

Langerhans' Cell Distribution in Drug Eruption

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The number in and distribution of Langerhans' cells were studied in 11 patients with a maculopapular drug eruption. The Langerhans' cells (LC) were identified with a monoclonal antibody to OKT6 antigen, by employing an immunofluorescence technique. Skin biopsies were taken from lesional and non-lesional skin during the acute stage of the disease. LC in the lesional biopsies increased in number by 66% ($p < 0.001$) and displayed more intense staining and more prominent dendrites than did LC from non-lesional skin. Control biopsies, taken from identical sites at least 4 weeks after the eruption disappeared, exhibited a cell distribution similar to the non-lesional acute stage ($p = N.S.$). Delivery of drugs via the circulation and their distribution into the skin may cause a type IV immune reaction due to LC activation by a drug-carrier complex. **Key words:** Antigen presenting cell; Monoclonal antibody OKT6.

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Drug allergy is an ubiquitous problem in modern medicine (1) that accompanies the life span increase and increasing drug consumption. Drug intake carries an overall 1-3% risk of causing an allergic reaction and drug-induced hypersensitivity occurs in 6-10% of all drug reactions (2). Cutaneous drug eruptions are one of the clinical manifestations of this immunologic response. The appearance of a maculopapular rash is characteristic of almost half of these cases and is the most common sign of drug allergy (3). The immune system is involved through different mechanisms in the pathophysiology of drug eruption (4, 5).

Langerhans' cells (LC) are local migratory dendritic cells

having immunological functions (6). LC are in the centre of an 'epidermal Langerhans' cell unit' and function in symbiosis with the surrounding keratinocytes. LC secrete interleukin-1 (7) and are involved in the sensing and amplification of the immune response. The antigen presenting ability and its specific HLA-DR marker implicate the involvement of these cells in a variety of lymphocyte-mediated skin diseases. Variations in the number, distribution and morphology of LC have been found in various diseases, such as allergic contact dermatitis, viral infections (8), lichen planus and cutaneous T cell lymphoma (9). The involvement of LC in the drug eruption reaction has not yet been conclusively established.

A prospective study of patients affected by drug eruption was performed. The distribution of LC was studied in acute and healed stages of the disease. The results may help us to understand the pathophysiologic mechanism active in drug eruption.

MATERIALS AND METHODS

Patients

Eleven patients (7 females and 4 males) were included in a prospective study. Subjects were enrolled for the study if they fulfilled the following preconditions: (1) a maculopapular skin eruption excluding other skin lesions such as urticaria, purpura and vasculitis, (2) an anamnesis of drug intake and timing of the rash consistent with a drug eruption, (3) a positive withdrawal test within 4 weeks of ceasing medication. All patients underwent a blood cell count and routine laboratory tests (Sequential Multiple Analyzer 12 including, among other tests, glucose, alkaline phosphatase and creatinine); their values were within the normal limits. None of the subjects had fever or arthropathy.

Punch biopsies (4 mm) were performed no later than 24 h from cessation of drug intake. All biopsies were taken from an identical location, the lateral aspect of the thigh. During the acute stage of the disease, biopsies were performed on uninvolved (group A) and lesional (group B) skin. At most 4 weeks from the cessation of the eruption, a control biopsy (group C) was taken.

Table I. Summary of clinical data.

Case number	Sex/age	'Suspected' drug	Drug eruption (days of intake)	Previous exposure to drug	Other drug intake ('unsuspected')
1	F/21	Sulfamethoxazole and Trimethoprim	10	-	
2	F/70	Sulfamethoxazole and Trimethoprim	8	+	Nifedipin, carbidopa, levodopa
3	F/65	Doxycycline	3	+	Codein, mebhydrolin
4	F/20	Minocycline	21	-	
5	M/90	Amoxicillin	4	+	Furosemide, isosorbide dinitrate
6	F/67	Cloxacillin	8	+	Diltiazem
7	M/77	Acetylsalicylic acid	15	+	Isosorbide dinitrate, metoprolol
8	F/55	Fenbufen	11	-	Oestradiol, oestriol
9	F/21	Fenbufen	13	-	
10	M/72	Hydrochlorothiazide and Amiloride	5	+	Indomethacin, isosorbide dinitrate
11	M/71	Amiodarone	10	-	Furosemide, isosorbide dinitrate, diltiazem

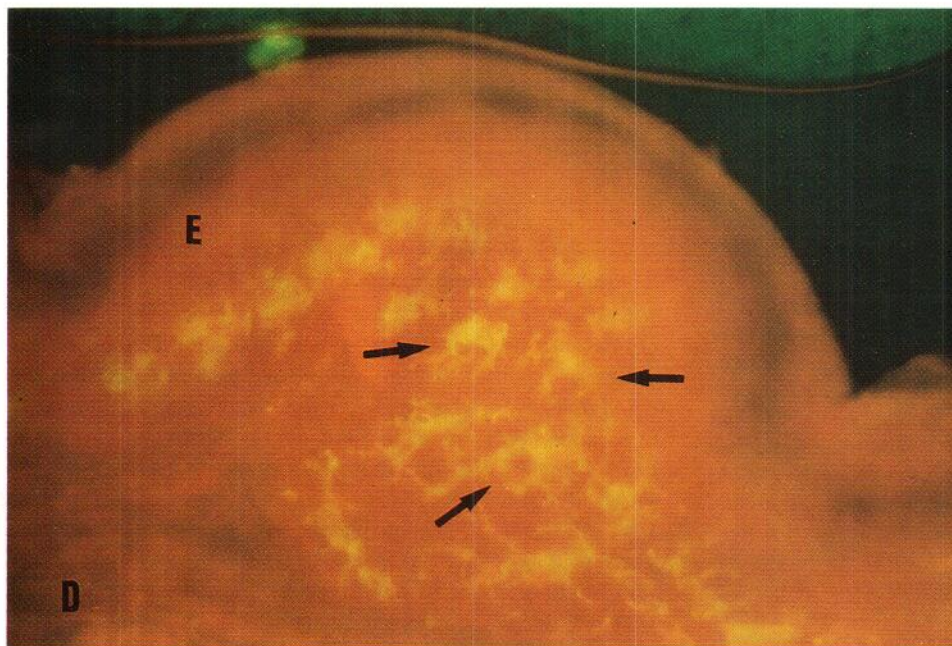


Fig. 1. Lesional skin during the acute stage of drug eruption. Note the Langerhans' cell increase in apparent size, number and prominence of dendrites as compared with those in non-lesional skin. Arrows depict LC; E and D delineate the epidermis and dermis, respectively (OKT6 staining, $\times 400$).

Immunofluorescence technique

The LC were identified by anti-T6 (OKT6) monoclonal conjugated antibody (Ortho Immunodiagnostic Systems, Raritan, NJ). The staining procedure was based on the technique of Haftek et al. (10). Immediately after excision, skin biopsies were frozen in liquid nitrogen and kept at -70°C for 4 months, at most. Frozen sections were cut at $4-5\ \mu\text{m}$ and fixed in cold acetone for 10 min at -20°C . Sections were counterstained with Evans Blue and incubated in a humidified chamber with OKT6 antibodies at 37°C for 60 min and rinsed in phosphate-buffered saline three times for 5 min. Finally, the section was mounted in glycerine on a slide and viewed under a Zeiss fluorescence microscope. Dendritic cells with a dark nucleus and a bright cytoplasm were counted. An ocular square grid covering $0.0226\ \text{mm}^2$ of skin section under $\times 40$ magnification was used. Statistical analyses were carried out using the paired Student's *t*-test.

RESULTS

Details of the patients and their medication are presented in Table I. Antibiotics and anti-inflammatory agents were the most frequent 'suspected' causes of drug eruption (6 and 3 subjects, respectively). The LC count in the lesional biopsies increased by 66% ($p < 0.001$), as shown in Table II. This reproducible increase in the number of LC in the epidermis was found irrespective of the age and sex of the patient. The

Table II. Counting of Langerhans' cells¹ (LC) in the epidermis of subjects with drug-induced eruption (LC/mm²).

Group	LC count in epidermis (mean \pm S.D.)	<i>p</i> * as compared with controls ^a
A. Non-lesional (acute stage)	423 \pm 35	N.S.
B. Lesional (acute stage)	707 \pm 31	<0.001*
C. Controls (normal skin)	427 \pm 24	

^aControl biopsies taken from the same patients at an identical location at least 4 weeks after the rash disappeared.

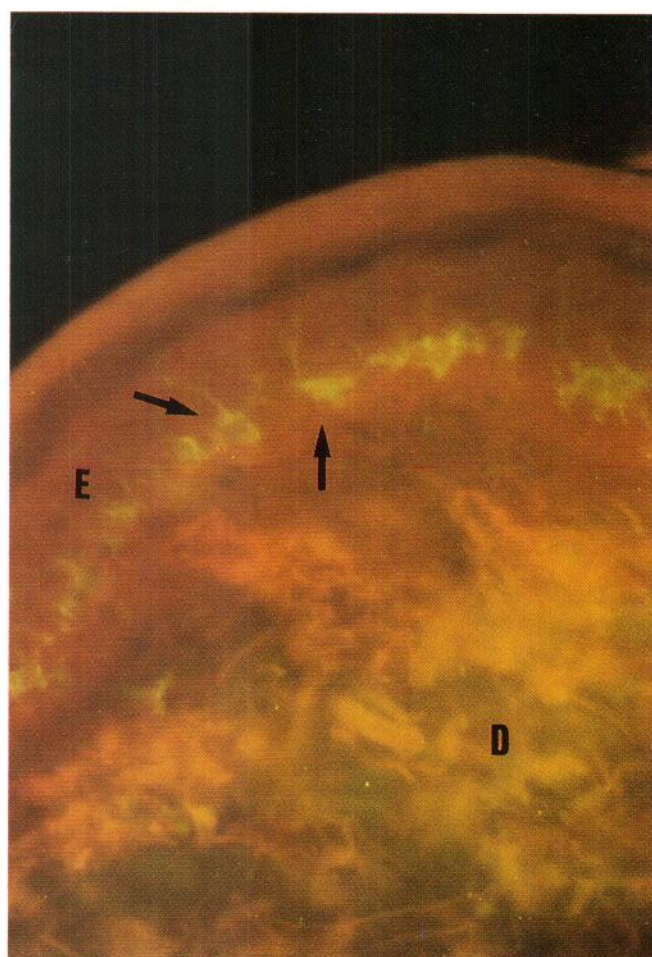


Fig. 2. Langerhans' cells (arrows) in non-lesional skin of a patient affected by drug eruption, stained with OKT6 monoclonal antibody. Regularly distributed positively stained cells in the epidermis (E) (OKT6 staining, $\times 400$).

microscopic findings in lesional (Fig. 1) and uninvolved skin (Fig. 2) are presented. The cells display an increase in staining intensity and cell body. The dendrites are more prominent and thicker than in non-lesional skin. These changes in the involved skin are most conspicuous in the suprabasal area.

In order to determine whether a patient with drug eruption has an increase in LC count in uninvolved skin during the acute stage, control biopsies (group C) were performed at least 4 weeks after disappearance of the eruption. Indeed, the control LC numbers of group C were almost identical with the uninvolved skin during the eruption ($p = \text{N.S.}$) and their LC appearance was comparable to that of non-lesional skin (group A).

DISCUSSION

In the present study we investigated the LC distribution in a group of selected patients with drug eruption. During the acute stage of the disease, the involved skin of these patients presented morphological changes in LC and a 66% increase in numbers, as compared with the subjects' non-lesional skin. The LC displayed thickened dendrites and a large cell body as compared with normal controls. By contrast, skin biopsies taken either during the rash period from the uninvolved areas, or from the subjects after healing, disclosed an almost equal LC distribution.

Antibiotic or anti-inflammatory drugs (6 and 3 patients, respectively) were assumed to be the agents causing the eruption. These drugs are well known inducers of skin reactions (11). In all cases the cessation of medication stopped the clinical response within one month. For obvious reasons, we preferred to defer a rechallenge with the 'suspected' drug.

The observed LC increase and conformational changes during the rash may be due to a primary event, e.g. the exposure of epidermal cells to a specific drug via the peripheral blood circulation and the subsequent formation of a low molecular weight drug-macromolecule complex (4). Indeed the carrier-hapten complex may activate the local LC, which can take up and process a hapten antigen intracellularly (12). We may infer that the primarily induced allergic reaction is a class IV cell induced immune response. The limited clinical response confined to the skin, and the maculopapular appearance of the rash, are atypical of class III immune reactions. Furthermore, the normal blood counts and the exclusion of patients with urticaria from the study constitute evidence against a class II or I allergic reaction, respectively.

In a related condition, fixed drug eruption, patients showed a tendency to have fewer LC in the acute stage of the phenomenon (13). The small number of subjects precluded the drawing of any firm conclusions. Patients with drug-induced Erythema Multiforme display an increase in the number of T6+ staining before the basal unit destruction (14). These results are comparable to those of the present study and may reflect a similar activation of cells in both entities.

A difference in LC distribution in non-lesional skin versus

control healthy biopsies might have been expected, in view of the "turned on" state of the entire peripheral "guardian" system, i.e. the LC, during the rash stage. Surprisingly, no such changes have been observed, at least when applying a rigid inclusion criteria protocol, as described. Hence, this study is limited by a lack of knowledge concerning LC distribution during the course of a drug eruption, information which should correlate the duration and dynamics of the LC response in the immune system with the clinical signs of the disease.

The application of our method as a routine diagnostic test in the diagnosis of skin eruption due to drugs, "the great mimickers of our era" (15), is cumbersome. Furthermore, the test is not especially specific, as various other diseases considered in the differential diagnosis, e.g. viral infections, also increase the Langerhans' cell count. The present findings should contribute to the understanding of drug eruption pathophysiology. The LC involvement in the drug eruption is demonstrated and a class IV immune reaction is suggested to underlie the response.

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