Both Epidermal Dendritic Cell Populations, Langerhans' Cells and Thy 1$^+$ Dendritic Cells are Simultaneously Stained by an Lyt-1 Monoclonal Antibody

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Both Langerhans' cells (LC) and Thy 1$^+$ dendritic epidermal cells (DEC) are bone marrow-derived epidermal cells that are thought to play an important role in immune responses. Despite their several similarities, the surface phenotypes of both cells appear to be almost mutually exclusive. We found that an anti-Lyt-1.2 monoclonal antibody that recognizes solely an epitope on certain T cells can crossreact with a shared epitope on both cell types. The morphological details of LCs and Thy 1$^+$ DECs stained by the anti-Lyt-1.2 MAb was superior to those stained by either anti-I-A or anti-Thy-1 MAb. The availability of this MAb enables us to simultaneously view both cell populations in the same sections.

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In addition to their morphological similarities, Langerhans' cells (LCs) and Thy 1$^+$ dendritic epidermal cells (Thy 1$^+$ DECs) have some features in common: both are bone marrow-derived epidermal cells (1, 2) and are sensitive to ultraviolet (UV) irradiation and glucocorticosteroids (3). However, the surface phenotypes appear to be almost mutually exclusive except Ly-5 leukocyte common antigen (4): LCs are Ia$^+$, Thy-1$^-$, Ly-5$^+$, Lyt-1$^-$23$^-$, L3T4$^{+/-}$, sIg$^-$, FcR$^+$, asioalo GM$_1^-$ and S-100$^+$, while Thy 1$^+$ DECs are Ia$^-$, Thy 1$^+$, Ly-5$^+$, Lyt-1$^-$23$^-$, L3T4$^-$, sIg$^-$, FcR$^{+/-}$, asioalo GM$_1^+$ and S-100$^-$. It is widely accepted that LCs play a central role as critical antigen presenting cells in the induction of contact sensitivity (5), while Thy 1$^+$DECs have been postulated to be involved in the down regulation of contact sensitivity (6). The contrast in the possible functional role between both cells rather suggests that localization patterns of these cells in the epidermis may not be independent of each other, but be intimately regulated by each other. This view is substantiated by a recent finding that the ratio of the LC to Thy 1$^+$ DEC influences the intensity of contact sensitivity (7). Nonetheless, studies addressing the relationships of both populations in their distributions have been few in number, probably due to the lack of antibodies by which the morphologic details of both cells can be simultaneously stained.

In an attempt to identify the epidermal invasion of murine cloned T cells locally injected, a variety of monoclonal antibodies (MAbs) to the antigens that are expressed on the surface of the T cells but not on the epidermal cells, were tested for their ability to specifically stain the invading T cells in the epidermal sheets (8). During these experiments, we noted that in addition to the T cells, epidermal dendritic cells can be also stained with an anti-Lyt-1.2 MAb obtained from Cedarlane Laboratories (Ontario, Canada), but not with other commercially available Lyt-1.2 MAb.

We report here on the reactivity of both epidermal dendritic cells in situ with the Lyt-1.2 MAb which was thought to recognize solely an epitope on T cells.

MATERIALS AND METHODS

Animals

Female C57BL/6 (B6), C3H/He and BALB/c mice were obtained from Charles River Japan, Inc. and B6-Ly-1^a/cy (B6-Ly 1^a) mice were obtained from Jackson Laboratory, Bar Harbor, ME. They were used predominantly at 8–16 weeks of age.

Monoclonal antibodies

Details of the monoclonal antibodies (MAb) used in this study are listed in Table I. These MAb preparations were diluted 1:20–1:40 in phosphate buffered saline (PBS) containing 3% bovine serum albumin and 0.1% sodium azide.

Immunofluorescence studies on epidermal sheets

Epidermal sheets were separated from the dermis by incubation in 0.02% EDTA for 2 h at 37°C. The epidermal sheets were fixed in acetone at room temperature for 20 min and stained by an indirect immunofluorescence method, as described previously (8). Briefly, the sheets were incubated overnight at 4°C with the appropriate MAb. After several washes, they were incubated for 60 min at 37°C with the appropriate fluorescein isothiocyanate (FITC)- or tetramethylrhodamine isothiocyanate (TRITC)-conjugates. For double labeling, this procedure was done with TRITC-conjugates, followed by incubation with FITC-conjugated anti-Thy 1.2 or anti-I-A. The stained specimens were mounted in glycerin-PBS and viewed through a fluorescence microscope. MAb of

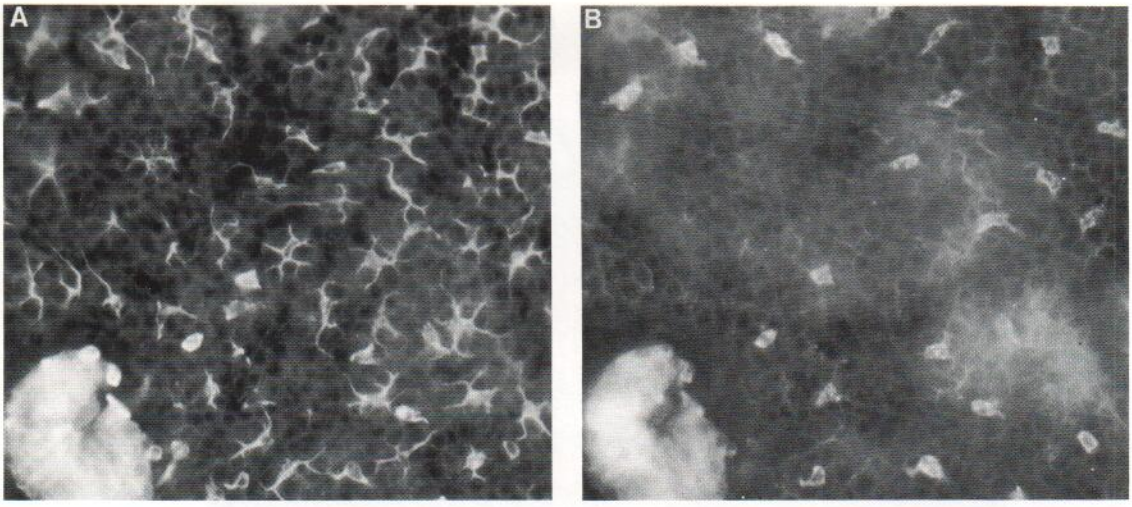

Fig. 1. Double immunofluorescence of a B6 ear epidermal sheet stained consecutively with the anti-Lyt-1.2 MAb followed by TRITC-goat anti-mouse IgG (A) and the FITC-anti-Thy 1.2 MAb (B), ×80. Thy 1.2 antigens are found on some, but not all Lyt-1.2 positive cells.

the same isotype having irrelevant specificities were used as controls.

RESULTS AND DISCUSSION

To determine whether the anti-Lyt-1.2 MAb (Cedarlane) could stain both LCs and Thy 1^+ DECs, the epidermal sheets from B6 mouse ear skin were first exposed to the Lyt-1.2 MAb followed by TRITC-conjugated goat anti-mouse IgG and then either to FITC-anti-Thy-1.2, or to FITC-anti-I-$A^{b,k}$. The results of double staining clearly showed that cells positive for the Lyt-1.2 MAb (Fig. 1 A) are also positive for Thy 1.2 (Fig. 1 B) and for I-A antigens (data not shown). The results of dopa-staining followed by the immunofluorescence procedure revealed that the Lyt-1.2 MAb is not reactive with melanocytes (data not shown). The morphological details of Thy 1 DECs and LCs stained by the anti-Lyt-1.2 MAb was superior to those stained by either the anti-Thy-1.2 or the anti-I-A MAb.

Table I. *Monoclonal antibody panel*

Specificity	Isotype	Source
Thy 1.2	rIgG2b	Becton Dickinson, Mountain View, CA
Lyt 1.2	mIgG2b	Cedarlane Laboratories, Ontario, Canada
Lyt 1.2	mIgG2b	New England Nuclear, Boston, MA
Lyt 1.2	mIgM	Meiji Institute of Health Science, Tokyo, Japan
Lyt 1.1	mIgG2a	Cedarlane Laboratories, Ontario, Canada
Lyt 2.1	mIgG3	Cedarlane Laboratories, Ontario, Canada
Lyt 2.2	mIgM	Cedarlane Laboratories, Ontario, Canada
I-$A^{b,j,k,s}$	mIgG2a	Meiji Institute of Health Science, Tokyo, Japan
I-A^k	mIgG2a	Cederlane Laboratories, Ontario, Canada

That this Lyt-1.2 staining is not specific for Lyt-1 antigen expressed on T cells was determined by the following experiment: the Lyt-1.2 MAb also reacted with both epidermal dendritic cells from mouse strains with other Lyt-1 allelic determinant (Lyt-1.1), such as C3H/He (Fig. 2) and B6-Ly 1^a and; two other commercially obtained anti-Lyt-1.2 MAbs did not bind to both epidermal dendritic cells in situations where they would have been expected to do so. Double-staining experiments using all Lyt MAbs revealed that all of I-A^+LCs and Thy 1^+ DECs displayed strongly the Lyt-1.2 reactivity but were negative with the three other Lyt MAbs (Lyt-1.1, 2.1 and 2.2) obtained from the same company, regardless of mouse strains used. The findings that as far as splenic T cells were characterized, an allotypic distribution of the Lyt-1.2 antigen was demonstrated with the use of this Lyt-1.2 MAb and that this Lyt-1.2 MAb, as well as other Lyt-1.2 MAbs, have been shown to precipitate a

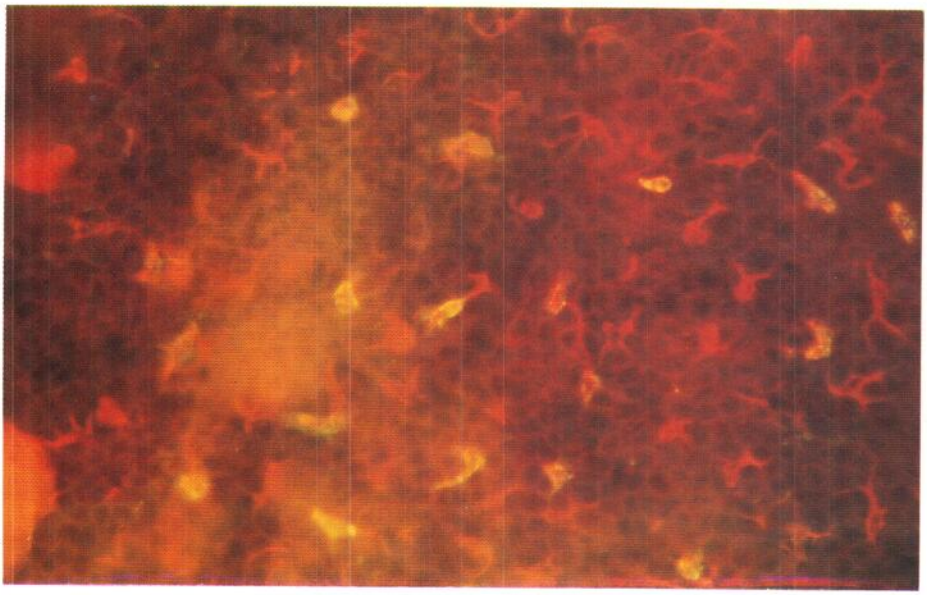

Fig. 2. Two-colour staining with the antiLyt-1.2 MAb and anti-Thy 1.2 MAb on a C3H/He ear epidermal sheet, ×80. LCs were stained with the anti-Lyt-1.2 MAb followed by TRITC-goat anti-mouse IgG, but not with the FITC-anti-Thy 1.2 MAb, resulting in a red colour. Thy 1 DECs were stained doubly with the anti-Lyt-1.2 MAb followed by TRITC-goat-anti-mouse IgG, and with the FITC-anti-Thy 1.2 MAb resulting in a yellow colour.

67-kD polypeptide identical to that previously determined for Lyt-1 on T cells, rather suggests that the antigens on LCs and Thy 1^+ DECs recognized by this MAb are not the same as found on the T cells. A similar staining pattern was also observed with another batch of this MAb, but not with the isotype-matched control MAb with irrelevant specificities. These results exclude the trivial possibility that the staining pattern demonstrated with this MAb was simply due to an accidental contamination with other MAb.

The only other MAb in the mouse that is shown to react specifically with both dendritic epidermal cells is Ly-5 MAb. In this regard, the Lyt-1.2 staining seemed to be comparable to that of Ly-5. However, the Lyt 1.2 MAb stained both dendritic epidermal cells much more intensely and evenly than did anti-Ly 5 MAb (data not shown). These findings suggest that this MAb may have a higher affinity to surface antigens that are expressed exclusively on bone marrow-derived epidermal cells. Although the nature of antigens defined by this Lyt-1.2 MAb is not known at present and the reactivity is considered to be fortuitous, the availability of this MAb that enables us to simultaneously view both cell populations in the same sections will give us more insight into the relationships between ICs and Thy 1^+ DECs.

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