for the blood tests of each patient in G₁ and G₂ are reported in Table 1. Leukocytosis was observed, especially in group G₃, consisting mainly of increased numbers of segments and eosinophils. Eosinophilia was detected in both G₁ and G₂.

Table 2 presents the mean values (± SD) of the relative (percent) values for total T cells (CD₄⁺), helper T cells (CD₄⁺), cytotoxic T cells (CD₈⁺) and B lymphocytes (CD₂⁺⁺) of patients (G₁ and G₂) and controls (G₃).

Table 3 presents the mean values of the absolute values (mm³) for total T cells (CD₄⁺), helper T cells (CD₄⁺), cytotoxic T cells (CD₈⁺), B lymphocytes (CD₂⁺⁺), CD₄⁺⁺/CD₈ ratio, and lymphocyte blast transformation index for patients (G₁ and G₂) and controls (G₃).

By comparing the three groups of individuals
TABLE 2

<table>
<thead>
<tr>
<th>GROUP</th>
<th>CD4⁺(%)</th>
<th>p</th>
<th>CD8⁺(%)</th>
<th>p</th>
<th>CD4⁺(%)</th>
<th>p</th>
<th>CD8⁺(%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₁ (n=10)</td>
<td>52.44±</td>
<td>p&lt;0.01</td>
<td>33.12±</td>
<td>p&lt;0.01</td>
<td>21.00±</td>
<td>NS</td>
<td>13.66±</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>11.54</td>
<td>7.82</td>
<td>6.27</td>
<td>4.88</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G₂ (n=6)</td>
<td>48.66±</td>
<td>p&lt;0.01</td>
<td>26.50±</td>
<td>p&lt;0.01</td>
<td>17.66±</td>
<td>0.05&lt;p&lt;0.1</td>
<td>15.83±</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>11.32</td>
<td>12.24</td>
<td>5.27</td>
<td>9.57</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G₃ (n=14)</td>
<td>65.78±</td>
<td>44.61±</td>
<td>23.71±</td>
<td>18.33±</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.92</td>
<td>6.19</td>
<td>4.92</td>
<td>5.06</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

p = difference between the group and the control.

(G₁xG₂xG₃), it can be seen that the relative values (%) of the CD4⁺ and CD8⁺ lymphocyte subpopulations were lower in G₁ and G₂ than in the controls (G₃), and a tendency to lower relative values of the CD4⁺ lymphocyte subpopulation was observed in the patients in relation to the controls.

No differences were observed between the three groups in the absolute values of the CD4⁺, CD8⁺ and CD₈⁺ lymphocyte subpopulations. Also, no differences between groups were observed with respect to relative (Table 2) and absolute (Table 3) values of the B(⁴⁺)/CD₈⁺ lymphocyte subpopulations, CD₁₉⁺/CD₂⁺ ratio, and homologous BLT.

When the group of patients as a whole (G₁+G₂) was compared with the normal group (G₃), the same characteristics as reported above were observed. Patients had lower relative levels of CD4⁺ and CD8⁺ lymphocytes when compared to the controls, and levels of CD4⁺ lymphocytes equal to or lower than those of the controls.

There was no difference between normal individuals and patients in absolute values of CD4⁺, CD8⁺ and CD₈⁺ lymphocytes, relative and absolute values of B(⁴⁺)/CD₈⁺ lymphocytes, or CD₁₉⁺/CD₂⁺ ratio. BTI values were higher in patients than in controls.

The results of the indirect immunofluorescence test for G₁ and G₂ are presented in Table 4.

The titers obtained by the indirect immunofluorescence test before treatment were higher in G₂ than in G₁ patients. In two G₁ patients the indirect immunofluorescence tests were negative at all times.

The indirect immunofluorescence tests were negative in all control individuals.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>CD4⁺</th>
<th>p</th>
<th>CD8⁺</th>
<th>p</th>
<th>CD4⁺</th>
<th>p</th>
<th>CD8⁺</th>
<th>p</th>
<th>CD₁₉⁺/CD₂⁺</th>
<th>p</th>
<th>TBL</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₁</td>
<td>1902.22±</td>
<td>600.21</td>
<td>NS</td>
<td>1106.88±</td>
<td>415.78</td>
<td>NS</td>
<td>757.12±</td>
<td>291.02</td>
<td>NS</td>
<td>404.83±</td>
<td>102.45</td>
<td>NS</td>
</tr>
<tr>
<td>G₂</td>
<td>1401.50±</td>
<td>485.77</td>
<td>NS</td>
<td>790.31±</td>
<td>357.94</td>
<td>NS</td>
<td>503.50±</td>
<td>217.12</td>
<td>NS</td>
<td>471.50±</td>
<td>317.36</td>
<td>NS</td>
</tr>
<tr>
<td>G₃</td>
<td>1596.71±</td>
<td>810.99</td>
<td>1095.77±</td>
<td>499.30</td>
<td>524.21±</td>
<td>274.68</td>
<td>460.25±</td>
<td>220.26</td>
<td>0.34</td>
<td>66.26±</td>
<td>41.06</td>
<td>NS</td>
</tr>
</tbody>
</table>
TABLE 4
Distribution of the titers obtained by indirect immunofluorescence in the two patient groups before and during treatment.

<table>
<thead>
<tr>
<th>GROUP 1(G₁)</th>
<th>WEEK OF TREATMENT</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1ˢᵗ  2ⁿᵈ  3ʳᵈ  4ᵗʰ  8ᵗʰ  1₂ᵗʰ  1₆ᵗʰ  2₀ᵗʰ  2₄ᵗʰ  2₈ᵗʰ  3₂ᵗʰ  3₆ᵗʰ</td>
</tr>
<tr>
<td>PRE-TREATMENT</td>
<td>-    -    -    -    -    -    -    -    -    -    -    -</td>
</tr>
<tr>
<td>1/80</td>
<td>2/80  1/40  1/20  1/20  1/20</td>
</tr>
<tr>
<td>1/20</td>
<td>1/20  1/20  1/20  1/20  1/20</td>
</tr>
<tr>
<td>1/640</td>
<td>1/160 1/320 1/160 1/80 1/40</td>
</tr>
<tr>
<td>1/640</td>
<td>1/160 1/160 1/160 1/80 1/40</td>
</tr>
<tr>
<td>1/320</td>
<td>1/40  1/40  1/20  1/20  1/40</td>
</tr>
<tr>
<td>1/80</td>
<td>1/160 1/320 1/160 1/80 1/40</td>
</tr>
<tr>
<td>1/80</td>
<td>1/80  1/40  1/20  1/20  1/20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GROUP 2(G₂)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1/320</td>
<td>1/640 1/320 1/320 1/640 1/80 1/20 1/20 1/20 1/20 1/40</td>
</tr>
<tr>
<td>1/2560</td>
<td>1/1280 1/320 1/320 1/160 1/80 1/80 1/20 1/20 1/20 1/40</td>
</tr>
<tr>
<td>1/320</td>
<td>1/320 1/320 1/320 1/80 1/80 1/80 1/40 1/40 1/40 1/20</td>
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<td>1/640</td>
<td>1/320 1/320 1/320 1/80 1/40 1/40 1/20 1/20 1/20 1/40</td>
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<tr>
<td>1/640</td>
<td>1/1280 1/320 1/320 1/160 1/160 1/40 1/40</td>
</tr>
<tr>
<td>1/1280</td>
<td>1/640 1/320 1/320 1/160 1/640 1/20</td>
</tr>
</tbody>
</table>

* Times when clinical worsening occurred.

COMMENTS:
Median of group G₁ before treatment: Md = 1/80
Median of group G₂ before treatment: 1/320 < Md < 1/640
Statistical analysis by the "U" test: U = 6; p<0.05; therefore the serologic titers were higher in group G₁.

DISCUSSION

The blood counts performed on the patients demonstrated the presence of leukocytosis, especially in group G₁, mainly represented by an increase in number of segments and eosinophils. The leukocytosis observed in G₁ patients occurred before any type of treatment, contrary to the data reported by OLIVEIRA et al. 21, who observed leukocytosis in patients under corticoid treatment. The leukocytosis observed in the present study may be explained by the secondary infection often occurring in the exacerated areas.

Evaluation of cell immunity by determination and comparison of lymphocyte subpopulations in the three groups revealed that, if the relative values are considered, patients presented a decreased subpopulation of CD₄⁺ and CD₈⁺ lymphocytes and a tendency to reduced CD₈⁺ numbers when compared to the controls. However, if considered as absolute values, the results did not show a change in relation to normal controls.

No changes were detected in the B(CD₁₉⁺) subpopulation between G₁ and G₂ or between these groups and the normal controls.

These results partially agree with those reported by GUERRA et al. 13, who evaluated cell immune behavior by the rosette method in 30 individuals with active pemphigus foliaceus and detected decreased percentages of T lymphocytes and normal percentages of B lymphocytes. In lymphonodules, depletion of T cells was observed in the paracortical areas. The authors...
concluded that the findings probably reflected decreased cell immunity. These authors, however, did not mention patient white cell counts or amounts of T and B lymphocytes per mm³, but only reported percent values of these lymphocytes. Such values may reflect a false decrease in lymphocyte populations if the number of white cells were increased.

In the present study, if we only consider percent lymphocyte values, we may suggest that there was a certain degree of cell immune deficiency in the patients. This fact, however, is no longer relevant when absolute values show that lymphocyte numbers are preserved. CASTRO 4 also observed normal amounts of T lymphocytes.

BLT indices in the presence of PHA were higher in patients than in controls, (G₁+G₂+G₃), and did not differ when the three groups were compared with one another (G₁+G₂+G₃). These data indicate that the lymphocytes of patients had the ability to be normally stimulated by PHA, contrary to the report made by CASTRO 4 who observed a depressed response, while OLIVEIRA et al, in 1988 21, observed a heterogeneous behavior of lymphocyte subpopulations in treated and untreated patients.

CASTRO in 1987 4 also evaluated the cell immune behavior of ten patients with pemphigus foliaceous by delayed hypersensitivity skin tests and by skin sensitization to DNCB, suggesting the existence of nonspecific depression of the cell immune response in these patients.

In 1988, OLIVEIRA et al. 21 performed delayed skin immunity tests in seven patients and they concluded that cell immunity is preserved in pemphigus foliaceous.

In 1993, ZILLIKENS et al. 30 determined the levels of soluble interleukin-2 receptor in blister fluid and serum samples from pemphigus patients prior to treatment. They detected that activated mononuclear cells are present in lesional skin of pemphigus patients, and may contribute to the pathology of this disease.

Overall analysis of the cell immunity data observed in the present study permits us to conclude that the cell immune response of patients with pemphigus foliaceous is preserved.

As to humoral immunity, BEUTNER et al. in 1968 2 carried out serological studies on a group of 28 patients with Brazilian pemphigus foliaceous by the indirect immunofluorescence test, using rabbit and monkey esophagus as substrate. They detected the presence of circulating IgG class antibodies in a deposit model in the substrate, which was undistinguishable from that obtained with serum of patients with pemphigus vulgaris and other nonendemic forms of pemphigus. Inter cellular antibody titers were proportional to the severity of the disease and higher than those detected in cases of nonendemic pemphigus.

After this pioneering study, other trials were carried out using different substrates and buffer solutions, although without an absolute agreement in the results obtained 1, 14, 15, 21, 24. In the present study, several substrates were first used, such as skin and esophagus obtained at autopsy from adult and newborn humans, and mouse and rabbit skin and esophagus. Human foreskin removed soon after post mortem and immediately frozen proved to be better in terms of clear readings than mouse or rabbit skin and esophagus.

In 1987, EYRE & STANLEY 8 demonstrated that patients with pemphigus foliaceous present autoantibodies against a desmosome complex and associated proteins, with an epitope of calcium-sensitive conformation.

MATIS et al. 14, in 1987, performed the indirect immunofluorescence test on sera from patients with pemphigus vulgaris, pemphigus foliaceous and bullous pemphigoid disease using different substrates and buffers. The highest titers were obtained when using human skin as substrate and buffer containing calcium. It was suggested that this fact may occur because the antigen of pemphigus foliaceous, which may be a desmoglein (desmosome glycoprotein), needs calcium to stabilize or because calcium facilitates antigen binding to its antibody, or yet again because calcium protects the antigen against proteolysis.

In 1989, FRIEDMAN et al. 11 concluded that human skin is the ideal substrate and can be obtained from the prepuce, head, neck or anterior abdominal wall, and that TAS-calcium should be preferred as a buffer since it preserves the antigenic properties of the skin.

During the preliminary phase of the present study carried out to define the methodology to be used, the
data obtained agreed with those reported above with respect to substrate (human prepubic skin) and calcium buffer. The present results showed the absence of antibodies in all nonconsanguineous patient relatives.

CASTRO et al. in 1976 3 studied the presence of auto-antibodies by IIF in the serum of normal individuals and relatives or friends of patients with South American pemphigus foliaceus. Of the 43 serum samples analyzed, seven gave positive reactions with titers ranging from 1/10 to 1/1280.

The present results agree with those obtained by CUNHA in 1988 4, who reported the absence of antineptelial IgG class autoantibodies in consanguineous relatives of patients from the Franco da Rocha and Mairiporã region. This author also detected negativity of these autoantibodies among individuals living with the patients, their neighbors and blood donors donating blood at the University Hospital of FMUSP, residing in the urban São Paulo area. The present results also agree with those obtained by FRIEDMAN et al. 5 who, in addition to patient serum, also used serum from normal individuals from endemic areas to search for antineptelial autoantibodies. By using rat tongue as substrate, they detected positively reacting sera from normal individuals. However, when using human skin as substrate, these same sera showed negative reactions, i.e., absence of IgG class autoantibodies. The sera were then tested for Cascar antigens, a recently described murine cytokeratin, giving positive reactions. This shows that the use of rat tongue as substrate may lead to cross reaction, a fact that does not occur with the use of human skin, which provides more reliable results.

In the present study the IIF tests were positive in 100% of the patients with the disseminated form of the disease and in 80% of the patients with the localized form, with an overall 87.5% rate of positivity. These results agree with those reported in the literature 2, 4, 11, 18 in which test positivity ranges from 77 to 100% with the use of different techniques in patients with clinical manifestations of variable intensity.

The two patients with a negative IIF test before and during treatment with corticosteroids presented minimal pemphigus foliaceus lesions and this negativity may be explained by the low activity of the disease. These patients responded very well to treatment with corticosteroids, with no clinical worsening at any time during therapy.

The results also demonstrate that, in the localized forms of the disease, the median values before treatment titers was 1/80, whereas in the disseminated form the median was 1/320 to 1/640. The difference between these values was statistically significant, in agreement with the literature 2, 4, 8, 10, 11, 19. Thus, proportionality was observed between IIF titers and extent of the clinical signs and symptoms presented by the patients.

By analyzing the course of each patient, a fall in IIF titers over treatment can be detected, as well as increased titers at the time of clinical worsening of the patient.

The results permit us to conclude that in the present study there was correlation between the extent of cutaneous involvement and titers obtained by indirect immunofluorescence, although there was no borderline titer distinguishing the forms of the disease.

In this respect, standardization of indirect immunofluorescence is necessary to provide a specific semiquantitative laboratory parameter of prognostic and treatment-planning value in addition to clinical parameters.

RESUMO

Imunidade humor de células no pênfigo foliáceo endêmico

Foram avaliadas dezessete doentes portadores de pênfigo foliáceo endêmico, dez com a forma localizada da doença (Grupo G1) e seis com a forma disseminada (Grupo G2), com os objetivos de correlacionar o quadro clínico e laboratorial desses pacientes com o perfil imunológico dos mesmos, e verificar a relação dos títulos dos anticorpos antiepiderme circulantes, identificados pela imunofluorescência indireta, com intensidade da lesão e com a evolução das lesões em tratamento.

Foram realizados: hemograma completo, quantificação de subpopulação de células mononucleares por anticorpos monoclonais e estudo da transformação blastica de linfócitos e quantificação de anticorpos circulantes por meio da reação de imunofluorescência indireta.

Observou-se leucocitose principalmente no grupo G1, diminuição dos valores relativos das subpopulações de linfócitos CD4+ e CD8+ e tendência à diminuição dos
valores relativos da subpopulação CD₄⁺ nos doentes (Grupos G₁ e G₂).

Os índices de transformação blastica de linfócitos frente à fitohemaglutinina revelaram níveis mais elevados nos doentes (Grupos G₁ + G₂) que nos controles.

A reação de imunofluorescência indireta foi positiva em 100% dos doentes do grupo G₂ e em 80% do grupo G₁. A mediana dos valores dos títulos foi maior no grupo G₁ quando comparado com o grupo G₂.

A análise global dos resultados permite concluir que a imunidade celular está preservada, e que existe uma relação entre os títulos de anticorpos obtidos à reação de imunofluorescência indireta e extensão da lesão cutânea.

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