8. Streilein JW, Tigelaar RE. SALT: Skin-associated lymphoid tissues. In: Parrisch J, Kripke ML, Morison L, eds. Photoimmunology. New York and London: Plenum Medical, 1983: 95–132.
9. Nestor MS, Cochran AJ, Ahmed AR. Mononuclear cell infiltrates in bullous disease. J Invest Dermatol 1987; 88: 172–175.
10. Drijkoningen M, De Vos R, De Wolf-Peeters C, Degreef H, Desmet V. Immunoelectron microscopic investigation of HLA-DR-positive dendritic cells at the dermo-epidermal junction in skin disorders associated with the deposition of immune complexes. Ultrastruct Pathol 1986; 10: 123–128.
11. Lindberg M, Emtestam L. Dynamic changes in the epidermal OKT6 positive cells at mild irritant reactions in human skin. Acta Derm Venereol (Stockh) 1986; 66: 117–120.
12. Berman B, Chen VL, France DS, Dotz WI, Petroni G. Anatomical mapping of epidermal Langerhans' cell densities in adults. Br J Dermatol 1983; 109: 553–558.

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.)

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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 HN_2 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 1:5). 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, 2×15 min). Fluorochrome-conjugated second-layer antiserum (Nordic Immunology, goat anti-mouse Ig-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 40× objective magnification, 0.0625 mm^2 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 HN_2 therapy, were 20 days. In group A, two patients became sensitive within the first 4 weeks of HN_2 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 HN_2 treatment. In group B, they fell from the pretreatment value of 25.0±9.0/mm^2 (mean ± SD) to 14.7±4.7/mm^2 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/mm^2 (mean ± SD).

Table I. *Characteristics of the two patient groups*

	No. of pat.	Therapy	No. of patients biopsied			Mean days of HN_2 treatment until biopsy	No. of patients sensitive within first 4 weeks of HN_2 treatment
			Before therapy	After 10 PUVA	During HN_2		
Group A	5	HN_2 alone	5	–	5	20 (range 15–28)	2
Group B	21	PUVA prior to HN_2	21	18	–	–	0

Table II. *Enumeration of Langerhans' cells in involved psoriatic skin*
Results; mean ± SD/epidermal section surface unit (1 mm^2); n = number of patients biopsied

	After treatment	
	Group A	Group B
Before treatment (n=26)	At mean 20 applications of HN_2 (n=5)	After 10 PUVA (n=18)
25.0±9.0	24.1±8.5	14.7±4.7
Statistical significance between pretreatment vs. posttreatment	NS*	$p<0.001$**

* 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 HN_2 treatment alone, though they were decreased after 10 PUVA as reported by Baker et al. (6). We consider that HN_2 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 HN_2 (7). Tumour cells of histiocytosis X are probably more sensitive to HN_2 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% HN_2 in human; 1% azathioprine in mice.

Topically applied HN_2 has been demonstrated to be effective in the treatment of psoriasis (1), alike PUVA therapy. However, the commonest complication of HN_2 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/mm^2) 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 HN_2 even on the uninvolved skin adjacent to involved lesion. We may speculate that patients are sensitized to HN_2, in part, via adjacent uninvolved skin, that has a higher number of LCs than involved skin.

REFERENCES

1. Epstein E, Ugel AR. Effects of topical mechlorethamine on skin lesions of psoriasis. Arch Dermatol 1970; 102: 504–506.
2. Halprin KM, Comerford M, Presser SE, Taylor JR. Ultraviolet light treatment delays contact sensitization to nitrogen mustard. Br J Dermatol 1981; 105: 71–76.

3. Mauduit G, Silvestre O, Thivolet J. PUVA therapy prevents sensitization to mechlorethamine in patients with psoriasis. Br J Dermatol 1985; 113: 515–521.
4. Horio T, Okamoto H. Immunologic unresponsiveness induced by topical applications of hapten to PUVA-treated skin in guinea pigs. J Invest Dermatol 1983; 80: 90–93.
5. Haftek M, Faure M, Schmitt D, Thivolet J. Langerhans cells in skin from patients with psoriasis: Quantitative and qualitative study of T6 and HLA-DR antigen-expressing cells and changes with aromatic retinoid administration. J Invest Dermatol 1983; 81: 10–14.
6. Baker BS, Swain AF, Griffiths CEM, Leonard JN, Fry L, Valdimarsson H. Epidermal T lymphocytes and dendritic cells in chronic plaque psoriasis: the effects of PUVA treatment. Clin Exp Immunol 1985; 61: 526–534.
7. Thivolet J, Cambazard F, Euvrard S, Hermier C, Kanitakis J. Histiocytose langerhansienne cutanée isolée. Amélioration clinique et immunohistologique après applications locales de moutarde à l'azote. Ann Dermatol Vénéréol 1984; 111: 765–766.
8. Halliday GM, Knight BA, Muller HK. Reduction in murine Langerhans cell ATPase staining following topical but not systemic treatment with steroid and non-steroid immunosuppressants. Br J Dermatol 1986; 114: 83–89.
9. Stingl G, Katz SI, Clement L, Green I, Shevach EM. Immunologic functions of Ia-bearing epidermal Langerhans cells. J Immunol 1978; 121: 2005–2013.
10. Streilein JW, Toews GB, Bergstresser PR. Langerhans cells: Functional aspects revealed by in vivo grafting studies. J Invest Dermatol 1980; 75: 17–21.
11. Moss C, Friedmann PS, Shuster S. Impaired contact hypersensitivity in untreated psoriasis and the effects of photochemotherapy and dithranol/UV-B. Br J Dermatol 1981; 105: 503–508.
12. Christensen OB, Daniels TE, Maibach HI. Expression of OKT6 antigen by Langerhans cells in patch test reactions. Contact Dermatitis 1986; 14: 26–31.
13. Mackie RM, Turbitt ML. Quantification to dendritic cells in normal and abnormal human epidermis using monoclonal antibodies directed against Ia and HLA antigens. J Invest Dermatol 1983; 81: 216–220.
14. Ranki A, Lauharanta J, Kanerva L. Effect of etretinate on the distribution of Langerhans cells and T lymphocytes in psoriatic skin. Arch Dermatol Res 1984; 276: 102–104.

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

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Helander I, Aho HJ. Solid facial edema as a complication of acne vulgaris: treatment with isotretinoin and clofazimine. Acta Derm Venereol (Stockh) 1987; 67: 535–537.

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.