# The Murine (C3H/He) Epidermal Ia$^+$ Dendritic Cells (Ia$^+$DECs) and Thy-1$^+$ Dendritic Cells (Thy-1$^+$DECs) in Contact Hypersensitivity and Aging

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The contact sensitivity evaluated by the ear swelling test and the dynamic changes of epidermal Ia$^+$ dendritic cells (Ia$^+$DECs) and Thy-1$^+$ dendritic cells (Thy-1$^+$DECs) were studied in trinitrochlorobenzene (TNCB) sensitized different age group C3H/He mice after challenge. A significant increase of ear swelling was observed between 6 h and 10 days of both 8–10 week (wk) and 40–48 wk groups; the ear swelling indices of 8–10 wk group were significantly higher than those of 40–48 wk group from 18 h to 5 days. A significant decrease of the densities of Ia$^+$DECs from 18 h to 48 h, followed by a gradual increase reaching significant increase of the densities of Ia$^+$DECs from 5 days to 21 days in both 8–10 wk and 40–48 wk groups, was observed; the densities of Thy-1$^+$DECs significantly decreased from 18–48 h, followed by a gradual increase reaching a significant increase from 5 days to 21 days in both 8–10 wk and 40–48 wk groups. In the normal control groups, a significant decline of both Ia$^+$DECs and Thy-1$^+$DECs in the 40–48 wk group was observed. Results suggest that contact allergy may be diminished in aged mice. On the other hand, like Ia$^+$DECs, Thy-1$^+$DECs seem to be involved in the process of contact allergy. (Accepted October 24, 1988.)

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The importance of Ia$^+$ dendritic epidermal cells (Ia$^+$DECs) (Langerhans' cells) (LCs) has been established in delayed contact sensitivity. Among epidermal cells, Ia antigens are demonstrable only on LCs in normal epidermis (1–3). LCs selectively take up small molecules, some of which serve as haptens in delayed contact hypersensitivity (4). Furthermore, the surface density of LCs becomes a determining factor in the induction of either sensitization or hyporesponsiveness in delayed contact hypersensitivity (5, 6). On the other hand, studies of dynamic changes of Ia$^+$DECs after induction of delayed contact hypersensitivity by TNCB or DNCB have shown that there is a transient decrease in the number of Ia$^+$DECs (7, 8), which is followed by a gradual increase of Ia$^+$DECs densities after challenge (7). Thy-1$^+$ dendritic epidermal cells (Thy-1$^+$DECs) have been identified as a new epidermal subset in mice (9, 10). Available evidence shows that Thy-1$^+$DECs can express either T helper phenotype or natural killer phenotype and natural killer activity after Con-A stimulation, indicating that the Thy-1$^+$DECs may play a role in immune response (11, 12). Therefore, it would be also interesting to know the dynamic changes of Thy-1$^+$DECs in allergic contact sensitivity. The present study was undertaken to observe the dynamic changes of both Ia$^+$DECs and Thy-1$^+$DECs in C3H/He mice after induction of delayed contact hypersensitivity with regard to the aging.

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## MATERIALS AND METHODS

### *Animals*

Two different age groups of C3H/He mice ($Ia^k$, Thy-1,2) 8–10 week (wk), and 40–48 wk respectively were obtained from Japan Charles River Company and maintained in our own animal colonies.

### *Antigen*

2, 4, 6, Trinitrochlorobenzene (TNCB) was purchased from Sigma Chemical Company, St. Louise, MO.

### *Sensitization and elicitation of contact sensitivity*

Contact sensitization was induced according to a minor modification of a previously described method (5, 6). Briefly, mice were painted with 100 µl of 7% TNCB (4 : 1 acetone : olive oil) on the shaved dorsal body wall and the solution was air-dried. Five days after the epicutaneous painting, the ears were challenged with 50 µl of 1% TNCB in olive oil on each side of the ear. Four to eight mice were used in each experimental group and normal control group. The thickness of ears was measured using an engineer's micrometer (Mitutoyo, Tokyo, Japan) and the epidermis was separated for identification and enumeration of $Ia^+$ and Thy-$1^+$DECs.

For evaluation of the contact sensitivity, the ear swelling test was used (13). The ear swelling was expressed as a percentage according to the following formula:

$$\frac{\text{test ear} - \text{control ear}}{\text{control ear}} \times 100\ (\%)$$

### *Epidermis specimens*

Epidermal sheets were obtained by EDTA-separations. Details are given elsewhere (14). Mice were killed under ether anesthesia and their ears were amputated at the base. The free edges of the ears were cut away in 2–4 mm widths and mechanically split into dorsal and ventral sides (Ear D, Ear V). After a 2 h incubation in a buffered medium containing 20 mM EDTA (37°C) the epidermis could be removed as a sheet. The Ear D was used for identification and enumeration of Thy-$1^+$DECs while Ear V was used for $Ia^+$DECs.

### *Immunofluorescence staining*

The epidermal sheets were fixed in cold acetone for 30 min and rinsed for one hour in 6.7 mM phosphate buffered saline (PBS) (pH 7.3). Each specimen of epidermis was incubated overnight in one of the following commercial polyclonal or monoclonal reagents respectively: 1) unconjugated mouse Ia alloantiserum (A.TH-$K^sI^sD^d$ anti A.TL-$K^sI^kD^d$) (Cedarlane Laboratories Limited, Canada) diluted in 1 : 160 in PBS; 2) unconjugated monoclonal anti-Thy-1.2 (IgG 2b, K chain) (Cedarlane Laboratories Limited, Canada) diluted 1 : 800. After washing in 4 changes of PBS over 2 h specimens were incubated for 90 min (23°C) in one of the following fluorescein conjugated affinity purified second antibodies according to the content of first antibodies used. 1) $F(Ab')_2$ fragment goat anti-mouse immunoglobulins (IgA+IgG+IgM) (H and L chains specific) (Cooper Biomedical Inc., PA, USA) diluted in 1 : 20—for Ia alloantiserum. 2) $F(Ab')_2$ fragment goat anti-mouse immunoglobulin (IgG, H and L chains specific) (Cooper Biomedical Inc., PA, USA) diluted 1 : 20—for anti-Thy-1.2. All above mentioned working dilutions had been previously titrated out. After a second series of washes over 2 h, specimens were mounted, dermal side up on microscope slides and covered with 10% glycerol PBS and then a coverslip. Prepared specimens were examined with an Orthoplan fluorescence microscope equipped for epi-illumination (E. Leitz Inc., Germany).

### *Determination of the surface density of fluorescent cells*

Dimensions of the rectangular photographic grid were calibrated with a micrometer (Olympus, Tokyo, Japan). At ×40 the grid encompassed a surface of 0.15×0.24 mm or 0.035 $mm^2$ and each cell observed within that rectangle represented 28 cells/$mm^2$ surface area within the specimen. At ×25 each observed cell within the rectangular grid represented 11 cells/$mm^2$. In specimens with uniform density, 15–20 random fields were examined.

Student's *t*-test was used to assess the differences between groups. *p* value of less than 0.05 was considered significant.

## RESULTS

Epicutaneous application of 7% TNCB (100 µl in 4 : 1 acetone : olive oil) to the back of C3H/He mice induced sensitization in all of the animals. The time courses of the response of both

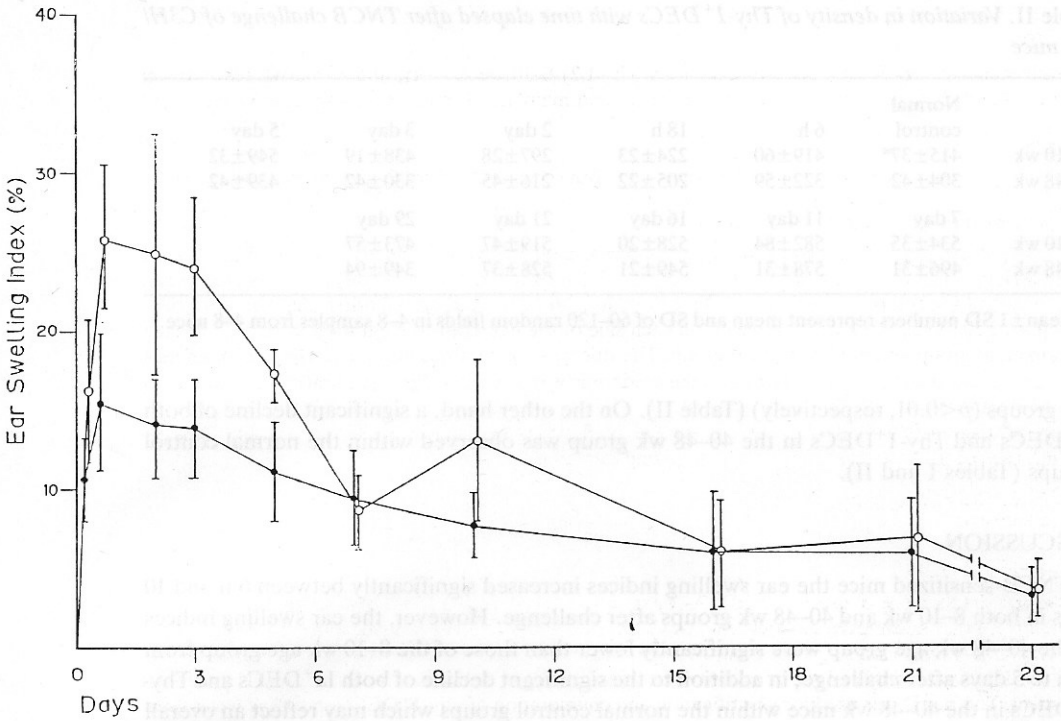


*Fig. 1.* Time course of ear swelling index after challenge. Mean±SD percentage increase in ear swelling index is shown. ○, 8–10 wk; ●, 40–48 wk.

8–10 wk and 40–48 wk age groups after challenge were assessed with ear swelling test (Fig. 1). A significant increase of ear swelling was observed between 6 h and 10 days ($p<0.05$ or $<0.01$, respectively) of both 8–10 wk and 40–48 wk age groups. The ear swelling indices of the 8–10 wk age group were significantly higher than those of the 40–48 wk group from 18 h to 5 days ($p<0.05$ or $<0.01$, respectively). Challenge with 1% TNCB on the ear resulted in a significant decrease of the densities of Ia⁺DECs from 18 h to 48 h both in 8–10 wk and 40–48 wk groups ($p<0.01$, respectively), then followed by a gradual increase, reaching a significant increase of the cell densities from 5 days to 21 days both in 8–10 wk and 40–48 wk groups ($p<0.01$, respectively) (Table I). The densities of Thy-1⁺DECs significantly decreased from 18 h to 48 h ($p<0.05$ or $<0.01$, respectively), followed by a gradual increase reaching a significant increase from 5 days to 21 days after challenge both in 8–10 wk and 40–48 wk

Table I. *Variation in density of Ia⁺DECs with time elapsed after TNCB challenge of C3H/He mice*

| | Normal control | 6 h | 18 h | 2 day | 3 day | 5 day |
|---|---|---|---|---|---|---|
| 8–10 wk | 792±41[a] | 830±75 | 352±35 | 480±49 | 824±12 | 1 092±50 |
| 40–48 wk | 578±37 | 616±72 | 299±31 | 386±41 | 607±16 | 846±49 |
| | 7 day | 11 day | 16 day | 21 day | 29 day | |
| 8–10 wk | 1 069±42 | 1 076±69 | 1 128±36 | 1 029±45 | 858±39 | |
| 40–48 wk | 793±59 | 799±12 | 784±46 | 817±35 | 577±34 | |

[a] Mean±SD numbers represent mean and SD of 60–120 random fields in 4–8 samples from 4–8 mice.

Table II. *Variation in density of Thy-1$^+$DECs with time elapsed after TNCB challenge of C3H/He mice*

| | Normal control | 6 h | 18 h | 2 day | 3 day | 5 day |
|---|---|---|---|---|---|---|
| 8–10 wk | 415±37[a] | 419±60 | 224±23 | 297±28 | 438±19 | 549±32 |
| 40–48 wk | 304±42 | 322±59 | 205±22 | 216±45 | 330±42 | 439±42 |
| | 7 day | 11 day | 16 day | 21 day | 29 day | |
| 8–10 wk | 534±35 | 582±84 | 528±20 | 519±47 | 473±57 | |
| 40–48 wk | 496±31 | 578±31 | 549±21 | 528±37 | 349±94 | |

[a] Mean±1 SD numbers represent mean and SD of 60–120 random fields in 4–8 samples from 4–8 mice.

age groups ($p<0.01$, respectively) (Table II). On the other hand, a significant decline of both Ia$^+$DECs and Thy-1$^+$DECs in the 40–48 wk group was observed within the normal control groups (Tables I and II).

## DISCUSSION

In TNCB-sensitized mice the ear swelling indices increased significantly between 6 h and 10 days in both 8–10 wk and 40–48 wk groups after challenge. However, the ear swelling indices of the 40–48 wk age group were significantly lower than those of the 8–10 wk age group from 18 h to 5 days after challenge, in addition to the significant decline of both Ia$^+$DECs and Thy-1$^+$DECs in the 40–48 wk mice within the normal control groups which may reflect an overall depression of cell-mediated immunity in aging.

We confirmed that the densities of Ia$^+$DECs first decreased post challenge, but were followed by a gradual increase in TNCB sensitized mice (7). Nevertheless, the significant increase of Ia$^+$DECs was found from 5 days to 21 days even in the period that the ear swelling indices had already returned to normal in both 8–10 wk and 40–48 wk mouse groups. This may reflect the histologic changes persisting longer than the clinical manifestations.

The exact functional properties of Thy-1$^+$DECs, especially in relation to contact allergy, have not yet been clarified. However, there is some evidence indicating that Thy-1$^+$DECs may play a role in immune responses (11, 12). In the present study, a similar dynamic change pattern of Thy-1$^+$DECs was demonstrated both in the 8–10 wk and 40–48 wk mouse groups; after challenge the densities of Thy-1$^+$DECs first decreased and then increased gradually, and significant higher densities persisted for a longer period. It may be speculated that Thy-1$^+$DECs could somehow be also involved in the pathogenesis of contact allergy.

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