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Long Term Effect on Epidermal Dendritic Cells of Four Different Types of Exogenous Inflammation

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In the present study we produced allergic, irritant, ultraviolet and liquid nitrogen inflammation in human volunteers. Biopsies were taken from each test site and adjacent normal skin as control 4–5 weeks later. The monoclonal antibodies OKT 6 and HLA-DR were used for light microscopic detection of Langerhans' cells (LC). At all 4 post-inflammatory test sites the number of epidermal LC (OKT 6 positive as well as HLA-DR positive) were significantly increased compared to normal skin. Also, the density of dermal dendritic cells was increased in post-inflammatory test sites. The increased number of epidermal LC seems to be a result of inflammation and not a specific event strongly related to certain cutaneous inflammatory disorders. The present investigation supports the theory that epidermal LC play a role in skin homeostasis. (Received November 7, 1986.)

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The number of epidermal Langerhans' cells (LC) increases, decreases or stays unchanged in allergic and irritant patch test reactions (1–9). The substance applied, the test method, the time for biopsying and the method used to identify LC may influence the outcome. In different inflammatory dermatological disorders an increased number of LC have been reported (10–17), suggesting that LC play a role in the pathogenesis of these conditions.

Utilizing fluorescence- (L-dopa) and electro-microscopy (18) and T6 labelling (19), the number of epidermal LC is found significantly increased in 4–8 weeks old allergic patch test sites in nickel hypersensitive subjects.

We have investigated whether the increased number of epidermal LC in old allergic patch test reactions is specific for that reaction or may follow inflammation induced in other ways.

MATERIAL AND METHODS

Patch testing

Five male subjects, mean age 50.4 years (39–60), with known hypersensitivity to potassium dichromate (2 subjects), nickel (1 subject), thiuram-mix (1 subject) and rhus allergen enrolled in this part of the study after giving informed consent. The allergens were applied on the inner aspect of the right forearm in big Finn Chambers® (12 mm) on Scanpore® in the following concentrations: potassium dichromate 0.1%, nickel sulphate 5%, thiuram-mix 1%, all in petrolatum, and rhus allergen 1% in alcohol. 50 µl 3% sodium lauryl sulphate (SLS) in water was micropipetted onto paper discs placed in big Finn Chambers® and immediately applied on the inner aspect of the left forearm. The subjects were instructed to remove the test material after 48 hours' application and return for a test reading 24 hours later. The allergic reactions were scored: + = erythema and infiltration, ++ = erythema, infiltration and papules, +++ = erythema, infiltration, papules and vesicles/exudation. The toxic reactions were scored: + = marginated erythema with slight infiltration, ++ = marginated erythema with strong infiltration, +++ = marginated erythema, strong infiltration and exudation.

Irradiation with UVB and freezing with liquid nitrogen (LN_2)

Five other subjects (the 2 authors and 3 other colleagues), mean age 35.6 years (28–47), participated in this part of the study. Covering the rest of the body a 2×2 cm area on the inner aspect of the left forearm was exposed to 300 mJ/cm² in a Waldmann UV 8001K phototherapy cabin equipped with 13 Sylvania F 75/85 W/UV21 fluorescent bulbs emitting a spectrum of 230–365 nm with a maximum at approximately 310–315 nm. The irradiance was measured with a Waldmann UV-meter as 2.3 mW/cm² at the skin surface. The inner aspect of the right forearm was sprayed continuously with LN_2 from a Cryowen® at a distance of 1.5 cm for 10 sec.

Biopsying

Following induction of inflammation the center of the reaction was marked with an indelible pen, and the marking was kept visible by remarking at appropriate intervals until the subjects returned for biopsy 4–5 weeks later. During that period the subjects were instructed to avoid topical corticosteroids; the test area was chosen to minimize exposure to overt sunshine. Following local anaesthesia (Xylocain® 10 mg/ml) a 3 mm punch biopsy, avoiding the area marked with the indelible pen, was obtained from each area and adjacent normal skin as control. The specimens were immediately frozen in LN_2 and stored at −70°C until embedded and cryosectioned. 10 μm thick non-continuous full length sections were cut from each biopsy and reacted with 1/40 dilution of OKT 6 (Ortho Diagnostics, Raritan, NJ, USA) and HLA-DR (Becton Dickinson, Sunnyvale, CA, USA) monoclonal antibodies, respectively. The antibodies were visualized with an avidin-biotin-immunoperoxidase assay (Vectastain® ABC Kit, Burlingame, CA, USA) as previously described (19). Sections without incubation with primary antibody were run as control. Also, 5 μm thick sections were fixed by dipping in ether/alcohol (1 : 1), dried and stained with hematoxylin and eosin.

The interfollicular epidermis of at least 4 sections were counted blind by use of a calibrated eyepiece at 400× magnification for each antibody; only cells with a clear nucleus and at least 2 dendrites were counted as positive. The number of LC was expressed as LC/mm (10 μm thick section). Also, the density of dendritic looking positive dermal cells was estimated and graded: 3 = high, 2 = moderate, 1 = low.

Statistical differences were calculated by Wilcoxon signed rank matched pairs test.

RESULTS

The clinical reaction of allergic and irritant patch testing and the density of epidermal and dermal T6 as well as HLA-DR positive cells in 4–5 weeks old test sites (including control sites) are illustrated in Table I. All 5 subjects reacted with a strong (+++) allergic test

Table I. *Patch test reactions and density of epidermal and dermal T6 as well as HLA-DR positive cells 4–5 weeks after allergic and irritant patch testing in 5 male subjects*

	Allergic site					Irritant site					Control site			
				Dermal density					Dermal density				Dermal density	
Subject + hypersensitivity	Patch test reaction	T6 epid.	HLA-DR epid.	T6	HLA-DR	Patch test reaction	T6 epid.	HLA-DR epid.	T6	HLA-DR	T6 epid.	HLA-DR epid.	T6	HLA-DR
1. 52 years Thiuram	+++	17.6	13.6	2	3	++	16.5	11.5	2	2	12.6	9.4	1	1
2. 39 years Rhus	+++	16.8	11.6	3	3	+++	16.8	12.0	2	2	10.3	5.8	0	1
3. 58 years Chromate	+++	16.5	11.5	3	3	+	12.6	9.3	2	1	6.9	4.1	1	1
4. 60 years Nickel	+++	18.4	13.1	2	2	++	19.7	13.4	2	2	8.4	3.0	1	1
5. 43 years Chromate	+++	18.6	14.1	2	3	+	13.6	8.9	1	1	10.6	6.7	1	1

reaction, whereas the irritant test reactions varied in intensity. Epidermal dendritic cells stained with both antibodies were regularly distributed in the epithelial sections and easy to count. The number of epidermal T6 positive cells were always and rather proportionally increased compared with HLA-DR positive cells in all sections counted. The density of epidermal T6 and HLA-DR positive cells was significantly increased ($p<0.05$) in 4–5 weeks old allergic as well as irritant test sites, respectively. No difference between the number of epidermal dendritic cells was observed comparing allergic and irritant test sites. The number and density of dermal lympho-histiocytic perivascular cell infiltrates were increased at old patch test sites as identified in hematoxylin-eosin staining. Also, the number of positive dendritic T6 and HLA-DR positive cells was more pronounced at old patch test sites (allergic as well as irritant) than in control sites (Table I).

The results from the other part of the study following irradiation with UVB and freezing with LN_2 are illustrated in Table II. Also, in these types of postinflammatory reaction the number of epidermal T6 and HLA-DR positive cells was significantly increased ($p<0.05$) compared to normal skin. No difference was observed between UVB and LN_2 test site. Especially, at the LN_2 site positively stained dendritic cells were noticed in the dermis (Table II).

DISCUSSION

We have used two different monoclonal antibodies (T6 and HLA-DR) to identify epidermal and dermal LC. Both markers provide a specific and sensitive probe for epidermal LC. As earlier reported (20, 21), we also found that the T6 antibody consistently identifies more LC than similar antibodies against Ia-like antigens. This observation holds true in all specimens investigated, post-inflammatory and normal control skin sites. In the present study, inflammation was induced in 4 different ways. The 4 acute inflammatory reactions differ in pathogenesis and clinical morphology (degree of erythema, infiltration, marking, lesions e.g.). Postinflammatory, 4–5 weeks old changes were mostly not visible except for a couple of subjects with a slight red-brown colour at the sites of allergic patch testing, slight hyperkeratosis at the site of freezing with LN_2 and slight hyperpigmentation following UVB irradiation. Apart from these early and late morphological clinical variations, a significantly increased number of epidermal LC was found in all 4 postinflammatory skin sites. It is remarkable that epidermal LC are also enumerated following a relatively high dose of UVB (300 mJ/m^3), which deplete LC surface markers (22–24). However, LC

Table II. *Density of epidermal and dermal T6 as well as HLA-DR positive cells 4–5 weeks after exposure to UVB and LN_2*

	UVB-site				LN_2 site				Control site			
			Dermal density				Dermal density				Dermal density	
Subject	T6 epid.	HLA-DR epid.	T6	HLA-DR	T6 epid.	HLA-DR epid.	T6	HLA-DR	T6 epid.	HLA-DR epid.	T6	HLA-DR
1. 42 years	13.0	4.6	1	1	19.8	12.9	2	2	12.6	8.3	1	1
2. 47 years	19.2	14.1	1	2	18.9	14.0	2	3	15.6	13.2	1	1
3. 30 years	14.2	11.2	1	1	15.5	10.9	2	2	11.1	10.3	1	1
4. 28 years	13.5	10.2	1	2	16.1	10.8	2	2	13.0	9.8	0	1
5. 31 years	11.8	7.5	1	2	11.9	10.1	1	2	5.4	3.7	0	1

markers are usually recovered within 3 weeks (25–27). One investigation even indicated that ATPase positive epidermal dendritic cells show a higher density 24 days after UVB exposure (26).

Positive patch test reactions in nickel hypersensitive subjects result in increased numbers of epidermal LC at 4–8 weeks old test sites (18, 19). The present investigation shows that this observation is not a result of nickel hypersensitivity as such, but follows positive patch test reactions to other contact allergens as well. Also, the present investigation indicates that the increased number of epidermal LC in old allergic patch test sites is not a specific phenomenon for this reaction, but can be induced by other types of exogenous inflammation. Therefore, an increased number of epidermal LC seems to be a non-specific event following inflammation. As a consequence, one should be warned of taking enumeration of epidermal LC in an inflammatory or post-inflammatory dermatological disorder as a sign that LC necessarily play a pathogenetic role in that condition. Because of the affinity of LC for keratinizing epithelia, it has been speculated that LC, beyond the function of antigen presentation, have a role in regulating epidermal homeostasis (28–30).

From a hypothetical point of view, the results of the present investigation support the theory that LC play a role in skin homeostasis. However, it is still unclear as to whether the increased number of epidermal LC is a result of formation of LC locally, activation of cell membrane determinants or migration to the epidermis of new LC.

The density of T6 as well as HLA-DR positive cells in dermis was increased in all the postinflammatory test sites but especially in allergic, irritant and LN_2 sites. This phenomenon has been described earlier following allergic patch test reactions (31, 32). Again, this finding does not seem to be specific for allergic contact dermatitis but may follow different types of inflammation.

In conclusion: Early as well as late stages of inflammation induce dynamic changes in epidermal LC. The specificity of such changes is at present uncertain and further morphological studies are needed to investigate the role of LC in different inflammatory cutaneous disorders.

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