Peripheral lymphocyte subpopulations in recurrent aphthous ulceration

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Peripheral lymphocyte subsets—T-helper (CD4+), T-suppressor/cytotoxic (CD8+), and naive/virgin T cells/natural killer cells (CD45RA)—were studied quantitatively in 30 patients with recurrent aphthous ulceration (RAU) and 29 sex- and age-matched RAU-free control donors. The CD4+ percentage was significantly lower in the patients than in the control group ($P < 0.0001$), whereas CD8+ and CD4/CD8 ratio figures did not differ significantly between patients and controls. The CD45RA+ counts were significantly higher in the patient group ($P < 0.01$). The study supports previous investigations with regard to the demonstration of immunologic disturbances in RAU patients. Whether the imbalance is primary or secondary with regard to the basic etiology remains to be resolved. □ *Aphthae; natural killer cells; oral disease; oral medicine; stomatitis; T lymphocytes*

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The etiology of recurrent aphthous ulceration (RAU) remains unresolved. However, previous studies of peripheral blood lymphocyte subsets have indicated a general immunologic imbalance in the patients as compared with RAU-free control populations (1–3). A decreased T helper to T suppressor/cytotoxic (CD4+/CD8+) cell ratio has been a consistent finding. (Throughout this paper we have applied the current cluster of differentiation (CD) nomenclature to the cells detected by the various monoclonal antibodies used in the referred studies.) In two of the studies the reduced ratio was a result of both significantly increased CD8+ (OKT8+) and decreased CD4+ (OKT4+) cell counts (1, 2). In our previous study, however, the lowered ratio could only be assigned to an increase in CD8+ (OKT8+) percentages, as no significant differences between patients and controls were demonstrated in CD4+ (OKT4+) numbers (3). Furthermore, the changes in lymphocyte subset numbers in RAU patients are apparently unrelated to various disease stages, as no significant changes have been demonstrated between active disease (within the first 8 days of subjective RAU symptoms) and remission stages (2, 3).

Natural killer (NK) cells might be involved in the pathology of RAU, as increased numbers of CD11b+ (OKM1+) have been found in peripheral blood of the patients (4).

The purpose of the present study was to investigate further the T helper, T suppressor/cytotoxic, and NK cell percentages in patients with recurrent aphthous ulceration as compared with RAU-free controls.

Materials and methods

Population

The patient group consisted of 30 patients with 'minor' RAU (18 women and 12 men), with a mean age of 36.0 years (range, 18–67 years). Before the study the diagnosis was in all cases confirmed by objective examination of at least one recurrence, and lesions were verified as 'minor' aphthae in accordance with traditional classification (5). The estimated average number of recurrences the

previous year was 12.1 (range, 3–30), and the mean duration of RAU experience was 18.8 years (3–41). General diseases and extraoral manifestations along with RAU were absent, and objectively, all patients were in physical and psychologic good health, and oral diseases apart from RAU were not found.

The control group consisted of 29 voluntary blood donors from the University Hospital Blood Bank with a negative RAU history. The control group was matched with the patient group with regard to sex (17 women and 12 men) and age ($\bar{x}$ = 37.5 years; range, 19–65 years).

The protocol was approved by the local ethical committee, and informed consent to participate was obtained in accordance with The Declaration of Helsinki II. Peripheral blood samples from the patients were obtained consecutively, disregarding disease stage. All blood samples from patient and control groups were collected between 0800 h and 1000 h, to minimize the circadian variation.

Methods

The blood samples were examined for T helper (CD4+), T cytotoxic/suppressor (CD8+), and NK (CD45RA+) cell percentages. Lymphocytes were isolated after 30 min of centrifugation on Lymphoprep® at 20°C. After being washed three times with RPMI-1640, 10^6 lymphocytes were labeled with 10 µl of the following 1:20 diluted mouse monoclonal antibodies for 30 min at 4°C: OKT4 (CD4), OKT8 (CD8) (Ortho Pharmaceuticals, Raritan, N.J., USA), or Leu-18 (CD45RA) (Becton Dickinson, Mountain View, Calif., USA). After renewed washing, the cells were incubated for 30 min with 100 µl 1:30-diluted fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse-Ig (anti-kappa and anti-lambda) (Dakopatts, Copenhagen, Denmark). Finally, the washed cells were stored in 150 µl 1% formalin at 4°C and quantified by flow cytometry in a fluorescence-activated cell sorter (FACScan, Becton Dickinson).

Statistical analysis

Mann–Whitney's U-test was used for comparing numbers from the patient and control groups. *P* values below 0.05 were considered significant.

Results

The percentages of OKT4+ cells were significantly different in the two examined

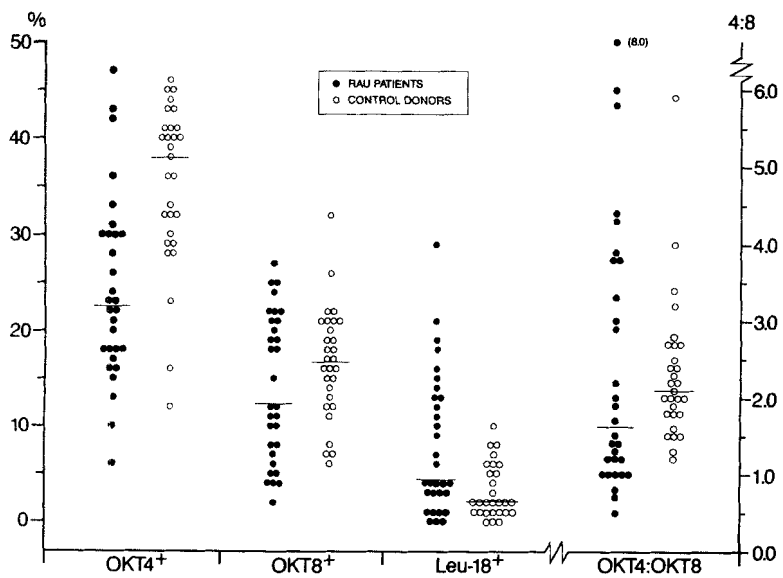

Fig. 1. Percentages of OKT4, OKT8, and Leu-18-positive cells and OKT4/OKT8 ratio in patients with minor recurrent aphthous ulceration (RAU) and matched control donors. Horizontal lines indicate the medians.

Table 1. Statistics of OKT4+, OKT8+, and Leu-18+ percentages and the OKT4/OKT8 ratio in the peripheral blood of 30 patients with recurrent aphthous ulceration and 29 matched control donors

	OKT4+	OKT8+	4/8	Leu-18+
Mann–Whitney U-test	$P < 0.0001$ (↓)†	NS*	NS	$P < 0.01$(↑)

* NS = not significant.
† (↓) (↑) direction of the numbers in the patients as compared with the controls.

groups ($P < 0.0001$) (Fig. 1, Table 1), and the median was 41% lower in the patient group than in the control group. No statistically significant differences were demonstrated in OKT8+ counts, and even though OKT4/OKT8 ratios tended to be lower in the patient group, the differences were not significant (Fig. 1, Table 1).

Leu-18+ percentages were significantly different in the two groups ($P < 0.01$), with a 110% increase of the group median in RAU patients as compared with control donors (Fig. 1, Table 1).

Discussion

The decreased CD4+ counts in the patient group is in agreement with two earlier studies (1, 2) but disagrees with another investigation, in which CD4+ figures did not differ significantly between patients and controls (3).

Irrespective of disease stage, a previous study including 33 RAU patients and 25 controls showed significantly higher CD8+ counts in the patient group (1). This finding was not confirmed in the present study. In an earlier study of 20 minor-RAU patients, in which disease stage was taken into consideration, significantly increased CD8+ counts in the patients were demonstrated in both active and remission stages as compared with 19 matched controls (3). Concordingly, Savage et al. (2) did not demonstrate any significant difference in CD8+ counts between active and remission periods in 15 RAU patients, although the numbers tended to be higher during remission.

The trend towards a reduced CD4/CD8 ratio seen in the present study is in line with previous investigations in which significantly decreased ratios in RAU patients compared with controls have been reported during both active (3) and remission stages of RAU (2, 3).

On balance, all studies demonstrate features of immunosuppression in RAU patients as compared with RAU-free sex- and age-matched subjects; two of the previous three investigations have shown decreased CD4+ counts (1, 2), and all three studies show increased CD8+ counts (1–3) and decreased CD4/CD8 ratio (1–3). Subpopulation figures may fluctuate with disease stage, with reduced CD4+ counts being more pronounced during early clinical activity (2) and CD8+ counts further increased during remission (2). This tendency seems to be reflected in the tissue, in which CD4+ cells have been reported to prevail in the preulcerative stage of RAU lesions, whereas CD8+ cells are dominant at the time of ulceration (6). Although precautions must be taken when trying to parallel subpopulation numbers of peripheral blood with lesional numbers, it is noteworthy that one study has demonstrated similar proportions of CD4+ and CD8+ counts in peripheral blood and RAU lesions (4).

The two previous investigations of NK percentages in peripheral blood of RAU patients have been contradictory. One study demonstrated no significant differences in CD11b+ (OKM1+) or CD57+ (Leu-7+) counts in 15 RAU patients as compared with controls and, furthermore, no differences between active and remission stages (2). In the other study of eight RAU patients the CD11b+ (OKM1+) counts were significantly higher in the patient group (4).

NK cells constitute a heterogeneous group

of cells with regard to functional properties and cell surface phenotype (7–9). Few markers are expressed on all NK cells, and none are specific for NK cells, since they also appear on other cells (mainly T cells, but some also on monocytes or granulocytes) (9). Whereas CD11b and CD57 seem to have higher affinity for the larger-sized T cells within the previously activated (resting) memory/effector pool, CD45RA seems to have the highest expression within the naive (virgin, not yet activated) T-cell pool (7, 10, 11). Therefore, the presented results on CD45RA percentages probably cannot be directly compared with the previous CD11b and CD57 figures. The involvement of subsets within the NK population in the pathology of RAU does, however, seem possible, as increased antibody-dependent cellular cytotoxicity (ADCC) has been reported during the early ulcerative stage (ulcers at 1–3 days) in RAU patients as compared with controls (12). At any other stage of disease (prodromal, healing, remission), ADCC in the patients did not differ from that in controls (12). Furthermore, CD57+ (Leu-7+) cells have been detected in preulcerative and early ulcerative RAU lesions but not in the more developed lesions (6). The wide dispersion of CD45RA+ numbers in the patient group in the present study might thus reflect different disease stages, with the high counts possibly obtained during early RAU activity.

Altogether, complicated immunologic disturbances seem to be involved in the pathology of RAU. But it still remains unknown whether the imbalances are primary or secondary with regard to the basic etiology.

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References

1. Kayavis I, Daniilidis M, Albanidou-Farmaki E, Vergoulas G, Polymenidis Z, Papanayotou P. T-lymphocyte subsets in recurrent oral ulceration. A preliminary study. J Oral Med 1987;42:198–200.
2. Savage NW, Mahanonda R, Seymour GJ, Bryson GJ, Collins RJ. The proportion of suppressor-inducer T-lymphocytes is reduced in recurrent aphthous stomatitis. J Oral Pathol 1988;17:293–7.
3. Pedersen A, Klausen B, Hougen HP, Stenvang JP. T-lymphocyte subsets in recurrent aphthous ulceration. J Oral Pathol Med 1989;18:59–60.
4. Greenspan JS, Daniels TE, Shillitoe FJ, Olson JA. Mononuclear cell subpopulations in recurrent aphthous ulceration enumerated using monoclonal antibodies, flow cytometry and the ABC technique [Abstract 1507]. J Dent Res 1982;61:346.
5. Lehner T. Autoimmunity in oral diseases with special reference to recurrent oral ulceration. Proc Royal Soc Med 1968;61:515–24.
6. Savage NW, Seymour GJ, Kruger BJ. T-lymphocyte subset changes in recurrent aphthous stomatitis. Oral Surg Oral Med Oral Pathol 1985;60:175–81.
7. Roitt I, Brostoff J, Male D. Immunity to tumours. In: Roitt I, Brostoff J, Male D, eds. Immunology. Edinburgh: Churchill Livingstone, 1986:18.1–16.
8. Rager-Zisman B, Bloom BR. Natural killer cells in resistence to virus-infected cells. Springer Semin Immunopathol 1982;4:397–414.
9. Klein J. Immunology. Boston: Blackwell Scientific Publishers 1990:43–6.
10. Sanders ME, Makgoba MW, Shaw S. Human naive and memory T-cells: reinterpretation of helper-inducer and suppressor subsets. Immunol Today 1988;9:195–9.
11. Beverley PCL. Is T-cell memory maintained by crossreactive stimulation? Immunol Today 1990; 11:203–5.
12. Greenspan JS, Gadol N, Olson JA, Talal N. Antibody-dependent cellular cytotoxicity in recurrent aphthous ulceration. Clin Exp Immunol 1981; 44:603–10.

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