generalized endocrine disorder. From our study, DHEA is clearly the single most sensitive hormonal assay in this regard, while the addition of testosterone and androstenedione measurements does increase the discovery of patients with abnormally androgen levels. These patients with androgen abnormalities deserve thorough endocrinologic evaluation in an effort to treat both the acne and the endocrinological disorder.

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Spontaneous Cell-mediated Cytotoxicity (SCMC) in Patients with Alopecia Universalis

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Abstract. The spontaneous cell-mediated cytotoxicity (SCMC) of unfractionated lymphocytes was determined in 5 patients with alopecia universalis (AU). The target cells used were K562 and Chang cell lines. All patients had an increased SCMC as compared with age- and sex-matched donors. The results indicate that SCMC against K562 cells is more pronounced than that against Chang cells.

Human lymphocytes are known to express at least four kinds of cytotoxicity:

1. Cytotoxic activity of T lymphocytes previously sensitized against the target cells.

2. Cytotoxicity exerted by lymphocytes in the presence of humoral antibodies directed against relevant antigens on the target cells (antibody dependent cell-mediated cytotoxicity = ADCC).

3. Spontaneous cell-mediated cytotoxicity (SCMC) with no known presensitization history against the target cells, performed without addition of external antibodies. The lymphoid cells mediating this spontaneous or natural cell-mediated cytotoxicity have been termed natural killer (NK) cells.

4. Cytotoxicity induced by mitogens such as phytohemagglutinin (PHA) or concanavalin-A (Con-A). Lymphocytes activated by PHA or Con-A lyse target cells of allogeneic and syngeneic origin.

In our previous studies we found that the ADCC of non-fractionated lymphocytes was higher in patients with both alopecia areata (AA) and alopecia universalis than in normal controls (4). In the present study we looked for spontaneous cell-mediated cytotoxicity (SCMC) in patients with alopecia universalis.

MATERIALS AND METHODS

Patients and controls

Five patients with AU from our earlier study (4) were examined (Table I). The normal controls were matched according to age and sex.

Lymphocyte preparations

Lymphoid cells were separated from heparinized venous blood by gradient centrifugation on a layer of Ficoll-Isopaque. The interphase cells were collected and washed three times. Phagocytic cells were removed with a magnet after treatment of cell suspension with carbonyl iron. Then the lymphocytes were suspended in RPMI 1640 with 10% AB serum and glutamine.

Target cell lines

Two cell lines were used, viz. K562 derived from a human myeloid leukemia and Chang cells from human liver. The K562 were grown as a suspension culture in RPMI 1640 medium with 10% of inactivated fetal calf serum (FCS) and antibiotics. Chang cells were grown as monolayers in Eagle's minimal essential medium supplemented with Earle's salts (MEM), glutamine, 10% FCS, and antibiotics. The cell lines were maintained at 37°C in a humidified 5% CO_2 air atmosphere.

Assay of ^{51}Cr release

One million target cells suspended in 0.5 ml of RPMI 1640 containing 10% of FCS were labelled by incubation for 1 hr at 37°C with 0.075 ml of Na_2 $^{51}CrO_4$ (approx. 75 μCi, specific activity 250–500/μCi/μg) (The Radiochemical Centre, Amersham, England). They were then washed three times with buffered saline solution pH 7.2–7.4 (BSS) and resuspended in RPMI 1640 with 10% AB serum. Ten thousand labelled cells were transferred to Ellerman tubes, together with various numbers of lymphocytes. The final lymphocyte–target ratios (a/t ratio) thus obtained were 100:1, 50:1 and 25:1 respectively. The volume was adjusted to 0.6 ml with medium. To some tubes intended for measurements of spontaneous release, only target cells and medium were added. After centrifugation at 4 000 g for 10–12 sec, the cells were incubated for 4 h at 37°C followed by centrifugation for 45 sec at 2 500 *g*. The radioactivity of 0.2 ml of supernatant and the remaining 0.4 ml was determined with a gamma spectrometer and expressed as cpm.

A specific isotope release was calculated as % release with lymphocytes, reduced by per cent of spontaneous release. The spontaneous release did not exceed 21%. All the cultures were set up in duplicate.

RESULTS

The results of SCMC as expressed as a percentage of specific ^{51}Cr release from K562 and Chang cells in the presence of lymphocytes of patients with AA as well as of age- and sex-matched normal donors are shown in Table I.

It can be seen that there was a significantly higher SCMC in the patients with AA than in normal donors. This was true for both the above-mentioned cell lines. However, as far as Chang cells were concerned, the difference was significant only for highest lymphocyte–target cell ratio. Furthermore the significance was lower than that obtained with the corresponding ratio for K562 cells. Thus it seems that SCMC was more pronounced with K562 as target cells than with Chang cells.

DISCUSSION

The nature and mode of SCMC is not yet fully understood. Present evidence indicates that NK cells comprise several apparently different types of T and non-T non-B cells with Fc receptors for IgG. It was demonstrated that both the effector cells of ADCC and SCMC possess similar surface characteristics and it has been suggested that SCMC is partly an ADCC reaction, mediated by antibodies either arming the lymphocytes already in vivo or being produced during the assay (8). Thus addition of Fab fragments of immunosorbent-purified rabbit antibodies against human immunoglobulin inhibited the SCMC of HP-column-fractionated lymphocytes (Helix Pomatia haemagglutinin coupled to Sepharose 4B) in varying degree (7). On the other hand there is also substantial evidence that SCMC is not merely a special type of ADCC, as Fc receptors for IgG are not involved in the NK lytic mechanism although these receptors are present on NK cells (2). ADCC, on the other hand, requires these receptors. In mice, NK cells have their own markers (asialo-GM1 antigen) and it can be mentioned that nude mouse—an animal with a defect reminiscent of Alopecia Universalis—has high SCMC. Furthermore it was shown that SCMC and ADCC against cells growing in monolayers are mediated by different mechanisms, the former requiring monocyte help and the latter not (10). There is also a report on dissociation in the activity of ADCC and SCMC in Crohn's disease (1) and in kidney allograft recipients the NK cells were reported to be very sensitive to immunosuppressive therapy, whereas ADCC remained normal (9). In our studies, however, higher ADCC and SCMC were both found in the patients with AA. These findings were well in line with the view that ADCC and SCMC are closely related and that at least a part of SCMC may be an ADCC reaction.

There is evidence available suggesting that NK cells may recognize specific antigens on their target cells. In mice studies on the established cell lines and clones of NK cells proved that antigen receptors on NK cells are not clonally distributed and it is possible that NK cells either recognize common

Table 1. *SCMC of lymphocyte in AA patients and normal controls*
AU=alopecia universalis

Case no.	Sex	Age	Diagnosis	Chang cell line					
				Patient			Control		
				25: 1[a]	50: 1	100: 1	25: 1	50: 1	100: 1
1	F	52	AU	17[b]	26	39	0	0	4
4	M	19	AU	12	19	31	7	13	17
5	M	39	AU	31	43	64	15	29	28
7	M	31	AU	8	15	20	0	8	4
11	M	35	AU	8	10	20	3	6	9
Mean ±S. D.				15.2 ±9.6	22.6 ±12.8	34.8 ±18.2	5 ±6.3	11.2 ±10.9	12.4 ±10.2
P value				>0.05	>0.05	<0.05			

[a] Lymphocyte: target cell ratio.
[b] % of specific isotope release from target cells.

structures on NK-sensitive targets or else carry many receptors—each with a different specificity (3). The existence of two different types of SCMC, namely the monocyte dependent against target cells growing in monolayers and the monocyte independent against cells in suspensions suggests that the SCMC mechanism may vary with the type of target cell used in the assay (10). The quantitative difference between the results obtained with K562 and Chang cells might reflect these different SCMC mechanisms.

It has been reported that there is a general lack of SCMC in persons carrying HLA-A3, -B7 haplotype (1) and -B18, -B_w35 haplotype (9). As a high frequency of HLA-B12 was found in the patients with AA (6) and the patients examined by us had more pronounced SCMC than controls, it would be interesting to investigate whether there is any relation between genetic factors and SCMC in this disease.

It is probable that in this way one will be able to learn whether the findings reported in this paper are involved in the pathogenesis of AA, or are only a secondary phenomenon.

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K562 cell line					
Patient			Control		
25: 1	50: 1	100: 1	25: 1	50: 1	100: 1
37	41	44	8	17	35
36	48	54	19	44	56
29	30	52	0	12	14
38	44	66	0	11	19
24	30	57	14	24	34
32.8	38.6	54.6	8.2	21.6	31.6
±6.1	±8.2	±8	±8.4	±13.5	±16.4
<0.01	<0.025	<0.025			

Cytoplasmic Tubular Structures in the Diagnosis of Oral Discoid Lupus Erythematosus

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Abstract. Biopsies from oral lesions of 5 patients with discoid lupus erythematosus (DLE), 5 with lichen planus (LP), 5 with leukoplakia (LEUK) and 3 patients with uncertain diagnoses termed DLE?, LP? were examined in the electron microscope for presence of cytoplasmic tubular structures (CTS) in vascular endothelium. Five vessels were examined in each of two randomly selected sections from each biopsy, without knowledge of the clinical diagnosis. All five DLE biopsies showed presence of CTS in 4–9 out of 10 vessels, as compared with none of 10 vessels in biopsies from LP or LEUK, the difference being significant. Further, CTS occurred in 4 out of 10 vessels in one DLE?, LP? biopsy from a patient with serologic signs of systemic lupus erythematosus. As CTS seem to be absent in oral lesions of LP and LEUK, which are the most important differential diagnoses for oral DLE, the identification of CTS in vascular endothelium using electron microscopy may be an auxiliary diagnostic procedure in oral DLE.

Key words: Lupus erythematosus, discoid; Lichen planus; Leukoplakia; Oral manifestations; Tubular structures

Cytoplasmic tubular structures (CTS) in vascular endothelial cells is a characteristic feature of skin lesions of discoid lupus erythematosus (DLE) and systemic lupus erythematosus (SLE), but CTS may also occur in other conditions (1–3). The CTS have been reported under various terms, such as paramyxovirus-like structures (3), tubuloreticular inclusions (2) and intra-endothelial tubular aggregates (1). The origin of CTS is not known. Recently such structures have been described in oral lesions of DLE (5). The diagnostic significance of the presence of CTS in the oral mucosa is not known either.

The purpose of the present study was to examine the frequency of the presence of CTS in oral lesions of DLE, compared with the frequency of CTS in oral lesions of lichen planus (LP) and leukoplakia (LEUK), which are the most important differential diagnoses for oral DLE. Furthermore, the relation between presence of CTS and deposits of immunoglobulins at the basement membrane zone is examined.

MATERIAL AND METHODS

The material consisted of representative 5 mm punch biopsies from oral lesions of buccal mucosa of each of the following groups of patients: 5 DLE, 5 LP, 5 LEUK, and 3 DLE?, LP? Patient data are given in Table 1. The diagnosis was based on clinical and histopathologic features. The DLE patients were diagnosed as described earlier (4). The biopsies were randomly selected from among the 10 cases presented earlier (5). The LP patients all had lichen planus of the reticular type and the LEUK patients had homogeneous leukoplakia. Both groups fulfilled the WHO criteria (7). The patients designated DLE?, LP? had oral lesions which clinically as well as histopathologically had features common to DLE and LP, but a definite diagnosis could not be made. Skin lesions were absent in these patients.

Biopsies from all patients had at the same time been submitted for direct immunofluorescence (IF) study (16). The biopsies were processed for electron microscopy as previously described (5). From each biopsy two tissue blocks (termed A and B) were chosen and from each block

Table 1. *Patient data*

Diagnosis	*N*	Sex	Age in years, range
DLE	5	4 F, 1 M	31–40
LP	5	3 F, 2 M	38–81
LEUK	5	4 F, 1 M	29–74
DLE?, LP?	3	2 F, 1 M	27–69