Medullary but Not Cortical Thymic Epithelial Cells Present Soluble Antigens to Helper T Cells

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Summary

Thymic epithelial cell lines (TECs) were established from newborn C57BL/6 mice. They were classified into two types (medullary and cortical TECs) by using the monoclonal antibody (Th-3) that recognizes the meshwork structure of thymic cortical epithelial cells. Antigen-presenting activity of each TEC was determined by using ovalbumin-specific, I-Ab-restricted helper T cell lines. It was demonstrated that the medullary but not the cortical TECs functioned as antigen-presenting cells. This is the first evidence for the functional difference between the cortical and the medullary TEC.

The thymic environment is the main site of T cell maturation/differentiation (1-5). On arrival in the thymus, bone marrow-derived T cell precursors mature into thymocytes that undergo intrathymic selection. It is generally believed that cortical thymic epithelial cells (TECs)1 are the main cell type that mediate positive selection, the process of selecting clones capable of self MHC-restricted antigen recognition, whereas medullary macrophages/dendritic cells or TECs are responsible for negative selection, the process of clonal elimination of autoreactive cells (6-8). There has been, however, no direct information that accounts for the functional difference of cortical and medullary TECs. In this report we demonstrated that cortical TECs are incapable of presenting soluble antigens to mature helper T cell lines while medullary TECs can function as APC, corroborating the proposed distinct roles played by these two types of TECs in selecting T cell repertoires.

Materials and Methods

Mice. Male and female C57BL/6 mice were purchased from Charles River Japan Inc. (Shizuoka, Japan) and were mated and reared in our specific pathogen-free mouse colony.

Establishment of TECs. Thymi obtained from newborn to 2-wk-old mice were digested in PBS containing 0.25% trypsin and 0.02% EDTA, and were suspended in Ca2+-free MEM. The cell suspension (10⁴-10⁵ per dish) was then plated on 4,000-rad irradiated Swiss 3T3 cells (7 x 10⁴ per dish) in 60-mm dishes (3802; Falcon Labware, Oxnard, CA) at 35°C in a CO2 (5%) incubator. The culture dishes were washed with PBS containing 0.02% EDTA to remove 3T3 cells and thymic fibroblastic cells, and then were washed with PBS containing 0.25% trypsin and 0.02% EDTA to make a suspension of TEC. Under an inverted microscope, single or clusters of the TECs were picked up and transferred to 24-well culture plates (3847; Falcon Labware) coated with 4,000-rad irradiated Swiss 3T3 cells and cultured in Ca2+-free MEM at 37°C. This cloning procedure was repeated several times to obtain TECs.

Antibodies. Th-3 mouse mAb against mouse thymic cortical epithelial cells was produced in our laboratory (9). M5/114 rat mAb against mouse I-Ab (10) was supplied by Dr. Uchida (NIH, Tokyo, Japan). Rabbit anti-human keratin antibody was purchased from Dakopatts (Glostrup, Denmark).

Immunocytochemistry. The established TECs were cultured on eight-hole heavy Teflon-coated slides (Bokusui Brown, New York, NY). The slides were washed twice with PBS(+) at room temperature (RT) and then fixed with 3.7% paraformaldehyde in PBS(−) for 5 min at RT. They were washed three times with PBS(−) at 4°C, and refixed with acetone for 5 min at 4°C. Then the slides were reacted with primary antibody for 2 h at RT, washed twice with chilled PBS(−) at 4°C, and reacted with FITC-conjugated second antibody for 2 h at RT. The slides were washed with chilled PBS(−), and then mounted in PBS(−)-glycerin (1:9). The immunofluorescence photographs were taken on a Nikon Optiphot equipped with an MRC-500 laser confocal scanning microscope (Japan Bio-Rad Lab. Co. Ltd., Tokyo, Japan).

Assay for APC Activity. OVA-specific, I-Ab-restricted helper T cell lines, MD-23 and KT-17, were used as indicator T cells. TECs were cultured in the presence of IFN-γ (100 U/ml) for 48 h to augment the expression of MHC class II molecules. They were irradiated at 10,000 rad and were used as APC. 10⁴ IFN-γ-

1 Abbreviation used in this paper: TEC, thymic epithelial cell.
pretreated TECs were cocultured with 10^4 helper T cells in the presence of various concentrations of antigen, OVA, for 24 h in a 96-well flat-bottomed microculture plate (0.2 ml/well) (25860; Corning, Cambridge, MA). Culture supernatant (0.1 ml) of each cultured well was assayed for IL-2/IL-4 content by measurement of [3H]thymidine incorporation by an IL-2/IL-4-dependent cell line, CTLL-2. Standard errors were generally <5% of the mean and so have not been included in the Fig. 3 and Table 1.

Results and Discussion

We have recently produced a mAb, Th-3, that specifically reacts with the meshwork structure of epithelial cells in the thymic cortex (9). This mAb is, therefore, expected to classify TECs into cortical and medullary type (see below). We have established 42 TEC lines from the thymus of newborn C57BL/6 mice and morphologically characterized them to be of epithelial nature by their cobblestone appearance (11). Among them, TEC1-4C18 and TEC1C6 were chosen for further experiments, because of their morphological appearances representing cortical (with thick cytoplasm and distinct cell borders, as shown in Fig. 1 a) and medullary TEC (with thin/broad cytoplasm and indistinct cell borders, as shown in Fig. 1 b), respectively. These two TEC lines were positive with antikeratin antibody showing fine filamentous structures (Fig. 1, c and d), and had typical desmosomes and tonofibrils in electron micrographs (data not shown). They were then examined for reactivity with Th-3 mAb. As shown in Fig. 1, e and f, TEC1-4C18 was positive with Th-3 mAb, whereas TEC1C6 was negative. Fig. 1 g confirmed that the Th-3 mAb reacts specifically with the meshwork structure in the thymic cortex but does not react with thymic medulla. According to these results and our previous observations (9, 11), it was reasoned that TEC1-4C18 was derived from epithelial cells in the thymic cortex and that TEC1C6 was derived from those in the thymic medulla.

We next examined the antigen-presenting activity of each TEC line by using OVA-specific, I-A^-restricted helper T cell

Figure 1. The phase contrast microscopy (a and b) and the immunofluorescence microscopy (c-g) of TEC1-4C18 and TEC1C6. (a) TEC1-4C18 showed a cobblestone pattern and distinct cell borders due to thick cytoplasm (scale bar, 20 μm). (b) TEC1C6 also showed a cobblestone pattern and relatively indistinct cell borders due to thin cytoplasm (scale bar, 20 μm). (c) TEC1-4C18 was positive with antikeratin antibody showing finely filamentous pattern (scale bar, 10 μm). (d) TEC1C6 was also positive with antikeratin antibody showing finely filamentous pattern (scale bar, 10 μm). (e) TEC1-4C18 was positive with Th-3 mAb (see the text) showing finely filamentous pattern (scale bar, 10 μm). (f) TEC1C6 was negative with Th-3 mAb (scale bar, 10 μm). (g) The frozen section of C57BL/6 mouse thymus stained with Th-3 mAb. The cortex was positive with the Th-3 mAb showing the meshwork pattern (left half), but the medulla was negative (right half) (scale bar, 50 μm).
lines. TEC lines were stimulated in vitro with IFN-γ for 48–72 h to induce a high level of class II MHC antigens (11). Fig. 2 illustrates the expression of class II MHC on each TEC line before and after the IFN-γ treatment. Despite comparable expression of class II MHC molecules by the two TEC lines, there is a clear difference between TEC1-4C18 and TEC1C6 in their ability to present the soluble antigen to two independently established OVA-specific, I-Aβ-restricted helper T cell lines, MD-23 (Fig. 3a) and KT-17 (Fig. 3b).

Other TEC lines were then examined for their phenotypes and the ability to present OVA by using the MD-23 as indicator cells. Table 1 summarizes the data obtained by analyzing 10 TEC lines, including TEC1-4C18 and TEC1C6. The immunostaining studies using Th-3 indicated that the five lines (TEC1-4C18, TEC1-2, TEC1-2C1, TEC1C8, and TEC1C9) were of cortical nature, and the other five lines (TEC1C6, TEC1-3, TEC2-3, TEC3-10, and TEC1-2C2) were of medullary nature. Without a single exception, only medullary-type TEC lines showed the antigen-presenting activity, whereas cortical-type TEC lines failed to present soluble antigen to helper T cells. Since stimulation of the OVA-specific helper T cell line was determined by the level of IL-2 produced in the culture supernatant, the observed failure in presenting OVA antigen of cortical-type TEC can hardly be explained by lack of costimulatory lymphokine. The failure of cortical-type TEC to function as APC was not based on a single dose of TEC used in the culture, since essentially the same results were obtained by using a wide range of TEC cell numbers.

Table 1. Medullary but Not Cortical Thymic Epithelial Cells Are Able to Present Soluble Antigens to Helper T Cells

| APC activity (IL-2 production by MD-23) | Thymic epithelial cell | Classification | No antigen | 250 µg/ml antigen |
|----------------------------------------|------------------------|----------------|------------|------------------|
| 1-4C18                                 | Cortex                 | 96             | 178        |
| 1-2                                    | Cortex                 | 120            | 95         |
| 1-2C1                                  | Cortex                 | 171            | 427        |
| 1C8                                    | Cortex                 | 101            | 158        |
| 1C9                                    | Cortex                 | 113            | 134        |
| 1C6                                    | Medulla                | 83             | 47,902     |
| 1-3                                    | Medulla                | 119            | 20,902     |
| 2-3                                    | Medulla                | 158            | 19,026     |
| 3-10                                   | Medulla                | 196            | 22,709     |
| 1-2C2                                  | Medulla                | 147            | 18,792     |

APC activity of TEC lines were assayed by the method described in Materials and Methods ([3H]Tdr uptake by CTLL-2). The TEC lines stained by Th-3 mAb are considered to be derived from the thymic cortex. The other TECs are considered to be derived from the thymic medulla (see Fig. 1).
mediated by either lymphokines or by certain accessory mole-
cules. This possibility, nevertheless, is unlikely, since the stim-
ing of the T cell line was assessed by IL-2/IL-4 production,
which is generally known to be fully triggered by solely cross-
linking (multi-valent ligand binding) of the TCR-T3 com-
plex (15). The third possibility is the blockade of peptide
binding to class II MHC molecules expressed on the surface
of cortical-type TEC. This model predicts inability of these
cells to present peptide fragments. Such a blockade could be
due to occupancy of the groove of surface class II MHC mole-
cules by something such as an invariant chain or by internal
self-peptide, in case the cells do not synthesize invariant chains.
It is also possible that some unidentified molecules are bound
on the surface class II MHC molecules enforcing a tertiary
structure not suitable for peptide binding. The fourth and
the most simple explanation is the differential expression of
the MHC class II molecules in the cortical and in the medul-
lar TECs. Interestingly, two groups have reported the ex-
pression of a novel MHC class II molecule in thymic medulla
but not cortex (16, 17). This novel MHC class II molecule
might be involved in the presentation of soluble antigens to
helper T cells, particularly in the thymic environment.

It may sound rather surprising that the cortical-type TEC
lines failed to present soluble antigens to helper T cells,
because it is generally believed that the thymic cortex is the
microenvironment where the positive selection of MHC-
restricted nominal antigen recognition structure of T cells
is operated. One assumption is that class II MHC antigens
expressed on cortical TEC lines are free of invariant chain
(ii) and preoccupied by self-derived peptides, which might
determine the "self-MHC restriction," and therefore antigenic
peptides derived from exogenous nominal antigen are unable
to be presented by cortical TECs. It would be advantageous
for the host immune system that this mechanism might pre-
vent an undesirable stimulation by too many exogenous an-
tigens that may permeate the thymic cortex (18).

In conclusion, we classified TEC lines, which we recently
established from the thymus of newborn C57BL/6 mice, into
cortical and medullary TEC by immunostaining with Th-3
mAb, and then demonstrated that the medullary TEC lines
are able to present soluble antigens to helper T cells, whereas
the cortical TEC lines are unable to do so. These results
strongly suggest that the cortical and the medullary TEC
may play functionally distinct roles in the selection of the
T cell repertoire.

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