Acta Derm Venereol (Stockh) 1989; Suppl 144: 64–66

Langerhans Cell Antigen Presentation and Interleukin-1 Production in Atopic Dermatitis

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We studied Langerhans cell (LC) antigen-presenting capacity and epidermal cell and monocyte interleukin-1 (IL-1) production in 23 atopic dermatitis (AD) patients and 24 healthy controls. Six of the atopics had previously had severe disseminated cutaneous Herpes simplex virus (HSV) type 1 infection but the HSV-induced T cell proliferation was intact in these patients. Five of the AD patients were allergic to birch pollen and had experienced exacerbations of their eczema during the pollen season. The birch pollen induced specific T cell proliferation in two of these 5 AD patients and one of these two also showed a positive patch test reaction to birch pollen. Epidermal cells and monocytes of AD patients produced significantly less IL-1 than those of healthy controls. Further studies are needed to examine whether the impaired IL-1 production in AD is due to a primary defect or results from mediators such as prostaglandins and histamine. ***Key words: Interleukins; Birch pollen; Herpes simplex virus; Atopic eczema.***

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Hereditary and environmental factors and immunological abnormalities play a role in the etiopathogenesis of atopic dermatitis (AD). Recently both type I and type IV hypersensitivity reactions have been connected with AD, and epidermal Langerhans cells (LC) may participate in the development of clinical symptoms of AD (1–5). LC present antigens to T cells (6) and both LC and keratinocytes are capable of releasing immunomodulatory cytokines such as interleukin (IL-1), which contributes to the activation of a variety of cells (7). We therefore examined whether LC functions and the ability of epidermal cells and monocytes to produce IL-1 are abnormal in patients with AD.

MATERIALS AND METHODS

Subjects

The study populations consisted of 23 AD patients and 24 healthy subjects. Within the last year, six of the patients had had at least one severe disseminated cutaneous Herpes simplex virus type I (HSV-1) infection. Five AD patients were included in the study because they had experienced exacerbations during the birch pollen season. All these subjects had positive prick tests and RAST to birch pollen and the proliferation tests were performed in the pollen-free season.

Isolation of cells

Mononuclear cells were obtained by Ficoll-Isopaque centrifugation of venous blood. These cells were used for isolation of T lymphocytes and monocytes. Monocytes were purified on the basis of their adherence to plastic surfaces and T lymphocytes by rosette formation with aminoethylisothiouronium bromide-treated sheep erythrocytes as described before (8). The average purity of monocytes was 94% as assessed by nonspecific esterase staining. T cell populations contained $<0.2\%$ monocytes, $<5\%$ OKT7-positive cells (B lymphocytes) and $>90\%$ OKT3-positive cells (T lymphocytes).

Suction blisters were raised on the uninvolved abdominal skin of patients and healthy subjects. Epidermal sheets were treated with 0.25% trypsin and 0.01% DNase I to obtain crude epidermal cells. Langerhans cells were isolated by attaching them to IgG-coated erythrocyte monolayers in tissue culture dishes (9). Crude epidermal cells contained on the average 2.4% LC and the purity of LC-enriched cells was about 80% as assessed on the basis of OKT6-positive cells.

Langerhans cell – T lymphocyte cultures

T lymphocytes supplemented with 5% LC were stimulated in cultures with HSV-1 (Behringwerke AG, Marburg, FRG) and birch pollen (Aquagen-SQ, Allergologisk Laboratorium A/S, Copenhagen, Denmark) antigens. The cells were suspended in 20% autologous plasma-RPMI 1640 at a density of 0.25×10^6 cells/ml and 0.1-ml volumes of this suspension were pipetted per well of V-bottomed microplates. Thereafter RPMI 1640 (controls) or various concentrations of HSV-1 and birch pollen antigens in RPMI 1640 were added to the plates. The cultures were terminated after 6 days of incubation. Sixteen hours before harvesting 0.125% μCi of iododeoxyuridine was added per well. The uptake of the isotope was measured with a gamma counter. The results were expressed as stimulation indices (SI). SI = uptake of isotope in stimulated culture/uptake of isotope in nonstimulated control culture.

Epidermal cell and monocyte cultures

For the production of IL-1 crude epidermal cells or purified monocytes (10^6 cells/ml) were incubated in RPMI 1640 supplemented with 5% autologous plasma or AB serum for two days. To enhance the elaboration of IL-1, lipopolysaccharide (LPS, final concentration 5 μg/ml), 4β-phorbol 12-myristate 13-acetate (PMA, final concentration 20 ng/ml) or formalin

Table I. *Generation of interleukin 1 by epidermal cells and monocytes from atopic patients and health controls*

Cells	Atopic patients		Healthy controls		Significance (Mann-Whitney U-test)
	n	IL-1 (U/ml)	*n*	IL-1 (U/ml)	
Epidermal cells	15	6.7 (1–14)[a]	9	13.3 (5–26)	$p<0.01$
Monocytes	19	19.8 (10–49)	21	38.4 (20–88)	$p<0.01$

[a] Mean (range).

treated *Staphylococcus epidermidis* bacteria (bacterium-to-cell ratio 2:1) were added to the cultures. IL-1 activity in the culture supernatants was determined as described by Luger et al. (10) using the thymocyte comitogenicity assay. Various concentrations of a standard IL-1 preparation (Genzyme, Norwalk, CT, USA) were also included and the results expressed as U/ml.

Patch tests

These were performed on the upper arm by using 0.1 ml of birch pollen allergen (10^5 SQ units/ml, Aquagen, Allergologisk Laboratorium A/S) in Finnchambers®, as described by Reitamo et al. (2). A diluent was used as a negative control. The tests were removed at day 2 and also read on day 3.

RESULTS

Both epidermal cells and monocytes from atopic patients produced clearly less IL-1 than those from healthy subjects ($p<0.01$, Table I). In addition, monocytes elaborated about three times higher IL-1 activities than epidermal cells both in atopics and healthy controls (Table I).

Table II shows the results on antigen presentation by epidermal LC from atopic and healthy subjects. The capacity of LC to present HSV-1 to T lymphocytes was intact in atopic patients with previous HSV infections. All these persons were seropositive to HSV-1 whereas there were a few seronegative individuals among other atopic patients and healthy subjects. The cells from the seronegative persons were not stimulatd with HSV in cultures.

Birch pollen appeared to be a weak lymphocyte stimulant in cultures. It induced T cell proliferation (SI $\geqslant 2.5$) in 2/5 patients with allergy to this antigen. The patch test to birch pollen was positive in 1/5 AD patients and showed an eczematous reaction with a maximum at day 3. This patch test-positive patient exhibited the highest in vitro response to birch pollen.

DISCUSSION

In the present study both monocytes and epidermal cells from AD patients produced lower IL-1 activities than the cells from healthy controls. These results are consistent with those of Mizoguchi et al. (11) who measured venous blood monocyte-derived IL-1 in atopics and normal controls. On the other hand, en-

Table II. *Presentation of Herpes simplex virus type 1 and birch pollen antigens to T lymphocytes by epidermal Langerhans cells*

Study group	Antigen[a]			
	HSV		Birch pollen	
	n	SI[b]	*n*	SI
Atopic patients				
With HSV infections	6	90.1 (48.2–180.9)		n.d.
With birch pollen allergy	5	32.6 (0.8–141.6)	5	2.1 (0.5–3.6)
Healthy controls	7	36.2 (2.3–130.4)	2	1.1 (0.9–1.3)

[a] Results with optimal antigen concentration (10^{-2}–10^{-3} dilutions of a stock preparation of HSV-1, 10–100 μg/ml of birch pollen allergen).
[b] Mean stimulation index (range).

hanced releasability of inflammatory mediators such as histamine, prostaglandin E_2 and leukotrienes B_4 and C_4 has been shown to occur in AD (12, 13). In vitro experiments indicate that both prostaglandin E_2 and histamine are able to suppress the elaboration of IL-1 (14–16), whereas leukotrienes B_4 and D_4 enhance IL-1 production (17). One explanation for the observed impairment in epidermal cell and monocyte production of IL-1 in AD might be the increased amounts of inflammatory mediators with IL-1 suppressive activity, but further studies are needed to confirm this.

We did not find any defect in the ability of epidermal LC to present HSV to T lymphocytes when our AD patients were investigated 1–12 months after the last episode of a severe HSV infection. However, some alterations seem to occur during the acute infection. El Araby et al. (18) and Vesley et al. (19) have measured HSV-induced proliferation of peripheral blood mononuclear cells in subjects with severe recrudescent HSV infections. These investigators reported diminished proliferative responses during the acute HSV attack and several months afterwards in patients with widely disseminated eczema herpeticum.

Several pieces of evidence suggest that environmental antigens may play a role in the pathogenesis of AD. Food, pollen, animal dander and house dust mite antigens have been shown to induce positive patch tests reactions and are suspected to exacerbate AD (2, 4, 5). We examined five such patients and found that the birch pollen antigen induced proliferation in 2/5 cultures and the patch test was positive in 1/5 cases. Our results suggest that the epidermal LC of at least strongly birch pollen allergic AD patients are capable of presenting this allergen to T cells, and such a mechanism could explain the AD exacerbations during the pollen season.

ACKNOWLEDGEMENTS

This study was financially supported by grants from the University of Tampere and the Paulo Foundation.

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