Effects of PUVA and Mechlorethamine Treatment of Psoriatic Patients on Epidermal Langerhans’ Cells

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Using OKT6 monoclonal antibody, we investigated the number of epidermal Langerhans’ cells (LCs) in involved skin from patients with psoriasis, before and after mechlorethamine (HN$_2$) or PUVA treatment. The number of LCs remained at about pretreatment number during three weeks of HN$_2$ treatment alone, though they were reduced after 10 systemic PUVA treatments. Therefore, in contrast to PUVA which influences LCs, HN$_2$ seems to have little effect on LCs. LCs in psoriatic plaques were, in number, 3-4 times less numerous than those in uninvolved, nontreated epidermis. Key words: Langerhans’ cells; Psoriasis; PUVA; Mechlorethamine. (Received April 3, 1987.)


Both PUVA therapy and topically applied mechlorethamine (nitrogen mustard; HN$_2$) (1), an alkylating agent among cytotoxic drugs, have been demonstrated to be effective in the treatment of psoriasis. The topical HN$_2$ can be easily applied by the patient himself, but most persons eventually develop contact hypersensitivity to the compound. In those patients, sensitization to HN$_2$ is prevented by PUVA (2, 3). Mechanisms possibly responsible for these results are the reduction of Langerhans’ cells (LCs) in the skin after UV radiation and/or induction of antigen-specific suppressor cells (4).

In the present study, we investigated the first possibility. Quantitative distribution of LCs in involved psoriatic epidermis was studied, before and after treatment of PUVA or HN$_2$. LC enumeration was also performed in uninvolved skin of patients.

MATERIAL AND METHODS

Patients and treatment schedules

Twenty-six patients with stationary psoriasis were included in the study. They were arranged into two groups. Five patients (group A) were treated with HN$_2$ alone. Twenty-one patients (group B) were treated with systemic PUVA followed by HN$_2$.

Group A. A freshly prepared aqueous solution of 0.02% HN$_2$ was applied daily to the skin lesions.

Group B. Ten systemic PUVA treatments (three times per week) were given before initiation of the daily applications of HN$_2$, as previously described (3). Subsequently, HN$_2$ alone was applied.
Skin biopsies

Biopsies from involved psoriatic skin (extensor surface of forearms) were performed before and after 10 PUVA treatments, and at various times during HN₂ treatment. Adjacent uninvolved psoriatic skin was also biopsied before treatment in 8 patients. Biopsies were immediately frozen in liquid nitrogen and stored in -70°C until used.

Indirect immunofluorescence

Monoclonal antibody OKT6 (Ortho Pharmaceutical Co., Raritan, NJ, USA), which is known to be specific for epidermal LCs, was used in this study (working dilution 1:3). Frozen sections of 4 μm were prepared, air-dried and fixed in acetone for 10 min at 4°C. Immunofluorescent staining was performed as follows: First-layer antiserum were added and the sections incubated for 45 min at 37°C. They were then washed in phosphate-buffered saline (pH 7.2, 2x15 min). Fluorochrome-conjugated second-layer antiserum (Nordic Immunology, goat anti-mouse lg labelled with FITC, dilution 1:20) was added next, incubated for 30 min at 37°C, and washed as above. Slides were mounted in buffered glycerin and were examined under a Zeiss fluorescent microscope.

Quantification technique

The method of quantitative evaluation of LCs in skin sections has been described previously (5). Briefly, epidermal LCs were specifically labelled by OKT6 antibody using the technique described above. Positive cells were counted by means of an ocular square grid covering, under 40x objective magnification, 0.0625 mm² of skin section surface. A total of 40 to 60 adjacent grid fields of epidermal sections were examined on 2 slides, each bearing 4 skin sections. Only dendritic cells exhibiting a bright fluorescence and a dark nucleus were counted as positive: isolated dendrites and partially fluorescent cells without dendrites were not scored.

Statistical analysis

This was performed using Student’s t-test and Mann-Whitney’s test, with p<0.05 indicating statistical significance.

RESULTS

The data in the two groups are summarized in Table I. The average number of days until biopsy, during the course of HN₂ therapy, were 20 days. In group A, two patients became sensitive within the first 4 weeks of HN₂ treatment (mean number of days, 26). Unfortunately, skin biopsy specimens were not obtained from these two patients just before and after onset of hypersensitivity.

Table II shows LC enumeration noted in involved psoriatic skin before and after treatment. The number of LCs in group A remained at about pretreatment values even during HN₂ treatment. In group B, they fell from the pretreatment value of 25.0±9.0/mm² (mean ± SD) to 14.7±4.7/mm² after 10 exposures of PUVA.

We also enumerated the number of LCs in uninvolved psoriatic skin (8 patients) before treatment: OKT6(+) cells, 92.7±19.1/mm² (mean ± SD).

Table I. Characteristics of the two patient groups

<table>
<thead>
<tr>
<th>No. of pat.</th>
<th>Therapy</th>
<th>No. of patients biopsied</th>
<th>Mean days of HN₂ treatment until biopsy</th>
<th>No. of patients sensitive within first 4 weeks of HN₂ treatment</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before therapy</td>
<td>After 10 PUVA</td>
<td>During HN₂</td>
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<tr>
<td>Group A</td>
<td>5</td>
<td>HN₂ alone</td>
<td>5</td>
<td>–</td>
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<tr>
<td>Group B</td>
<td>21</td>
<td>PUVA prior to HN₂</td>
<td>21</td>
<td>18</td>
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</tbody>
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Table II. LC enumeration in involved psoriatic skin

<table>
<thead>
<tr>
<th>No. of patients biopsied</th>
<th>Mean days of HN₂ treatment until biopsy</th>
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<tr>
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<td>Group B</td>
<td>21</td>
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</tbody>
</table>
Table II. Enumeration of Langerhans’ cells in involved psoriatic skin

Results: mean ± SD/epidermal section surface unit (1 mm²); n = number of patients biopsied

<table>
<thead>
<tr>
<th>Before treatment</th>
<th>After treatment</th>
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<tr>
<td></td>
<td>Group A</td>
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<td></td>
<td>At mean 20 applications</td>
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<td>of HN₂ (n=5)</td>
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<td>Before treatment</td>
<td>25.0±9.0</td>
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<tr>
<td>Statistical significance</td>
<td>NS*</td>
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<td>between pretreatment vs.</td>
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<td>posttreatment</td>
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* Mann-Whitney’s test. ** Student’s t-test.

DISCUSSION

In the present study, epidermal LCs of psoriatic plaques remained at about the level of pretreatment values during the course of HN₂ treatment alone, though they were decreased after 10 PUVA as reported by Baker et al. (6). We consider that HN₂ treatment has little effect on LCs.

On the other hand, the cellular infiltrate of histiocytosis X, which is composed of LCs, is reduced by topical HN₂ (7). Tumour cells of histiocytosis X are probably more sensitive to HN₂ than normal LCs. Halliday et al. (8) reported that azathioprine, another cytotoxic drug, caused a reduction in murine LCs when administered topically. The disparity of the effect on LCs between our study and that of Halliday et al. (8) might be due to the difference in methodology and drug used: 0.02% HN₂ in human; 1% azathioprine in mice.

Topically applied HN₂ has been demonstrated to be effective in the treatment of psoriasis (1), alike PUVA therapy. However, the commonest complication of HN₂ therapy is the development of allergic contact dermatitis. It is generally accepted that the epidermal LCs plays a central role in the contact hypersensitivity reaction (9). A local paucity of LCs reduces or modifies the processing and presentation of antigens coming in contact with these sites. This contention is supported by the hyporesponsiveness of transplanted tail grafts of C57BL/6 mice sensitized with dinitrofluorobenzene (10). Patients with psoriasis treated by PUVA were significantly less responsive to dinitrochlorobenzene than subjects untreated (11). Recent studies indicate that the staining intensity and number of epidermal LCs is increased in allergic contact dermatitis test sites compared to adjacent normal skin (12, 13). These results suggest that LCs density determines the induction of contact hypersensitivity. In our study, the number of LCs in adjacent uninvolved skin (OKT6: 92.7±19.1/mm²) is 4 times higher than that of involved skin (OKT6: 25.0±9.0). The similar results have been reported (5, 14). There is a possibility for patients to apply HN₂ even on the uninvolved skin adjacent to involved lesion. We may speculate that patients are sensitized to HN₂, in part, via adjacent uninvolved skin, that has a higher number of LCs than involved skin.

REFERENCES


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Solid Facial Edema as a Complication of Acne vulgaris: Treatment with Isotretinoin and Clofazimine

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We present two patients, a 20-year-old female and an 18-year-old male, who suffered from persistent solid facial edema as a complication of acne vulgaris. They were treated with isotretinoin with moderate response and thereafter with lymph massage with further response. The female patient also received clofazimine with good response. (Received February 27, 1987.)

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Persistent solid facial edema, as a complication of acne vulgaris is rarely seen and infrequently reported in the literature (1, 2, 3). It consists of periorbital, centrofacial, occasionally erythematous nonpitting swelling.

We present two patients, a 20-year-old female and an 18-year-old male, who were treated with isotretinoin with moderate response and thereafter with lymph massage with good response. The female patient also received clofazimine with moderate response.