Infiltration of Activated Eosinophils in the Skin Lesions of Atopic Dermatitis

Sir,

Eosinophils infiltrating atopic skin lesions are thought to be involved in or play an important role in the formation of atopic eczema (1). It has been reported that serum eosinophil cationic protein (ECP) is higher in infants and young children with atopic dermatitis than in control children (2). However, Kondo *et al.* (3) reported that no activation of eosinophils was noted in skin lesions of atopic dermatitis in infants. In this context, involvement of activated eosinophils in the skin lesions of atopic dermatitis is still controversial and whether or not the eosinophils are activated in atopic skin lesions has to be clarified.

In the present study, we investigated the number of activated eosinophils, both in the acute and subacute skin lesions of atopic dermatitis, using a monoclonal antibody, EG2, to the activated form of eosinophil. Findings revealed that the majority of eosinophils infiltrating eczematous lesions of atopic dermatitis were activated.

MATERIAL AND METHODS

Ten patients with atopic dermatitis, 9 men and 1 woman (aged 15–35 years; mean 24.8 years), diagnosed according to criteria of Hanifin & Rajka (4) were included in the present study. After informed consent was given, lesional skin biopsies were obtained from the eczematous skin lesions with lichenification on the extremities of each patient. According to histopathological findings, the biopsy specimens were divided into 2 groups. The "acute type" group consisted of 3 patients in which inflammatory cell infiltration in the epidermis and spongiosis of the epidermis predominated. The "subacute type" group consisted of 7 patients showing mainly acanthosis of the epidermis and chronic inflammatory cell infiltration in the dermis. At the time of skin biopsy, eosinophil numbers in the peripheral blood and ECP and IgE concentrations in the serum were examined. As controls, normal human skin samples were obtained during the surgical removal of benign skin tumors in 8 non-atopic, healthy individuals after informed consent was received.

The mouse monoclonal antibodies EG1 (Pharmacia, Uppsala, Sweden), an antibody to eosinophil extract that recognizes both activated and non-activated eosinophils, and EG2 (Pharmacia, Uppsala, Sweden), an antibody to the secreted form of eosinophil cationic protein, that recognizes only activated eosinophils (5), were used. After blocking endogenous peroxidase activity, 4-μm sections from the paraffin-blocks were incubated with EG1 diluted 1/150 or EG2 diluted 1/300 for 24 h at 4°C. Negative controls were performed with normal mouse IgG1 (DAKO A/S, Denmark). The sections were then incubated with biotin-conjugated rabbit anti-mouse immunoglobulins (DAKO A/S, Denmark) diluted 1/100 for 30 min followed by avidin mixed with biotin-conjugated peroxidase for 30 min (Vector Laboratories, Inc., Burlingame, CA). Color developed with incubation in 3,3′-diaminobenzidine solution containing 0.01% hydrogen peroxide.

Positively-stained eosinophils were counted in areas 3 mm^2 in size from the central portion of each immunostained section by superimposing a square grid over the dermis under 100× magnification.

RESULTS

Results of the eosinophil counts in the skin lesions, eosinophil counts in the peripheral blood and serum ECP and serum IgE concentrations are summarized in Table I. No immunostaining was observed in the negative control sections with normal mouse IgG1. Remarkably large numbers of EG2-positive activated eosinophils in acute lesions (34.0 ± 34.8 per 3 mm^2), and in subacute lesions (10.8 ± 7.6 per 3 mm^2) were observed compared with the number of activated eosinophils in the normal human skin controls (2.4 EG2-positive cells/3 mm^2 in 8 normal skin specimens). No significant differences in the numbers of activated eosinophils were observed in the skin lesions of the acute type and subacute type groups. The numbers of activated eosinophils in the skin lesions did not significantly correlate with eosinophil numbers in the peripheral blood, ECP concentrations, or IgE concentrations in the serum.

DISCUSSION

Several studies have shown a positive correlation between the eosinophil counts in the peripheral blood and the disease activity of atopic dermatitis (reviewed in (1)). In atopic dermatitis, it is hypothesized that eosinophils may be activated via several pathways including IL-5 produced by Th2 cells or by eosinophils themselves. Also the binding of IgE to high affinity IgE receptors on the cell surface of eosinophils may be involved in eosinophil activation. Eosinophil granule proteins secreted by activated eosinophils are thought to cause tissue damage and further inflammatory reactions (1). Eosinophils degenerate in the skin lesions of atopic dermatitis, presumably releasing eosinophil granule proteins (6), and are thought to be capable of degranulating mast cells. In fact,

Table I. *Results of the present study*

Skin lesions	Eosinophils in blood/ml	IgE (U/ml)	Eosinophil cationic protein (μg/l)	EG1-positive cells/3 mm^2	EG2-positive cells/3 mm^2
1. Acute	696	810	13.5	55.6	83.3
2. Acute	2,320	16,000	53.5	12.6	9.0
3. Acute	924	370	54.7	11.3	9.6
4. Subacute	604	800	33.4	14.6	5.3
5. Subacute	2,047	16,000	90.9	7.3	5.6
6. Subacute	1,960	8,600	48.4	14.3	12.6
7. Subacute	593	510	47.0	22.0	17.0
8. Subacute	750	16,000	10.7	1.0	1.0
9. Subacute	258	13,000	ND	12.3	11.0
10. Subacute	696	16,000	ND	23.0	23.0

ND; not done.

eosinophil granule proteins, reportedly, are distributed extensively in the lesional dermis in atopic dermatitis (7).

In the present study, we clearly demonstrated that large numbers of eosinophils, seen in both the acute and the subacute skin lesions of atopic dermatitis, are activated. Our present results may provide further evidence for eosinophil involvement in the formation of both acute and subacute skin lesions in atopic dermatitis.

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Acral Darier's Disease Successfully Treated with Adapalene

Sir,

Acral involvement is present in over 90% of the cases in Darier's disease, including keratotic papules, nail dystrophy, palmar pits and lesions closely resembling acrokeratosis verruciformis. Isolated papular acral lesions may represent an early and mild feature of the disease. However, reports in which acral lesions are the only manifestation are very rare: we have found only 4 reported cases in the literature (1–4). Therapeutic approach to Darier's disease with topical or systemic retinoids is usually effective, but these treatments are not always well tolerated (5). We report a case of isolated acral Darier's disease successfully treated with adapalene 0.1% gel.

CASE REPORT

A 22-year-old girl showed multiple, moderately itchy, keratotic papules on the back of both hands persisting since she was 10 years old. The slightly raised, skin-coloured, warty papules, 3–5 mm in diameter, associated with hyperkeratosis of the soles, worsening during summer season, were the only skin manifestations (Fig. 1). Nail changes and oral lesions were absent. A biopsy specimen from a papular lesion revealed hyperkeratosis, focal parakeratosis and suprabasal acantholytic lacunae and dyskeratotic cells with formation of round bodies and grains. An ultrastructural study showed intraepidermal suprabasal lacunae with reduced or absent desmosomal structures and altered desmosomal tonofilaments. In the granular layer, keratinocyte perinuclear vacuolization with peripheral tonofilament margination (round bodies) and subcorneal keratinocytes with intracytoplasmatic tonofilament aggregates (grains) were observed, suggesting Darier's disease features. No changes characteristic of this condition were observed in the family of this patient. Systemic retinoids were not administered due to the limited disease extension and the young age of the girl. Since treatment with topical steroids was ineffective and topical isotretinoin was too irritating, we started therapy with adapalene gel 0.1% once at bedtime. On the soles application of adapalene gel was preceded by a 7-day course of keratolytic topical therapy. Hand lesions cleared after 4 weeks of therapy, and the soles after 6 weeks of therapy, without any side effects. Application of adapalene was stopped after 2 more months of maintenance therapy without relapse. One month later a mild hyperkeratosis of the soles slowly appeared, which promptly resolved after a new cycle of therapy.

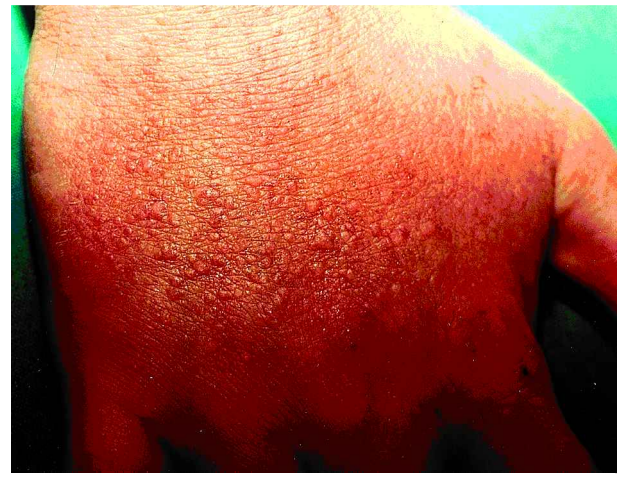

Fig. 1. Acral Darier's disease: hyperkeratotic papules on the back of the left hand before therapy.

DISCUSSION

Acral keratotic papules are frequently seen on the dorsa of hands and on the soles in Darier's patients; however, acral papules without other cutaneous manifestations are definitively rare (3–5). Our patient's family members showed no evidence of Darier's disease and the evolution of skin lesions during the years was steady. The acral Darier's disease first described in 1988 has been suggested as frusted form of Hopf acroker-