SHORT REPORTS

Decreased Natural Killer Cell Activity in Lichen ruber planus

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In lichen ruber planus (LP), a chronic inflammatory mucocutaneous disease of unknown etiology, there are clinical and laboratory signs for some immunological derangements. Recent investigations stated a predominance of suppressor/cytotoxic T lymphocytes and Langerhans' cells at the dermo-epidermal interface in lesional skin (1-3). On the other hand a reduced percentage of the suppressor/cytotoxic (T8 positive) T cell subset was revealed in peripheral blood in patients suffering from LP (3, 4).

Natural killer cells (NK) represent a subpopulation of lymphoid cells present in a broad range of mammalian species. There is now compelling evidence that NK may serve as effector cells in host resistance due to their spontaneous cytotoxic activity against a variety of tumour cells, microorganisms and cells infected by microbial agents (5, 6). Furthermore, NK may also act as immunoregulatory cells (7).

The monoclonal antibody OKT8 is a common marker for lymphocytes possessing suppressor activities and for lymphocytes with cytotoxic capacities as well (7, 8). It is well known that a fraction of NK also express T8 antigens (8). The aim of the present study was to investigate in vitro NK activities of peripheral blood lymphocytes in patients with LP, possibly getting new information on the pathogenetic role of such activities in this disease.

MATERIAL AND METHODS

Patients

Fifteen patients (7 females, 8 males) with clinically and histologically confirmed LP of skin and/or oral mucosa (LP n=11, LP exanthematicus n=4) were included in the study. The median age of the patients was 34 years with a range of 18-58. No patient had low peripheral blood total lymphocyte counts or had received topical or systemic immunosuppressive treatment for at least 3 months. Eleven healthy sex- and age-matched volunteers served as controls.

Effector cells and cryopreservation

Peripheral blood mononuclear cells were separated on Ficoll-Hypaque gradients according to the method described by Böyum (9). Adherent cells were removed from the mononuclear cell suspensions with the help of a nylon wool column. Non-adherent cells were washed twice with RPMI 1640...
(Grand Island Biological Company, Grand Island, New York, USA) supplemented with 10% fetal calf serum (FCS), penicillin, streptomycin, L-glutamine and Hepes buffer, counted and frozen (−70 °C) in a medium consisted of 75% RPMI 1640 and 25% DMSO. Cells were thawed on the day when the cytotoxic assay was performed, washed three times and adjusted to the appropriate concentration in the aforementioned culture medium of RPMI 1640. The viability of the lymphocytes was determined by Trypan-blue exclusion technique.

Cytotoxicity assay
Effector cell populations were tested against 51Cr labelled K 562 human myeloid cell line targets as described previously (10). Briefly, tumour cells were labelled with 100 µCi 51Cr as Na2CrO4 (specific activity 300–500 Ci/g, Amersham Buchler, Braunschweig, FRG) for 60 min at 37 °C. Triplicate determinations were made in round bottomed microtiter plates. Target cell to effector cell ratios varied from 1:10 to 1:40 with 2 x 10⁴ targets/well in a total volume of 0.2 ml RPMI 1640 containing 10% FCS served as culture medium. After an incubation period of 4 h at 37 °C the activity of cell-free supernatants was counted in a gamma counter.

Calculation of cytotoxic indices
Spontaneous release (SR) was defined as the counts per min (cpm) released from targets incubated with medium alone. The SR of the targets ranged 5–10%. Maximal release (MR) was determined by measuring cpm in the supernatants after detergent lysis (1% Triton X 100) of target cells. The cytotoxic index (CI) was calculated using the following formula:

\[
CI = \frac{cpm_{test} - cpm_{SR}}{cpm_{MR} - cpm_{SR}} \times 100
\]

For statistical analysis of the results the Wilcoxon-Mann-Whitney test was used.

RESULTS
The results are summarized in Table I. In all groups cytolitic responses corresponded proportionally well with the increase of the effector cell concentrations, indicating a proper assay system. Non-adherent mononuclear cells isolated from peripheral blood of patients with LP exanthematicus and LP showed reduced NK activities compared with those of healthy controls. Differences in NK activities (CI), concerning patients with LP exanthematicus and LP in comparison to healthy controls proved not to be statistically significant (p>0.05).

Table I. NK activity (CI) of peripheral blood lymphocytes in patients with LP exanthematicus (n=4), LP (n=11) and in healthy controls (n=11)

<table>
<thead>
<tr>
<th>Target: effector cell ratio</th>
<th>1:10</th>
<th>1:20</th>
<th>1:40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.9</td>
<td>2.6</td>
<td>3.5</td>
</tr>
<tr>
<td>2</td>
<td>0.1</td>
<td>0.5</td>
<td>1.5</td>
</tr>
<tr>
<td>3</td>
<td>1.4</td>
<td>2.7</td>
<td>4.7</td>
</tr>
<tr>
<td>4</td>
<td>5.0</td>
<td>7.3</td>
<td>12.3</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>1.85±1.08</td>
<td>3.26±1.43</td>
<td>5.50±2.36</td>
</tr>
<tr>
<td>LP, mean ± SE</td>
<td>7.72±1.32</td>
<td>14.80±2.95</td>
<td>22.47±3.65</td>
</tr>
<tr>
<td>Controls, mean ± SE</td>
<td>13.65±2.17</td>
<td>25.25±3.12</td>
<td>37.55±4.14</td>
</tr>
</tbody>
</table>
DISCUSSION

Recent in vitro and in vivo studies demonstrated, that epidermal changes seen in LP consist of continuous destruction of basal keratinocytes (11, 12, 13). Examinations of different lymphocyte subpopulations in cutaneous inflammatory infiltrate of active LP revealed the existence of T lymphocytes of both the helper/inducer and the suppressor/cytotoxic phenotype (1, 2). However, the dermo-epidermal interface, from the pathogenetic point of view probably the most important zone, showed a predominance of suppressor/cytotoxic T lymphocytes and Langerhans’ cells as well (3). Furthermore, a significant lymphocytotoxic effect of peripheral blood lymphocytes was observed on syngeneic oral epithelial target cells in LP (14). All these findings support the concept that immunological mechanisms may be involved in the pathogenesis of this disease.

In the present study we could show that patients with LP possess reduced NK activities compared with those of healthy controls. NK activity can be generally influenced by several signals (5, 10). γ-Interferon and interleukin 2 are potent activators of NK function (15, 16), while prostaglandins (PGE1, PGE2, PGA1, PGA2) and a suppressor lymphocyte subset are able to inhibit such activities (17, 18). It is conceivable, that decreased NK activity found in patients with LP is probably only a secondary phenomenon due to either low numbers of NK in peripheral blood of these patients and/or to one of the above mentioned inhibitory factors, always present in chronic inflammatory processes. Further investigations are in progress to get closer information about the origin of the decreased NK activity revealed in our patients with LP.

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REFERENCES

Milk Causes a Rapid Urticarial Reaction on the Skin of Children with Atopic Dermatitis and Milk Allergy

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Open skin challenge test with whole milk and its large and small molecular fractions was performed on intact skin of children with atopic dermatitis and suspicion of milk allergy. Of the 51 children challenged with milk 35 reacted within minutes with contact urticaria. The large molecular (m.w.> 10 000 d) fraction gave an urticarial reaction as often as whole milk, whereas the small molecular fraction gave only a few positive reactions. These were obviously caused by alpha-lactalbumin which was present only in small amounts in the small molecular fraction. These findings indicate that immediate contact allergy to relevant food allergens can be very common in children with atopic dermatitis and that the large molecular antigens readily penetrate children's skin. Key words: Milk allergy; Contact urticaria; Allergens. (Received September 10, 1985.)

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The penetration of different chemicals into human skin has been widely studied. Most of the studies have concerned substances with relatively low molecular weight such as steroids and other drugs used for the treatment of skin diseases or substances capable of causing contact dermatis.

Contact urticaria can be induced on human skin either by immunological or non-immunological mechanisms. Non-immunological contact urticaria is often caused by small molecular weight substances such as benzoic, cinnamic and sorbic acids. The testing of these substances is often performed using a modified chamber test method for patch testing. On the other hand, open test can also be used (1). In both tests the optimum time of recording the results is from 10 to 40 min after application of the test substance. In the adult neither stripping nor scratching the skin enhances the reaction.