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Comparative Study for the Differentiation of Allergic and Irritant Contact Dermatitis in Mice†

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=Abstract=**Our study was performed to compare the differences between allergic and irritant contact dermatitis in BALB/c mice. Allergic reaction was induced by a sensitizing regimen of 2,4-dinitro-1-fluorobenzene (DNFB) and irritant reaction by 10% sodium lauryl sulfate (SLS). The following differences were noted: 1) the mice with irritant reaction showed an earlier peak of ear swelling, 2) increasing number of Langerhans cells (LCs) in allergic reaction but decreasing number of LCs in irritant reaction was observed at 48 hr after challenge of DNFB or SLS, and 3) induction of Ia (+) keratinocytes was found only in allergic reaction. It was suggested that Ia (+) keratinocytes play an active role in the mechanism of allergic contact dermatitis.**

Key Words: *Allergic and irritant contact dermatitis, BALB/c mice, Langerhans cells, Ear swelling, Ia (+) keratinocytes*

INTRODUCTION

There have been studies to try to differentiate allergic reaction from irritant reaction by characterizing cellular infiltrations phenotypically, but the only difference reported was more cell infiltrates in allergic reaction (Reitamo *et al.* 1981; Scheynius *et al.* 1984) and, their main focus of interest was to observe the differences in T cell subsets.

Langerhans cells (LCs) are the bone-marrow-derived immune cells of the epidermis where they constitute approximately 2 to 4 percent of the total epidermal cell population (Stingl *et al.* 1978) and, LCs are critically needed for the initiation of the cutaneous immune response (Shelley and Julin 1976). Some conflicting data have also been revealed about LCs;Epidermal LCs have been reported to decrease in allergic (Kanerva L *et al.* 1987) and irritant (Ferguson *et al.* 1985, Mikulowska 1990) reactions but also to increase in both types (Nordlund and Ackles 1981; Scheynius *et al.* 1984; Christensen 1986). But these authors reported their results about LCs in a fixed time, e.g., 24 hr or 48 hr after the induction of allergic or irritant reactions and there have been few studies about the dynamic changes of LCs according to the time sequence.

Since LCs synthesize and express Ia antigens- in fact, in normal epidermis they are

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the only epidermal-cell population capable of doing so (Stingl *et al.* 1978)- they can be visualized by monoclonal antibodies directed against Ia moieties both in vertical tissue sections and in epidermal sheet. However, in certain immunologically mediated conditions including allergic contact dermatitis keratinocytes also express Ia antigens (Smolle 1985; Gawkrodger *et al.* 1987) and Scheynius and Fischer (1986) suggested that the presence of Ia antigens on keratinocytes may be of diagnostic significance for allergic reaction.

So far the immunopathological differences between allergic and irritant reactions have been unresolved. Our study was performed to evaluate possible differences between these two reactions in mouse epidermis in regard to the temporal changes in the number of LCs, and the induction of Ia antigens on keratinocytes. In addition, ear swelling response was also compared.

MATERIALS AND METHODS

6- to 8-week old female BALB/c mice were purchased from the Genetic Engineering Research Institute of Korea Institute of Science and Technology (KIST).

1. Induction of allergic and irritant contact dermatitis, and tissue procurement

Shaved abdominal wall skin was exposed to a sensitizing regimen of 2,4-dinitro-1-fluorobenzene (DNFB) (Sigma Chemical Company, St.Louis, Missouri). 30 μl of 0.5% DNFB in acetone:olive oil (4:1) were applied two times at a 24-hr interval. 5 days after sensitization allergic contact dermatitis of ear skin was induced by applying 30 μl of 0.2% DNFB solution on the dorsal surface of the ear. Irritant contact dermatitis was induced by applying 10% sodium lauryl sulfate (SLS) (Sigma Chemical Company, St.Louis, Missouri) in distilled water on the dorsal surface of the ear.

Biopsies were done from the ears of each group of 5 mice 6 hr, 24 hr, 48 hr, and 72 hr after the induction of each dermatitis to compare the number of LCs.

In order to observe Ia (+) keratinocyte and its expression time biopsies were done at 1 day, 2 days, 3 days, 4 days, 5 days, 7 days, and 9 days after the induction of each reaction.

2. Ear swelling test (EST)

For the evaluation of the contact sensitivity the thickness of both test and control ears was measured by an engineer's micrometer (Ozaki Co., Japan) until 4 days after the induction of each dermatitis. The ear swelling was expressed in percent according to the following formula:

$$\frac{\text{test ear}(\text{mm} \times 10^{-2}) - \text{control ear}(\text{mm} \times 10^{-2})}{\text{control ear}(\text{mm} \times 10^{-2})} \times 100$$

3. Epidermal separation, Langerhans cell and Ia (+) keratinocyte identification

Ear skin was separated from the underlying cartilage by blunt dissection. Epidermal sheets were separated from the underlying dermis by incubation in 0.02% EDTA solution at 37°C (Juhlin and Shelley 1977), fixed in cold acetone for 20 minutes, and stained with the method of avidin-biotin-peroxidase complex (ABC) briefly as follows.

Epidermal sheets were incubated with anti-I-A^d (1:50) (Becton Dickinson, Mountain View, California) overnight at 4°C and then reacted with 1:200 biotinylated anti-mouse IgG (Vector Laboratories, Burlingame, California), and avidin-biotin-peroxidase complex (Vector Laboratories, Burlingame, California) for 30 minutes at room temperature respectively. Development of the enzyme reaction was finally achieved using 3-amino-9-ethylcarbazole solution (Sigma Chemical Company, St. Louis, Missouri).

4. Enumeration of Langerhans cells and observation of Ia (+) keratinocytes

The number of Ia (+) dendritic Langerhans cells was counted at lower power (100×) with an Olympus OC-M eyepiece micrometer (Olympus Co., Japan) and expressed as mean numbers±SD/mm^2 according to the time. Keratinocyte expressing Ia positivity on its surface was described as positive or negative according to the time course. Statistical analysis was done by using Wilcoxon rank sum test, and the level of $P< 0.05$ was regarded as significant.

RESULTS

1. Ear swelling test

No appreciable response was noted at 6 hr, but considerable ear swelling (45.5±9.8%) was noted at 1 day, at a peak (76.2±12.6%) at 2 days and then decreased to 39.6±11.7% at 3 days, and to 22.5±9.7% at 4 days in allergic reaction. However, peak swelling (26.3±5.4%) was noted at 6 hr with a slight decrease to 17.7±3.5% at 1 day, 16.1±6.8% at 2 days, 19.4±5.8% at 3 days, and 6.2±4.6 at 4 days in irritant reaction. Ear swelling was more marked in allergic reaction than in irritant reaction (Fig. 1)

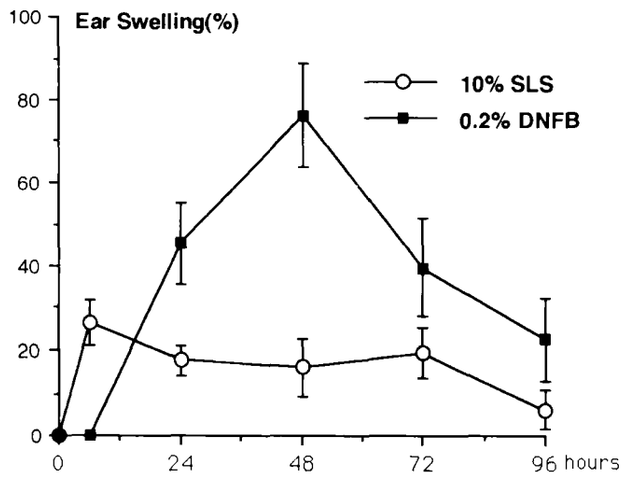

Fig. 1. Temporal changes of ear swelling in allergic and irritant reaction. Irritant reaction (10% SLS) showed earlier peak and less marked swelling.

2. Langerhans cells (Fig. 2)

The number of LCs significantly increased to 954.2±33.6/mm^2 at 48 hr ($P<0.05$), and significantly decreased to 763.6±60.8/mm^2 at 72 hr ($P<0.05$) compared with the control number (879.1±21.1/mm^2) in allergic contact dermatitis. In the irritant group the number of LCs decreased significantly at 6 hr, 24 hr, and 48 hr

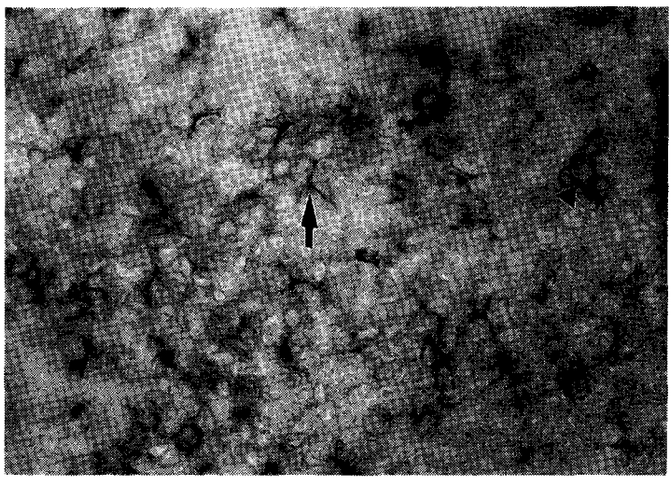

Fig. 2. Ia (+) dendritic Langerhans cell (arrow) and keratinocyte (arrowhead) showing Ia expression on its surface 4 days after induction of allergic contact dermatitis by DNFB.

Table 1. Temporal changes in the number# of Ia (+) Langerhans cells (LC)

Epidermal sheet	LC (No./mm^2)
control	879.6±21.1
DNFB challenged group	
6 h	931.2±37.7
24 h	879.8±25.1
48 h	954.2±33.6*
72 h	763.6±60.8*
SLS treated group	
6 h	719.8±94.1*
24 h	746.0±18.5*
48 h	731.6±45.3*
72 h	874.8±28.8

: This represents Mean (n=5)±SD of the Langerhans cells in 1 mm^2 of epidermal sheet.

* : $P < 0.05$ relative to control (non-challenged, vehicle-treated) group.

($P<0.05$) but normalized at 72 hr. Therefore increasing number of LCs in allergic reaction but decreasing number of LCs in irritant reaction was observed at 48 hr after challenge of DNFB or SLS (Table 1).

3. Ia (+) keratinocytes

Keratinocytes expressing Ia positivity were observed in allergic contact dermatitis 4 days after induction of the reaction (Fig. 2) but not in irritant contact dermatitis (Table 2).

DISCUSSION

Recently Gawkrodger *et al.* (1986) reported that the number of LCs in the epidermis increased by a third between 24 and 48 hr of the allergic reaction but was almost halved at 48 hr in the irritant reaction. This report is quite interesting in that the data revealed a time sequence analysis of the LCs 1 hr through 48 hr after the induction of each reaction contrary to the various conflicting results so far reported in a certain fixed time. Our results showed that the temporal changes in the number of LCs in allergic reaction were quite inversely related to those in irritant reaction, that is, increase in allergic reaction versus decrease in irritant reaction during the early observation period. So our data were quite similar to those of Gawkrodger *et al.* (1986), however, a direct comparison of various data may not be reliable because of differences not only in the specificity and sensitivity of the different LC markers applied but also in the method of inducing allergic or irritant reactions. DNFB was used to elicit allergic reaction in our study because the sensitizing regimen of DNFB was well established and standardized (Phanuphak *et al.* 1974).

Probably more importantly, the methods of making irritant reactions were quite different from each other and this seemed to produce very inconsistent results about LCs in the epidermis according to the studies. Various kinds of irritant materials have been applied and the optimal concentration of SLS inducing irritant contact dermatitis was reported to be 5% in humans when applied as an occlusive patch test using Finn Chambers (Willis *et al.* 1988). The concentration of 10% SLS was chosen in our study because our application was an open test. Lindberg and Emtestam (1986) reported that mild irritant stimuli caused an increase in the LCs at 48 and 96 hours after the exposure to 0.5% SLS. Although this report was different from ours in its obervation time and the concentration of SLS applied, this and other ultrastructural studies (Kanerva *et al.* 1984) indicated that quantitative alterations may be dependent on the primary irritant effect on the LCs by the substances applied. So it seems unreasonable that allergic reaction can be differentiated from irritant reaction solely by comparing the number of their LCs.

Baeck and Larsen (1982) reported that the ear swelling test is a simple and reproducible method for the evaluation of contact hypersensitivity in mice in quantitative terms. There have been no reports other than ours that have tried to differentiate allergic reaction from irritant reaction by comparing EST. EST in our allergic reaction showed a maximun response at 48 hr with a decrease at 72 hr and a significant drop at 96 hr. However, EST in our irritant reaction showed a maximum response at 6 hr with a decrease at 24 hr through 96 hr. The difference in the maximum response of EST between allergic and irritant reaction was noted

Table 2. Positivity of Ia expressing keratinocytes according to the time sequence

Epidermal sheet	Days after provocation						
	1	2	3	4	5	7	9
DNFB challenged	- (5)#	- (5	- (5)	+ (5)	+ (5)	+ (5)	+ (5)
SLS treated	- (5)	- (5)	- (5)	- (5)	- (5)	- (5)	- (5)

: The number in parenthesis represents the sample number.

and more prominent swelling of the ear was observed in allergic reaction.

γ-Interferon is known to be a potent inducer of HLA-DR antigens on keratinocytes (Basham *et al.* 1984) and the infiltrating activated T cells in the dermis may release γ-interferon and induce HLA-DR antigens on keratinocytes. Scheynius and Fischer (1986) reported that HLA-DR expression on keratinocytes was found in 9 of 14 allergic reactions 4 days after provocation with cobalt chloride, but not in irritant reactions. They cited a reference that also showed HLA-DR expression on keratinocytes 4 days after intradermal PPD injection, with a maximal distribution at 6 to 8 days. Gawkrodger *et al.* (1986) reported that no keratinocytes expressing HLA-DR were observed both in allergic (nickel sulfate) and irritant (dithranol) reactions until 3 days after the induction of each reaction.

However, Gawkrodger *et al.* (1987) reported that keratinocytes Ia expression began at 24 hr for dinitrochlorobenzene (DNCB) sensitization, and 24-48 hr for nickel-sensitive cases and even for anthralin irritant reactions. This report was quite inconsistent with their earlier report (1986) in that an earlier time sequence of keratinocyte Ia expression was noted and even irritant reaction caused keratinocytes to express Ia antigen and suggested that the positive rate and time factor of the appearance of Ia (+) keratinocytes seem to be dependent on the kinds of provocation materials and immunohistochemical methods to detect Ia antigen as well. In our study Ia (+) keratinocytes were induced in all 5 samples of allergic reactions 4 days after elicitation of allergic contact dermatitis but not in any sample of irritant reaction. So our result was consistent with that of Scheynius and Fischer (1986) in that Ia (+) keratinocytes were found only in allergic reaction 4 days after provocation.

In conclusion, allergic reactions showed differences compared with irritant reactions in our model in the following 3 ways: 1) EST showed early peak in irritant reaction, 2) increasing tendency of LCs was observed at 6 hr and 24 hr, and increasing number of LCs was observed at 48 hr in allergic reaction, but decreasing number of LCs was observed 6 hr through 48 hr in irritant reaction, and 3) induction of Ia (+) keratinocytes was found only in allergic reaction 4 days after provocation.

It was suggested that Ia (+) keratinocytes play an active role in the mechanism of allergic contact dermatitis. Further study for characterizing the functional differences of LCs and Ia (+) keratinocytes is required to elucidate the subtle differences between allergic and irritant contact dermatitis.

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