KAWASAKI MED. J. VOL. 13 : NO. 1, 43-44 (1987)

## Brief Note

# Two-Color Analysis of Mouse Epidermal Cell Suspension with Anti-Ia and Anti-Thy-1 Antibodies

*Accepted for Publication on January 6, 1987*

**Key words : Thy-1 positive epidermal cell — Ia positive epidermal cell — Langerhans cell — glass adherence**

The mammalian epidermis is a heterologous epithelium which is composed of ontogenetically and functionally diverse cell populations: keratinocytes, Langerhans cells, intermediate cells, melanocytes, Merkel cells and Thy-1 positive dendritic cells. In the experiment reported here, Langerhans cells (LC) and Thy-1 positive (Thy-$1^+$) cells were observed in mouse epidermal cell (EC) suspension by two-color method using fluorescent antibodies as the green-fluorescent stain for Ia antigen and the orange one for Thy-1 protein.

EC suspensions were prepared from the ear skin of C3H/He mice as described previously.[1] The cells were incubated in Eagle's MEM on cover slip for 2 hours at 37°C. The gas phase was $CO_2$ 5%; air 95%. Non-adherent ECs were collected by centrifugation of the medium at 200 g for 5 minutes. Adherent ECs on cover slip and smear section prepared from non-adherent ECs were exposed to 1:10 dilution (PBS) of anti-$Ia^k$ monoclonal antibody at 37°C for 30-minute incubation with 1:10 dilution of FITC-anti-mouse IgG. After PBS washes, the cells were reacted with 1:100 dilution of biotin-anti-Thy-1,2 monoclonal antibody at 4°C over night, followed by 25-minute incubation with 1:20 dilution of phycoerythrin (PE)-avidin at room temperature.[1] The specimens were mounted in glycerine buffer. Fluorescence was examined with a Nikon Fluorescence Microscope using a UV filter system.

TABLE. Mean frequency (%) and ratio with standard error of $Ia^{k+}$ and Thy-$1,2^+$ cells in adherent and non-adherent ECs of C3H/He mice

| Cells | Incidence (%) of | | |
|---|---|---|---|
| | $Ia^{k+}$ | | Thy-$1,2^+$ |
| Adherent | 5.4 ± 0.3 | | 4.0 ± 1.1 |
| | 61.5 ± 0.9 | : | 38.5 ± 0.9 |
| Non-adherent | 1.5 ± 0.2 | | 3.7 ± 0.4 |
| | 41.8 ± 1.6 | : | 58.2 ± 1.6 |

The EC suspensions prepared from the ear skin of mice were double-stained for Ia antigen (green) and Thy-1 protein (orange) (Figure). $Ia^k$ antigen was not simultaneously detected with Thy-1,2 protein on a cell. The cells with Ia antigen ($Ia^+$ cells) were generally larger than Thy-$1^+$ cells. A large part of the $Ia^+$ cells seemed to have a lobulated nucleus (Figure). The incidences of $Ia^+$

中川昌次郎，方　東植，武井洋二，植木宏明

and Thy-1$^+$ cells in adherent and non-adherent ECs were shown in Table. More than one hundred fluorescent cells were examined and ratios of Ia$^+$ and Thy-1$^+$ cells were also described in the Table. Ia$^+$ cells in adherent ECs were more frequent than those in non-adherent ECs. On the other hand, there was no significant difference in the incidences of Thy-1$^+$ cells between adherent and non-adherent ECs. This suggests that adherent potential of Ia$^+$ cells (presumably LCs) to glass surface is more intense than that of Thy-1$^+$ cells. It has been known that even if LC resemble monocytes and macrophages as to origin, surface membrane markers and function, LCs are less firmly adherent cells, at least *in vitro*.[2)]

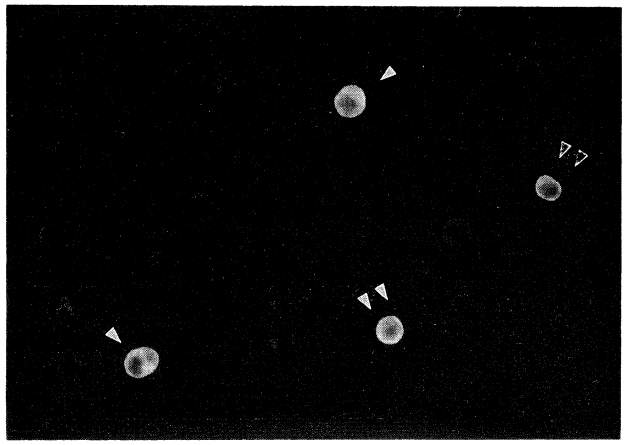

Figure. The smear section of non-adherent ECs prepared from C3H/He mouse epidermis were double-stained for Ia$^k$ antigen and Thy-1,2 protein. Fluorescein excitor demonstrates Ia$^+$ cells (arrow) and Thy-1$^+$ cells (arrows) (200×).

**Acknowledgments**

This work was supported, in part, by Kawasaki Medical School Grant (No. 61-407) for project research. We wish to thank Miss Y. Jinno for excellent technical assistance.

**Shojiro NAKAGAWA, Dongsik BANG, Yoji TAKEI and Hiroaki UEKI**

*Department of Dermatology,*
*Kawasaki Medical School,*
*Kurashiki 701-01, Japan*

**REFERENCES**

1) Bang, D., Nakagawa, S., Oka, D., Takei, Y., Jinno, Y. and Ueki, H.: The distribution of 2,4-dinitrophenyl groups on Thy-1 positive cells in the epidermis of mouse following skin painting with 2,4-dinitrochlorobenzene. Arch. Dermatol. Res. in print
2) Rasanen, L., Lehto, M., Reunala T. and Leinikki, P.: Enrichment of human epidermal Langerhans cells by attachment to erythrocyte monolayers. J. Invest. Dermatol. **86 :** 9-12, 1986