Lymphocyte Values in Patients with Recurrent Aphthous Ulceration

Vrijednosti limfocita u bolesnika s rekurentnim aftoznim ulceracijama

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Summary

The total number of T (CD2+)- and B (CD20+)- lymphocytes as well as T lymphocyte subpopulations CD4 (helper) and CD8 (suppressor) were examined in peripheral blood of patients with RAU, to determine their role in the onset of this disease. The study was carried out in two groups: a group of patients with acute stage of RAU and a control group of healthy subjects. Lymphocyte values were determined in peripheral blood by the method of flow cytometry. The obtained results showed decreased B-lymphocyte values in peripheral blood in patients with RAU in relation to healthy subjects. However, T-lymphocytes showed increased values in 1/3 of the patients, which is statistically insignificant. CD4 lymphocyte values were also increased and statistically insignificant, while CD8 lymphocyte values were significantly decreased in patients with RAU in relation to healthy subjects.

Key words: *RAU, lymphocytes, peripheral blood*

Acta Stomatol. Croat.
1995; 30:85—90

ORIGINAL PAPER

Received: July 5, 1995.
Primljeno: 5. srpnja 1995.

Introduction

Recurrent aphthous ulceration (RAU) occurs orally in 11 to 56 percent of the population. It is an oral mucosal disease which is very frequent among people susceptible to relapse (1). The onset of RAU is a result of an immunologic response induced by the Streptococcus antigen (2, 3). The mechanism of immunologic response is a result of cellular cytotoxicity which leads to oral necrosis and ulcer formation (4). This is encouraged by predisposing factors which decrease the immunologic reactivity of the body through oral epithelium (5, 6). Some studies suggest a changed immunologic systemic response of patients with RAU (7), particularly as a response to the human oral mucous extract and Streptococcus antigen (4, 8, 9). The lymphocyte function in RAU is examined according to Greenspan et al. (10), indicating a relation disorder in the lymphocyte subpopulation. T-lymphocyte activities, which are important for the lymphocytes role in cell cross-reactions as a characteristic, immunopathogenetic process in RAU, show various results. Landesberg et al. observed an increased CD4 cell function (11). Sciubba confirms that a local activity, particularly that of T-lymphocytes, is involved, and that relations between CD4 subpopulation and CD8 cells change, depending on the disease stage

(12). The B-lymphocyte function, which determines the immunologic response to various antigens, is linked to T-lymphocyte functions. There is a controversy on the B-lymphocyte activities in the onset of RAU (13—16). In this study, we investigated the number and activity of T- and B-lymphocytes in peripheral blood, and of the T-lymphocyte subpopulations (CD4 and CD8) in patients with RAU in the acute stage, to determine their role in the onset of the disease.

Materials and Methods

Clinical and laboratory examinations were carried out in two groups of subjects. The group of the affected population consisted of 51 patients, 25 males and 26 females, aged 13—75 years, in whom RAU relapses occurred every one or two months.

A control group consisted of 27 healthy subjects, 13 males and 14 females, aged 18—65 years.

Upon clinical registration of aphthous efflorescence types according to Lehner, which revealed 75 percent of the patients to have aphthae minores and 15 percent aphthae majores, the patients were referred for peripheral blood lymphocyte analysis. Total number of B-lymphocytes (CD20+) and T-lymphocytes (CD2+), and the values of inducer (CD4) and suppressor (CD8) subpopulations were determined.

The results were quantified and expressed as the total number of T-lymphocytes (CD2+), total number of B-lymphocytes (CD20+), and lymphocyte CD4 and CD8 cell subpopulations.

Statistical analysis of the results included determination of arithmetical means and standard deviations, compared to the respective values obtained in the control group of normal subjects. The level of significance was determined by the Student's t-test distribution.

The method of determination of the total number of T- and B-lymphocytes, and of the lymphocyte subpopulations in peripheral blood: the percentage of the total number of T- and B-lymphocytes, and of the T-lymphocyte subpopulations was determined by the method of flow cytometry at the Institute for Immunology in Zagreb. The technique of direct double marking of full blood leukocytes was employed, using the following monoclonal antibodies and isotype controls (Becton Dickinson Immunocytometry Systems, San Jose, California): anti-Leu-3a (CD4), anti-Leu-2a (CD8), anti-Leu-5b (CD2), anti-Leu-16 (CD20) and anti-Transferrin Receptor (CD71). Monoclonal antibodies and mouse immunoglobulins of the same isotypes were conjugated using fluorescein-isothiocyanate (FITC) and phycoerythrin (PE), respectively. Briefly, 100 μL fresh heparinized venous blood (5 IJ heparin/10 μL blood) were sampled in to test tubes containing 10 μL of anti-CD4 PE and anti-CD71 FITC, anti-CD8 PE and anti-CD71 FITC, and mouse IgG1 FITC and IgG2 PE. After gentle shaking, the test tubes were incubated for 15 min in dark, at room temperature. Upon incubation, erythrocytes were lysed by the addition of 2 ml of lysing solution (FACS Lysing Solution, Becton Dickinson) for 10 min at room temperature. The test tubes were centrifuged for 5 min at 300 G, the supernatant was removed and the cells rinsed twice with 1 ml PBS containing 0.1% sodium azide. After the second rising, the cells were resuspended into 0.5 ml PBS containing 1% paraform and analyzed by flow cytometry on a FACScan (Becton Dickinson). After the control sample had been passed through the double-parameter histogram having the size and granules allowing differentiation of lymphocytes, monocytes and granulocytes, an electronic fence was set around the lymphocyte population. All samples (including the isotype control) were submitted to the conditions set in this manner and an amount of 5,000 lymphocytes were analyzed. In the double-parameter histogram with green (FITC) and red (PE) fluorescence, a percentage of lymphocytes with one, the other and both marks was set (17).

Results

Figure 1 shows that the quantitative T-lymphocyte values were within the normal limits in 67.5% of the subjects without RAU, and exceeded the normal values in 32.5% of the RAU patients. The amount of B-lymphocytes did not show any deviation from the normal values in 97.5%, and higher values were observed in only 2.5% of the RAU patients.

No decreased values of T-lymphocyte subpopulations were observed in the patients with

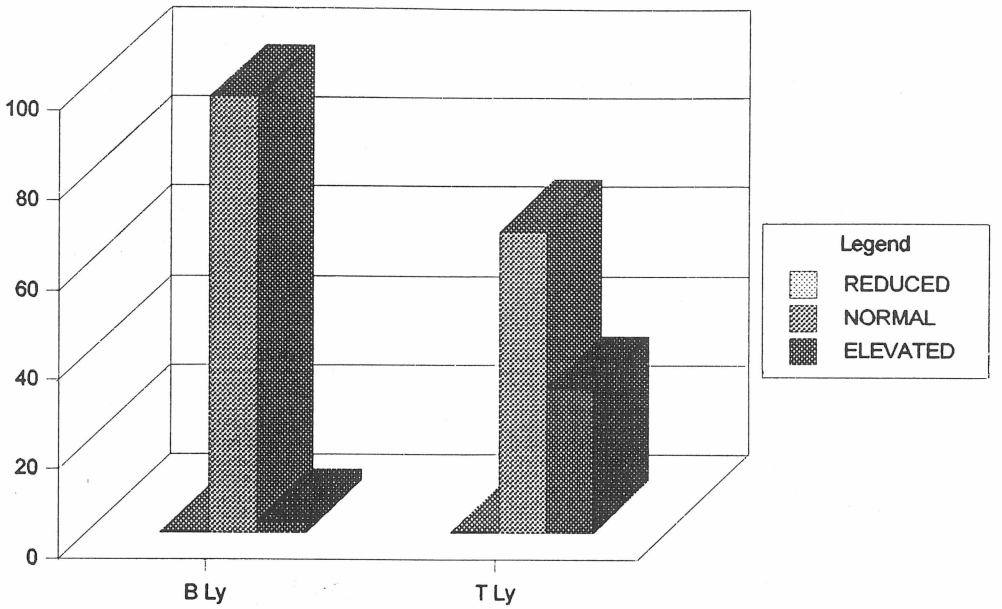

Figure 1. *Relation of the average presence of T and B lymphocytes in RAU*
Slika 1. *Omjer prosječne prisutnosti T i B limfocita kod RAU*

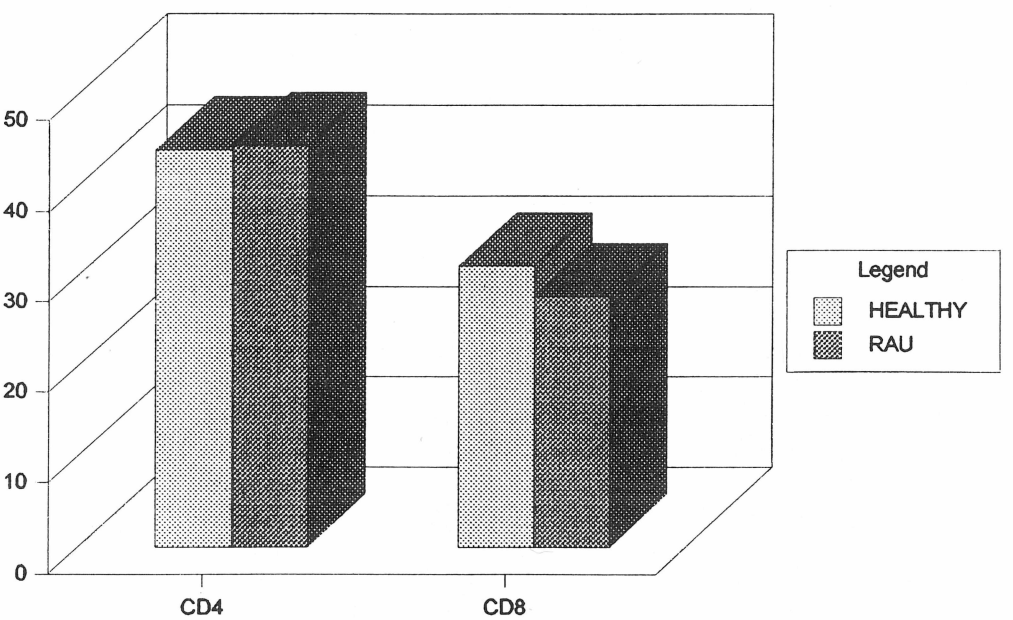

Figure 2. *Relation of the average presence of CD4 and CD8 lymphocytes in RAU*
Slika 2. *Omjer prosječne prisutnosti CD4 i CD8 limfocita kod RAU*

aphthous ulcerations. In 92.1% of the control subjects, normal values of CD4-lymphocyte cells were observed, whereas they were increased in 7.9% of the RAU patients. Normal values of suppressor CD8 T-lymphocytes were observed in 89.7% of the patients with RAU, whereas they were increased in 10.3% of the control subjects, as shown in Figure 2.

The mean values of T- and B-lymphocytes in the two groups showed slightly increased values of T-lymphocytes in RAU as compared to healthy subjects (Figure 3). The differences are not statistically significant ($p>0.05$). The mean values of B-lymphocytes showed significantly lower values in RAU than in healthy subjects ($p>0.05$).

The mean values of the CD4 and CD8 cells showed higher CD4-lymphocyte values in the patients with RAU ($p>0.05$) as compared to healthy subjects, but the difference was not statistically significant. The values of CD8 lymphocytes showed a reverse relationship, i.e. they were significantly lower in RAU as compared to healthy subjects ($p<0.05$) (Figure 4).

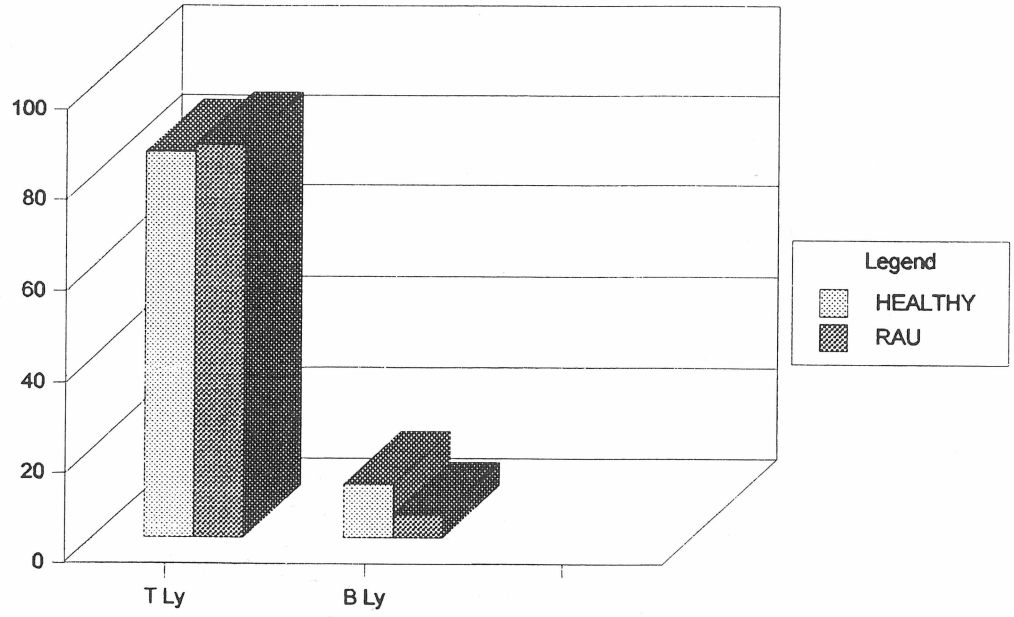

Figure 3. *The presence of T and B lymphocytes in patients with RAU*
Slika 3. *T i B limfocitne vrijednosti kod bolesnika s RAU u odnosu prema kontrolnoj skupini*

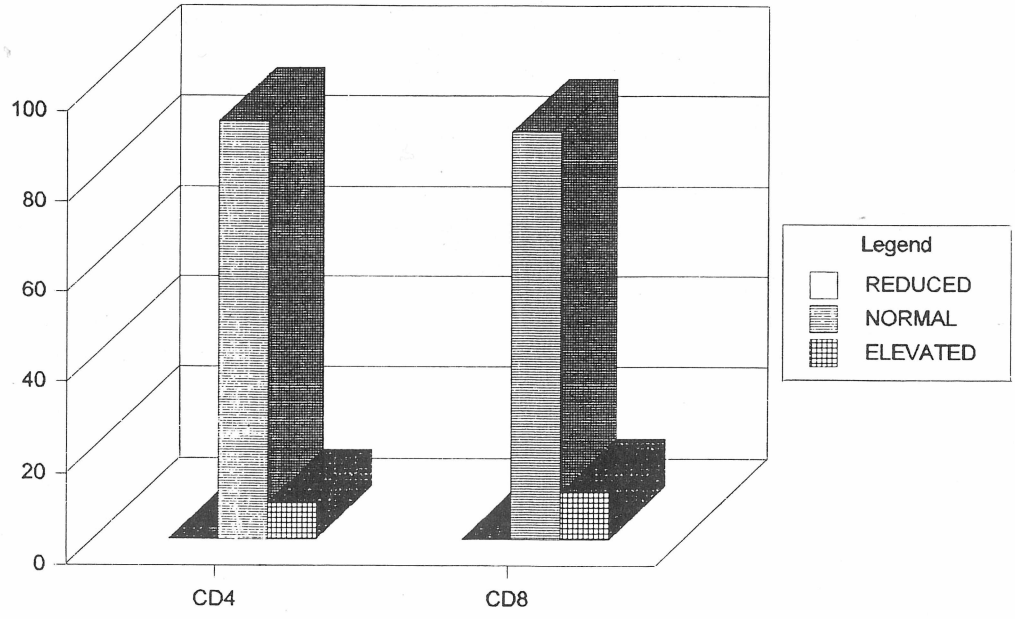

Figure 4. *The presence of CD4 and CD8 lymphocytes in patients with RAU*
Slika 4. *CD4 i CD8 limfocitne vrijednosti kod bolesnika s RAU u odnosu prema kontrolnoj skupini*

Discussion

In most subjects, peripheral blood B-lymphocytes were within the normal limits, but in a small number of RAU patients increased values of B-lymphocytes were found. B-lymphocytes were not always included in the pathogenetic process during the acute stage of the disease. Examinations carried out by Landesberg and Sciubba have shown that in RAU patients, a local immune response with T-lymphocyte activity is involved. Many authors report that no systemic immune defect could be detected (11, 12). T-lymphocytes showed increased values in the blood of patients in the acute stage of RAU, pointing to their important role in the pathogenesis of the disease (5, 10, 12, 14). The increased CD4 lymphocyte values during the acute stage of RAU indicate their increased activity at the time of the occurrence of oral ulcerations. The studies conducted so far have shown that the proportions of CD4 and CD8 vary depending on the stage of the disease (11, 12, 14). In our study, T-lymphocytes were increased as compared to the normal values. Most probably, an advanced stage of oral aphthous ulceration is associated with a CD8 lymphocyte decrease. Results obtained by other authors suggest that the changes in the activity of T-lymphocyte subpopulations depend on the stage of the disease (11, 12).

Conclusion

Normal or increased values of T-lymphocytes in peripheral blood were observed in RAU patients. In RAU patients, the total number of B-lymphocytes did not show any change. However, the values of T-lymphocyte subpopulations were changed in RAU patients. CD4 lymphocytes showed slightly increased values, whereas CD8 lymphocytes showed significantly lower values.

VRIJEDNOSTI LIMFOCITA U BOLESNIKA S REKURENTNIM AFTOZNIM ULCERACIJAMA

Sažetak

Ispitivan je ukupni broj T(CD2+) i B(CD8+) limfocita, kao i T limfocitnih subpopulacija CD4 (helpera) i CD8 (supresora) u perifernoj krvi bolesnika s RAU radi utvrđivanja njihove uloge u nastanku bolesti. Istraživanje je provedeno u dvije skupine ispitanika: skupini bolesnika s akutnim stadijem RAU i kontrolnoj skupini zdravih ispitanika. Limfocitne vrijednosti u perifernoj krvi ispitanika dobivene su metodom protočne citometrije.

Dobiveni rezultati pokazuju snižene vrijednosti B limfocita u perifernoj krvi bolesnika s RAU. Vrijednosti T limfocita povišene su u 1/3 bolesnika, što nije statistički znakovito. CD4 limfocitne vrijednosti također su povišene, statistički ne znakovito, dok su vrijednosti CD8 limfocita statistički znakovito snižene u bolesnika s RAU u odnosu prema zdravim ispitanicima.

Ključne riječi: *RAU, limfociti, periferna krv*

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