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Thymic epithelial cells of severe combined immunodeficiency (SCID) mice

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To characterize thymic epithelial cells of SCID(severe combined immunodeficiency) mice in comparison with those of Balb C mice, we did an immunohistochemical study using cortical and medullary epithelial cell specific monoclonal antibodies (MoAbs), Th-3 and Th-4, as well as gel electrophoresis and immunoblotting. The thymi of SCID mice were composed of epithelial cells and a few lymphocytes. Most epithelial cells were immunostained diffusely with Th-3, which indicated that they might be "cortical-type" epithelial cells. There were a few clusters of stellate cells with dendritic processes which were negative with Th-3 but stained strongly with Th-4. Cortical type epithelial cells and most of the Th-4 reacting cells were strongly immunostained with cytokeratin antibody MNF116. By immunoblotting, cytokeratin polypeptides No. 10 and 18 were detected in both SCID and Balb C mice; however, the relative amounts of each cytokeratin polypeptides were different. With immunohistochemical and immunoblotting results, we conclude ; 1) Th-3 and Th-4 are reliable markers for cortical and medullary thymic epithelial cells in SCID mice ; 2) disorganization of cells thymic structure is mostly due to maldevelopment of medullary epithelial and T lymphocytes ; and 3) the composition of cytokeratin subfamilies of SCID mice thymi may represent a phenotypic marker of the maldevelopment of medullary epithelial cells.

Key Words : *SCID mouse, thymus, epithelial cells, immunohistochemistry*

INTRODUCTION

SCID mice are genetically defective in the recombinase system and therefore can not produce functional T and B cells with antigenic receptors (Bosma et al., 1983 ; McCune et al., 1988). SCID thymi are rudimentary, being composed of mainly epithelial cells and remarkably decreased thymocytes. In addition, their cellular elements are not organized into cortex and medulla, resembling normal thymic medulla (Custer et al., 1985). The nature and development of the thymic microenvironment in association with a lack of T cell receptor positive thymocytes has been recently assessed in SCID mice (Shores et al,. 1991). In the present study, we used two monoclonal antibodies (Th-3 and Th-4) which were developed against the stromal tissues of C57BL/6 mice (Hirokawa et al., 1986) to characterize the thymic epithelial cells. We also undertook gel electrophoresis with immunoblotting to examine

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cytokeratin subfamilies with thymic proteins to compare SCID thymi with those of Balb C mice.

MATERIALS AND METHODS

Thymi from SCID and Balb C mice were snap frozen in liquid nitrogen, cut into 4-6 um thick slices and fixed in cold acetone for 10 minutes before immunohistochemical staining. After preincubation with 0.5% normal horse serum for 20 minutes at room temperature, sections were incubated with appropriately diluted MoAbs Th-3, Th-4, MNF116(DAKO-CK) for 40 minutes. We used a Vector ABC kit for immunohistochemical staining-(Hsu et al., 1981).

For one dimensional gel electrophoresis, thymic whole protein was made from 10 SCID thymi and 2 Balb C thymi. The thymi were solved in Laemli buffer and boiled for 5 minutes. The samples were loaded on 10% agarose gel. For electrophoretic separation of whole proteins, 12.5 % polyacrylamide gel was used. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and protein transfer onto nitrocellulose paper were done. Following preincubation in PBS containing 0.05% Tween 20, the membrane was incubated in the same solution containing monoclonal antibodies MNF116. After thorough washing, nitrocellulose paper was incubated with alkaline phosphatase-conjugated goat anti-mouse antiserum (Sigma, 1 : 1000) and then visualized with alkaline phosphatase substrate (Pierce, USA).

RESULTS

SCID thymi were small, usually comprising two lobes ranging from 1-5mm.
They were embedded in retrosternal fibroadipose tissue and had a fine fibrous capsule. Microscopically they were completely devoid of lymphocytic cortex and corticomedullary organization. They were composed of epithelial cells and a few lymphocytes. The epithelial components were large, ovoid cells with abundant cytoplasm and distinct cytoplasmic membrane. Their nuclei were vesicular with a small nucleolus. Lymphoid cells were small and had hyperchromatic nuclei. They were randomly scattered. No Hassall's corpuscle was developed (Custer et al., 1985 ; Choe et al., 1992).

Most stromal cells of the SCID thymi were immunostained diffusely with MoAb Th-3 (Fig. 1a) and small foci were positive with MoAb Th-4 (Fig. 1b). In Balb C mice, Th-3 reacted specifically with cortical epithelial cells. They were diffusely stained along the cell membrane and in the cytoplasm. They had long dentritic processes forming an epithelial network embracing thymocytes (Fig. 2a). In contrast, "cortical-type" epithelial cells of SCID mice were round and closely attached to each other. Long cytoplasmic processes were not discerned with Th-3 immunostaining and a labyrinthine network was not formed(Fig. 2b). Th-4 reacting cells were multifo-

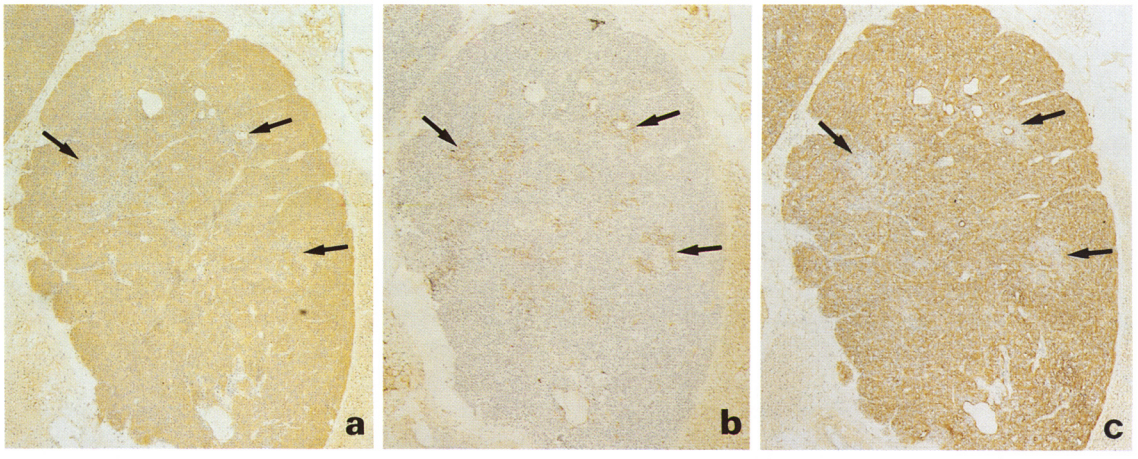

Fig. 1. Most SCID mice thymic stromal cells are diffusely immunostained with Th-3(a) and MNF116(c). There are a few foci negative with Th-3 and MNF116 but positive with Th-4(b) (arrows) (ABC, X 10).

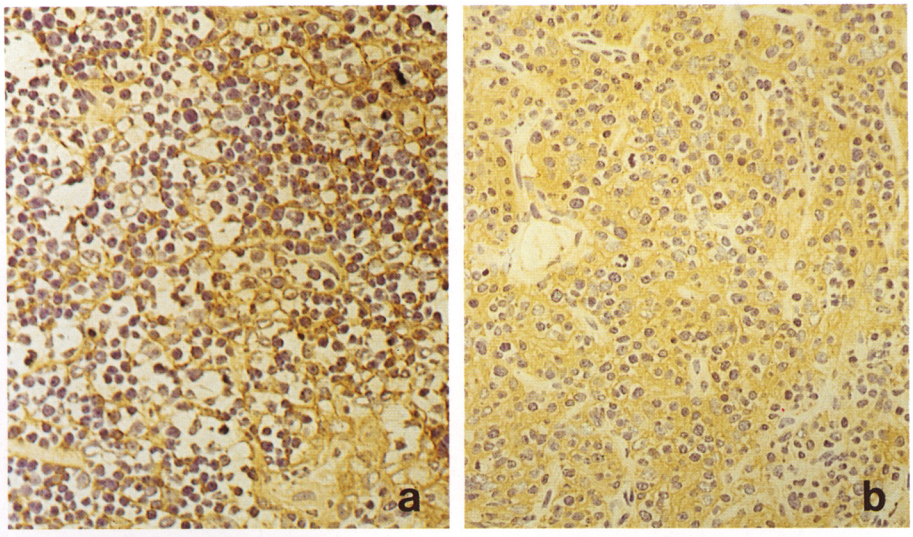

Fig. 2. Th–3 reacting thymic cortical epithelial cells of Balb C(a) and SCID(b) mice. Normal cortical epithelial cells have long cytoplasmic processes, forming an interwoven meshwork(a) (ABC, ×50). In contrast, Th–3 reacting SCID mice cortical type epithelial cells are round with no discernible cytoplasmic processes (ABC, ×25).

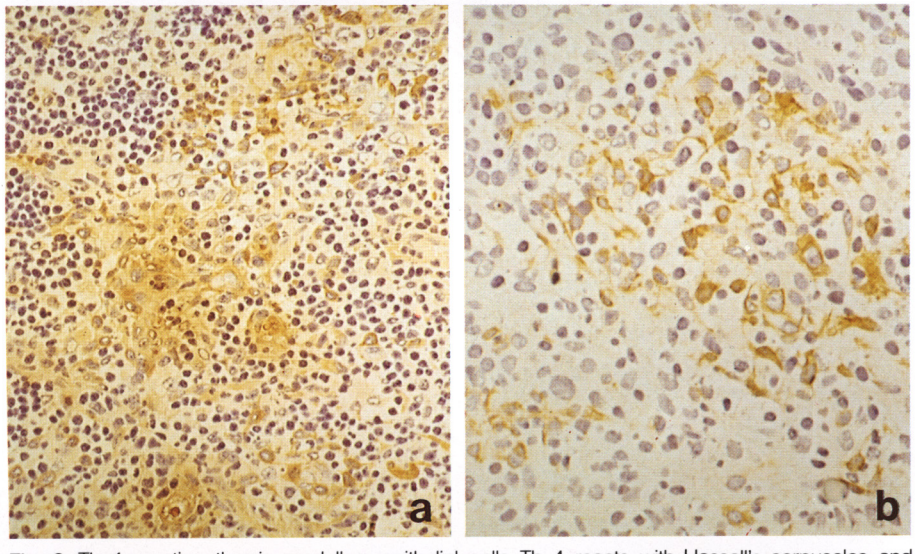

Fig. 3. Th–4 reacting thymic medullary epithelial cells Th–4 reacts with Hassall's corpuscles and medullary epithelial cells with short dendritic processes in Balb C mice(a) (ABC, ×25). SCID mice (b) Th–4 reacting thymic stromal cells of SCID mice also have short cytoplasmic processes (ABC, × 50).

cally aggregated but were not organized in SCID mice. Th-4 reacted only with medullary epithelial cells and Hassall's corpuscles in Balb C mice(Fig. 3a). They were stellate shaped with short cytoplasmic processes in SCID mice (Fig. 3b) like the medullary epithelial cells of Balb C mice (Fig. 3a). Both antibodies did not react with thymocytes.

By immunohistochemistry with MoAb MNF116,

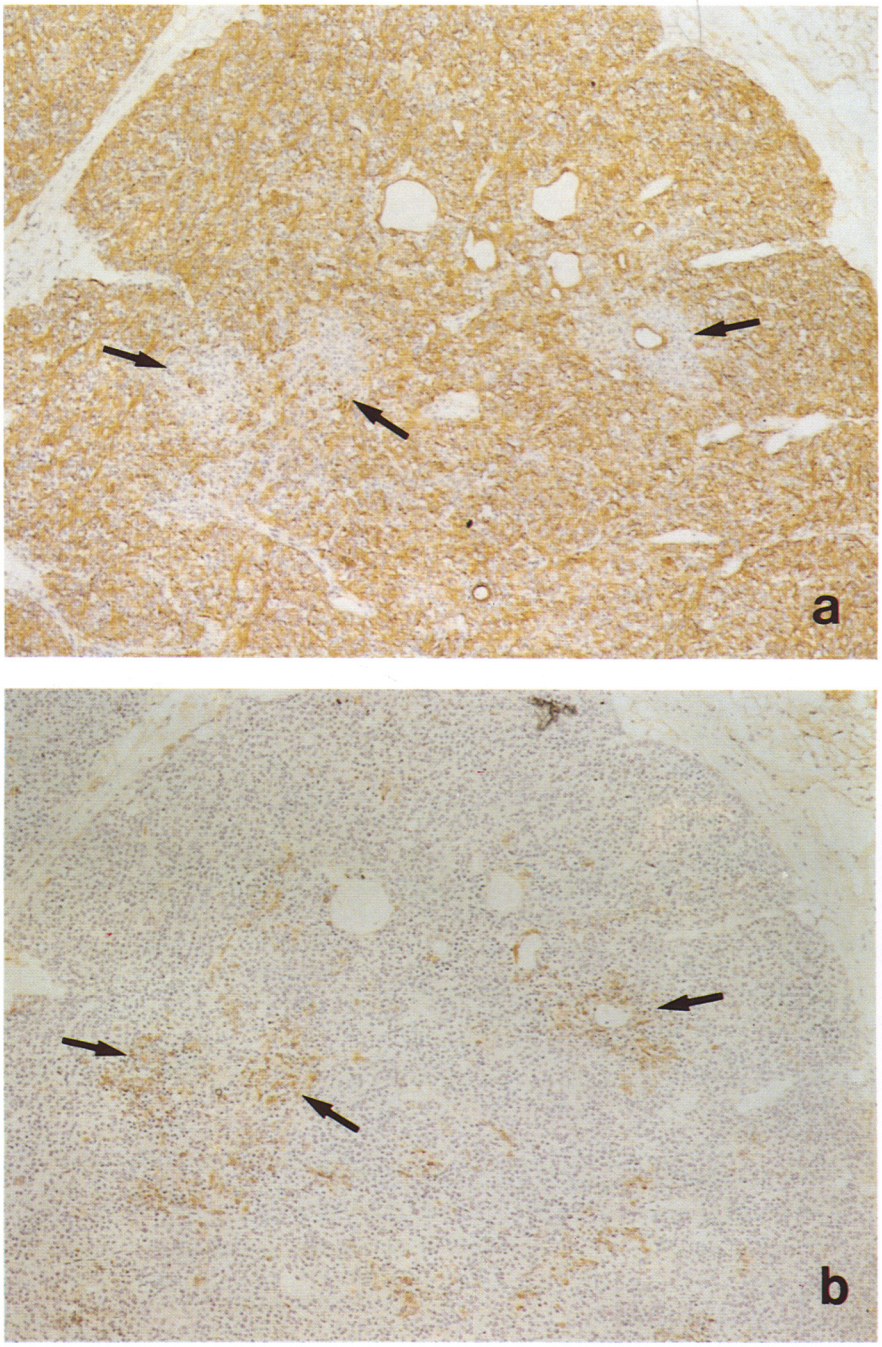

Fig. 4. SCID mice thymic epithelial cells are strongly stained with MNF116(a). A few clusters of stromal cells are not immunostained for cytokeratin but are positive with TH-4(b)(arrows) (ABC, × 16).

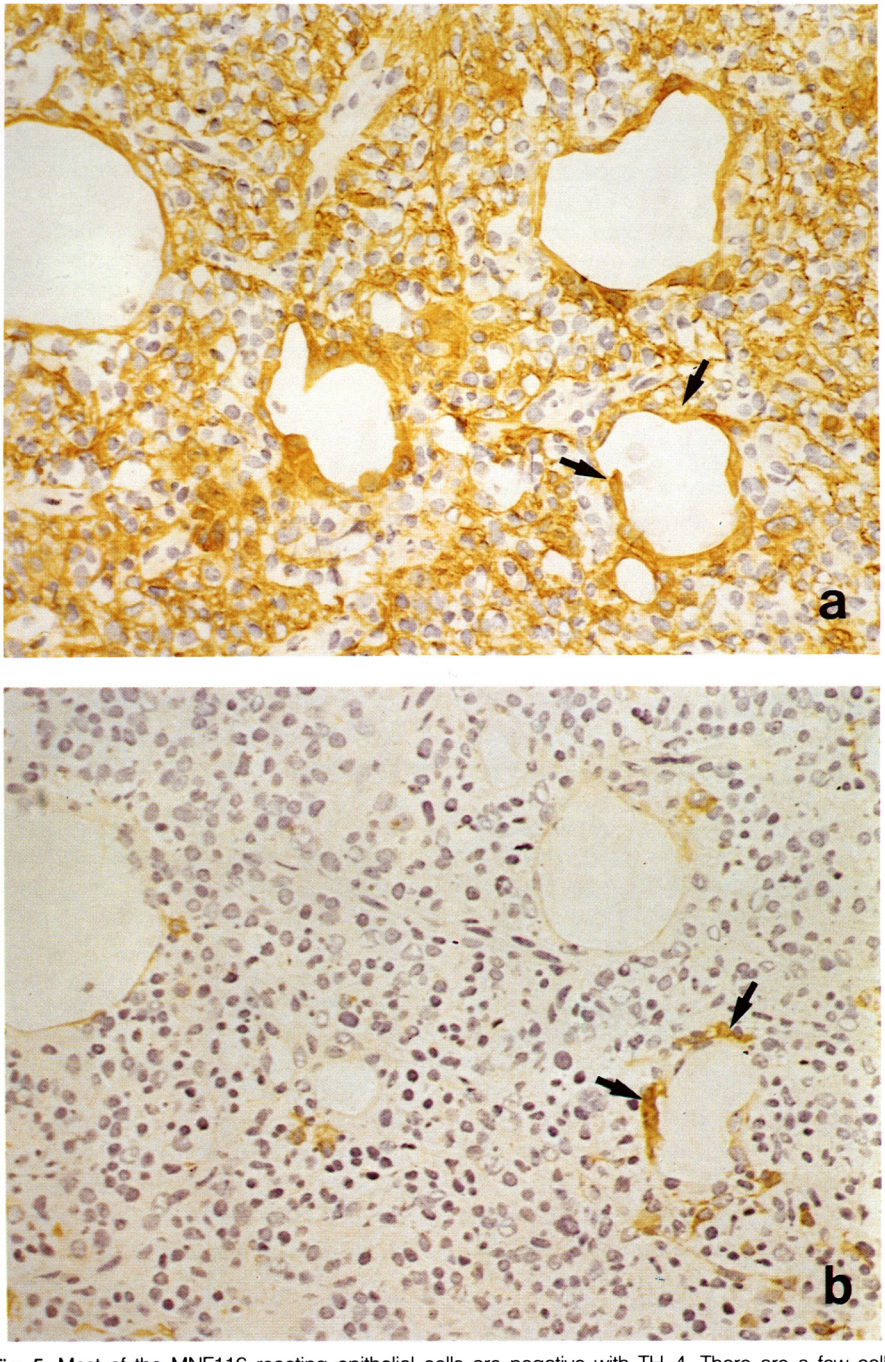

Fig. 5. Most of the MNF116 reacting epithelial cells are negative with TH-4. There are a few cells reacting with both MNF 116 and TH-4 antibodies (arrows) (ABC, ×80). a ; MNF116 b ; TH-4.

most SCID thymic stromal cells were strongly positive and revealed cytoplasmic processes (Fig. 4a). There were a few foci of MNF116-nonreacting stromal cells most of which were stained with MoAb Th-4 (Fig. 1c & Fig. 4b). There were two types of MNF reacting epithelial cells ; $MNF116^{+}/Th\text{-}3^{+}$ and $MNF116^{+}/Th\text{-}4^{+}$ cells(Fig. 5).

By one dimensional gel electrophoresis and immunoblotting, MoAb MNF recognized two polypeptides 56kd and 46kd, corresponding cytokeratins No.10 and No.18 with whole proteins of SCID and Balb C thymi. The density of blotted bands was somewhat different between SCID and Balb C mice. In SCID mice, the band at 56kd was denser than that at 46kd in contrast with those in Balb C mice (Fig. 6).

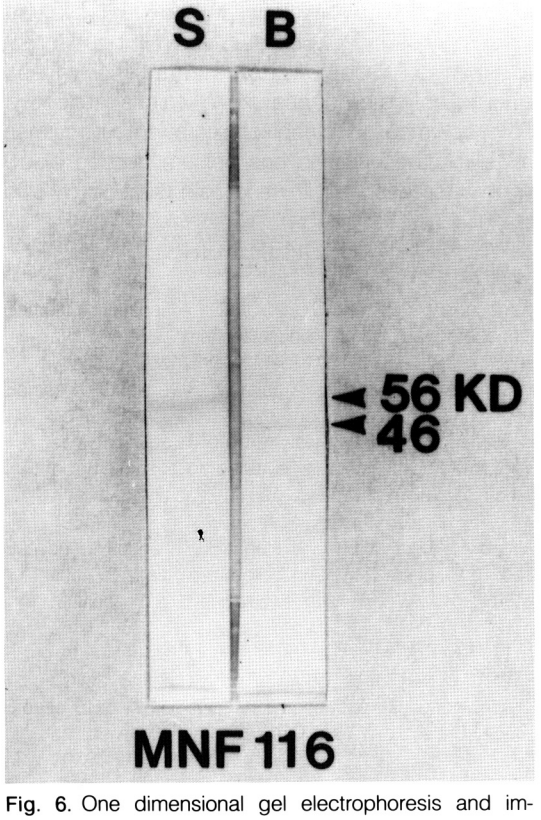

Fig. 6. One dimensional gel electrophoresis and immunoblotting Note two bands at molecular weight 56 and 46kd. Band density is different between SCID and Balb C mice.

DISCUSSION

In this study, we applied thymic epithelial specific monoclonal antibodies Th-3 and Th-4. Those antibodies are IgM in isotype, and the target antigens of Th-3 and Th-4 were not clarified. However, immunolocalization with immunoelectron microsopy showed that tonofibrils were positive with Th-3, suggesting a relationship of the antigen with cytokeratin polypeptide (Hirokawa et al., 1986). We detected coexpression of thymic stromal cells with anti-cytokeratin antibody and Th-3 in SCID mice. But staining patterns were quite different. While stromal cells were diffusely and weakly stained in the cytoplasm with MoAb Th-3, cytokeratin expression was characteristically filamentous. Difference of staining pattern may suggest that the Th-3 antigen is different from cytokeratin. In SCID mice, the cytological characteristics of epithelial cells could not be well discerned by light microscopy ; however, immunohistochemistry revealed different types of epithelial cells. So-called cortical type stromal cells of SCID mice were different from those of Balb C mice in shape and stainability. We could not discern cytoplasmic processes in Th-3 reacting stromal cells of the SCID thymus. Thus no labyrinthine meshwork was formed in SCID mice unlike in Balb C mice. These findings suggest that the cortical type stromal cells of SCID mice may be antigenetically similar to, but phenotypically different from those of Balb C mice. The phenotypic difference may be closely related to direct contact with thymocytes. Th-4 antibody reacted a few stromal cells which have short cytoplasmic processes in SCID mice. They were morphologically same as medullary epithelial cells of Balb C mice. Shores et al. (1991) also could not discern discrete cortical and medullary regions in the thymus of TcR-SCID mice by Thy-1 and did not indicate any morphologic differences between subtypes of epithelial cells.

Ontogenetically the SCID thymus may correspond to the human thymus of about 9 weeks of gestational age, because it mostly consists of epithelial cells and a few lymphocytes. The human thymus is rudimentary, being composed of epithelial cells before 9 weeks of gestational age (Hynes 1984). Thymocytes are present after 9 weeks and the corticomedullary junction can be detected by 15 weeks of gestational age. Thymic epithelial cells and lymphocytes interact with each other, playing an important role both in T cell development and

corticomedullary organization. Thymic stroma acts through direct interactions with thymocytes or production of humoral factors such as IL1 for T cell induction (Le et al., 1990). Shore et al.(1991) demonstrated corticomedullary organization in leaky SCID mice and restoration of medullary epithelial cells in SKR-SCID chimeras. Very early appearing TcR^+ thymocytes were responsible for inducing the maturation and organization of medullary epithelial cells. In this study, underdevelopment of Th-4 positive medullary epithelial cells in SCID mice may be closely related to the absence of functioning thymocytes.

Epithelial cells can be classified by their cytokeratin expression (Cooper et al., 1985). Monoclonal antibodies recognizing cytokeratins were recently applied to mouse and guinea pig thymi (Nicolas et al., 1985 & Nicolas et al., 1986). In this study, three subsets of thymic stromal cells were detected by cytokeratin expression. The first subset is Th-3 $^+$/MNF116$^+$ cortical epithelial cells. The other two subsets are Th-4 positive medullary ; a major one characterized by negative reaction with MNF116 and a minor one reactive with MNF116. Savino and Dardenne(1988) indicated that a number of mouse thymic epithelial cell subsets can be distinguished by cytokeratin expression ; CK $10^+/18^+$ cortical epithelial cells and three subsets of medullary epithelial cells. MoAb MNF116 used in this study represents cytokeratin no.10, 17 and 18. We could also detect two cytokeratin polypeptides by one dimensional gel electrophoresis and immunoblotting. They were cytokeratin no. 10 and 18. These findings indicate that SCID cortical epithelial cells are positive for cytokeatin no.10 or 18. It can be suggested that Th-4^+/MNF116$^+$ epithelial cells may have cytokeratin no.10 according to the results of Savino and Dardenne(1988). However, further study is necessary to examine specific expression of cytokeratin polypeptides with various antibodies, especially against cytokeratin no.19. Although an absolute amount of antigen can not be evaluated by one dimensional gel electrophoresis, the density of blotted bands represent relative amounts of antigen. Thus we could see the difference in relative amounts of cytokeratin no.10 and 18 between SCID and Balb C mice.

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