39, 44% vs. 3.3% in our controls. But since the frequence of HLA-B 38 was 11% vs. 3.5% in the controls (no significant difference), we thought it justified to refer to an increase in HLA-B 16.

It is always problematic to apply statistical methods to data of only few patients and even more dubious to draw conclusions on such evaluations. Hailey-Hailey's disease is a fairly rare disease, however, the pathomechanism of which could be easier assessed if all investigated cases were published.

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Circulating Lymphocyte Subsets in Patients with Alopecia areata

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Baadsgaard O, Lindskov R. Circulating lymphocyte subsets in patients with alopecia areata. Acta Derm Venereol (Stockh) 1986; 66: 266–268.

Lymphocyte subsets in peripheral blood of fourteen patients with patchy alopecia areata or alopecia universalis were estimated using monoclonal antibodies and immunofluorescence. The median percentage of circulating Leu 2a, 3a, 4 and 7 positive cells ("T-suppressor/cytotoxic", "T-helper/effector", total T-cells and killer and natural killer cells) were normal.
Key words: T-lymphocytes; Killer and natural killer cells. (Received March 14, 1985.)

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In spite of conflicting findings regarding cell-mediated immunity and auto-immune phenomena in alopecia areata patients, there is evidence indicating an immunologic abnormality as an etiological factor (1, 2, 3, 4, 5). On this background we have measured circulating lymphocyte subsets in patients with alopecia areata.

MATERIALS AND METHODS

Patients

Fourteen consecutive outpatients, 9 females and 5 males, between 16 and 71 years of age (median 32), participated in the investigation. Eleven patients suffered from patchy alopecia areata (AA) and 3

patients from alopecia universalis. Thirteen healthy age and sex matched individuals were used as controls.

Immunofluorescence studies

The mononuclear cells were isolated from freshly drawn heparinized whole blood by Ficoll-Hypaque flotation (Lymphoprep®), washed three times in Hanks' balanced salt solution (HBSS) and resuspended in RPMI 1640 (Gibco) with 10% v/v newborn calf serum (biocult).

Separate tubes with 10^6 blood mononuclear cells were incubated with each of the following monoclonal antibodies: Leu 4 (T-cells), Leu 2a ("T-suppressor/cytotoxic" cells), Leu 3a ("T-helper/inducer" cells), Leu 7 (killer + natural killer cells) (Becton Dickinson).

A second incubation was performed with $F(ab)_2$ fragments of FITC labelled rabbit anti-mouse immunoglobulin absorbed with human immunoglobulin. The cells were washed in HBSS three times after each incubation (6). Fluorescence microscopy was performed on the same day as the lymphocytes were prepared using a Carl Zeiss microscope with epiillumination. For each antibody two hundred cells were counted, and the proportion of cells with specific fluorescence was determined. The absolute number of cells in the subpopulation was calculated from the relative count and the routine leucocyte differential count. Control preparations included incubation without monoclonal antibody.

The Mann-Whitney rank sum test for unpaired data was used in the statistical evaluation of the results.

Auto-antibody screening

Auto-antibodies to smooth muscles, gastric parietal cells, adrenocortical cells and mitochondria were assessed using immunofluorescence. Further, auto-antibodies to thyroglobulin, thyroid microsomes and nuclear constituents were detected using hemaglutination tests and radio immunoassay. The total IgE was quantitated by a radio immunoassay technique. The mean of normal in our laboratory being 25 U/ml.

RESULTS

Auto-antibodies were found in 4 out of the 14 patients. Two had gastric parietal cell antibodies, two thyroid microsomal and adrenocortical antibodies. The total IgE was elevated from normal mean in 4 patients, however the values were within 1 SD.

The median percentage of circulating leu 2a, 3a, 4 and 7 positive cells was normal. Comparisons of median percentages of cell populations and median leu 3a/leu 2a ratios revealed no significant differences between total patient and normal controls (Table I).

We found no correlation between the presence of auto-antibodies or elevated total IgE and the distribution of lymphocyte subsets, the number of patients with these findings, however, was small.

DISCUSSION

Previous studies of patients with alopecia areata have shown a decreased percentage of T-cells detected with the E-rosette test (2, 7, 8, 3), but our findings of a normal percentage of leu 4 positive cells "T-cells" is in accordance with recent studies using monoclonal antibodies (MAB) (1, 4, 9, 10).

The data concerning the percentage of "T-helper/effector" cells and "T-suppressor/cytotoxic" cells in AA are conflicting. An early report, showing a high proportion of T-lymphocytes with receptors for IgG supposed to have suppressor cell functions (11) was confirmed in a study using MAB (9). However, other studies using MAB have demonstrated an unchanged (1) or even a decreased percentage of "suppressor/cytotoxic" cells (10, 4).

Our findings of a normal percentage of leu 2a, 3a and 4 positive cells are in agreement with a study comprising 60 patients with AA (1), which found a normal distribution of total T-cells, "T-suppressor/cytotoxic" cells and "T-helper/effector" cells. Furthermore, we

Table I. *Lymphocytes expressed as a percentage of total mononuclear cells in patients with alopecia areata*

AU = alopecia universalis, AA = alopecia areata, ND = not determined

	Diagnose	Leu 2a pos. cells	Leu 3a pos. cells	Leu 4 pos. cells	Leu 7 pos. cells	Leu 3a/2a ratio
1	AU	36	37	76	16	1.03
2	AA	22	41	66	ND	1.86
3	AA	30	24	50	ND	0.80
4	AA	24	42	60	ND	1.75
5	AA	34	19	52	32	0.56
6	AA	22	38	64	ND	1.73
7	AA	20	38	64	4	1.90
8	AA	22	43	67	8	1.95
9	AA	16	33	50	14	2.06
10	AA	25	48	80	2	1.92
11	AU	26	32	65	20	1.23
12	AA	36	42	70	10	1.17
13	AU	28	32	48	22	1.14
14	AA	26	52	78	10	2.00
Median of patients		26	38	65	12	1.70
Median of controls		24	43	66	10	2.10

detected a normal median percentage of leu 7 positive "killer" and "natural killer" cells (Table I). However, these normal phenotypical findings do not preclude a functional abnormality of the lymphocytes.

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