Dynamic Changes in the Epidermal OKT6 Positive Cells at Mild Irritant Reactions in Human Skin

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In the present study we induced mild irritant contact reactions by using 0.5% sodium lauryl sulphate (SLS) in distilled water or with distilled water in patch tests for 6 or 24 hours. The biopsies were taken at 6, 24, 48 and 96 hours. Light and electron microscopy were used to assess the irritant reactions produced and the monoclonal antibody OKT6 was used for the detection of the LCs. The number of the epidermal OKT6 positive dendritic cells was found to be increased at 48 and 96 hours after the exposure to SLS and at 96 hours in the water patch tests. It is concluded that mild irritant stimuli cause an increase in the LCs (OKT6 positive cells) and thus might influence and modulate the response to subsequent exposures to allergens. Key words: Patch tests; Electron microscopy; Monoclonal antibodies; Langerhans' cells. (Received June 14, 1985.)

MATERIAL AND METHODS

Material

Sixteen caucasians, 4 females and 12 males, with a mean age of 32 years (24-43) and without anamnestic or clinical signs of skin disease were included in the study. All had given their informed consent.

Irritant reactions

Irritant reactions were produced by using 0.5% sodium lauryl sulphate (SLS) in distilled water or with distilled water in patch tests according to Pirilä (15) (Finn Chambers, Epitest Ltd Oy, Helsinki.
Table I. Time schedule for the experimental design
0 h = the application of the patch tests

<table>
<thead>
<tr>
<th>Number of volunteers</th>
<th>Time for removal of patch tests (h)</th>
<th>Time for biopsies (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>24</td>
<td>24</td>
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<tr>
<td>4</td>
<td>24</td>
<td>96</td>
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Finland). Each person was tested with both SLS and water. The patch tests were applied for 6 or 24 hours (Table I) on the outer aspect of the thigh.

Biopsies
Following local anesthesia (intradermal injection of 0.1–0.2 ml of Xylocain® 10 mg/ml, ASTRA, Södertälje, Sweden) the punch biopsies (diameter 4 mm) were obtained from 4 persons at each interval (Table I). One biopsy was taken from the center of each patch test and a control biopsy was taken just outside the tested area. The biopsies were divided for further processing.

Assessment of OKT6 positive cells
The specimens were immediately frozen and stored at −70°C. Freeze sections were cut at −20°C to −30°C. The sections were thawed and exposed to a 1/100 dilution of OKT6 monoclonal antibodies (Ortho Diagnostic System Inc., Raritan, NJ, USA) and the antibodies were visualized with an avidin-biotin-immunoperoxidase assay (Vectastain® ABC kit, Vector Laboratories Inc., Burlingame, Calif., USA) as previously described (16). The sections were examined in a light microscope using the 400 power field. The total number of OKT6 positive cells as well as the distribution of the cells in the upper and lower half of the epidermis were counted per 4 mm length of interfollicular epidermis. Each dendritic OKT6 positive cell with a distinguishable nucleus was included in the count.

Light microscopy
Freeze sections from the biopsies used for the OKT6 staining were fixed with formaldehyde, dehydrated in ethanol and stained with haematoxylin and eosin.

Transmission electron microscopy
Biopsies from 6 and 24 hours were fixed with 2.5% glutaraldehyde in a 200 mosmol phosphate buffer, postfixed in 2% osmiumtetroxide in distilled water for 1 hour, dehydrated, and embedded in Epon. The ultrathin sections were stained with lead citrate and uranyl acetate and were viewed in a Philips EM 301 G at 60 kV.

Statistical methods
In the analysis of the quantitative results Student's paired t-test was used.

Table II. The total number of epidermal OKT6 positive cells in biopsies from control and tested skin at different time intervals
Mean and (SD) are given.

<table>
<thead>
<tr>
<th>Time</th>
<th>Controls</th>
<th>Water tests</th>
<th>SLS tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 h</td>
<td>41.5 (7.3) n=4</td>
<td>51.0 (10.8) n=4</td>
<td>59.8 (14.2) n=4</td>
</tr>
<tr>
<td>24 h</td>
<td>41.2 (8.8) n=4</td>
<td>65.8 (7.8) n=4</td>
<td>65.8 (5.8) n=4</td>
</tr>
<tr>
<td>48 h</td>
<td>39.0 (6.8) n=4</td>
<td>60.5 (14.5) n=4</td>
<td>64.6 (5.9)* n=4*</td>
</tr>
<tr>
<td>96 h</td>
<td>33.0 (2.2) n=4</td>
<td>50.3 (1.5)* n=3</td>
<td>51.3 (4.6)* n=4*</td>
</tr>
</tbody>
</table>

* p<0.01 compared to controls.
RESULTS
At the time of biopsy a mild erythematous reaction was seen at 24 and 48 hours in the SLS test sites and at 24 hours (1 person) and 48 hours (1 person) after distilled water exposure. A sparse cellular infiltrate was present in the dermis at all intervals both at SLS and at water exposure sites. At 24, 48, and 96 hours a few migrating cells were also observed in the lower part of the epidermis. There was a dilatation of the rough endoplasmatic reticulum, a finding which appeared more pronounced following the application of water.

The result of the quantitative analysis is given in Table II. A significant increase (p<0.01) in the number of the OKT6 positive epidermal cells was found at 48 and 96 hours after the SLS challenge. This was also found at 96 hours following exposure to water. Compared to the controls, in biopsies from the SLS and water test sites the OKT6 positive cells were more frequently found in the upper half of the epidermis (Fig. 1).

DISCUSSION
In the present study mild irritant stimuli were produced by applying patch tests with 0.5% SLS or with distilled water. Clinical assessment, light and electron microscopic evaluations confirmed that the reactions induced were weak.

The irritant reactions were associated with an increase in the number of the epidermal OKT6 positive cells with a concurrent intraepidermal redistribution of the cells with a polarisation towards the surface of the skin. These changes persisted for at least 3 days after the removal of the stimuli. For the quantitative analysis the OKT6 monoclonal antibody was utilized as it has been considered to have a high specificity for the epidermal LCs even under pathological conditions (14).

The changes in the OKT6 positive cell population within the epidermis may represent a passive “shedding” of defect cells in combination with a migration of cells from dermis. However, we found only minor ultrastructural changes in the LCs after the exposure to SLS and water for 6 and 24 hours. A more probable explanation is that irritant stimuli of the magnitude applied in this study cause a non-specific increase in the number of both inflammatory and immuno-competent cells. Such a hypothesis is in accordance with a recent analysis of contact reactions (13). It was suggested that the pattern of the cellular...
response, including that of the LCs, found at irritant and allergic contact reactions represents an increased readiness for further immune reactions.

In contrast to our findings, repeated applications of SLS have been reported to produce marked degenerative changes in the LCs and to reduce the total number of LCs in the epidermis as monitored by the L-dopa fluorescence method (9). At irritant reactions induced by dithranol in patch tests the number of LCs (OKT6 positive cells) was found to be unaltered (12). At these reactions the LCs displayed a combination of ultrastructural alterations which were interpreted as being signs of both degeneration and activation. It is thus probable that the initial response of the LC population at contact with irritant stimuli will depend on the degree of the cell injury inflicted.

We conclude that stimuli producing sub-clinical irritant contact reactions affect the LCs in such a way that the antigen processing capacity might increase and that the stimuli subsequently could influence and modulate the skin response at following exposures to allergens.

ACKNOWLEDGEMENTS

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REFERENCES