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A Monoclonal Antibody to Cell Surface Antigen of Human Thymic Epithelial Cell

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The cell surface molecule identified by a monoclonal antibody(TE-1) to human thymic epithelial cell showed the specificity for thymic epithelial cells of both the cortex and medulla. TE-1 reacted with the epithelial cells of normal thymus and thymoma in fresh frozen tissues. The antigen recognized by TE-1 was mostly confined to the cell surface membrane and arranged in reticular network with long processes between thymocytes. On immunohistochemical analysis, TE-1 did not recognize normal epithelial cells of the uterine cervix, skin and stomach, and neoplastic cells of squamous cell carcinoma and gastric adenocarcinoma, all of which were stained with anti-cytokeratin monoclonal antibody. Among the tumor cell lines tested with flow cytometry, most of epithelial and all of hematopoietic cell origin were not labeled with TE-1.

In summary, TE-1 appears to be a monoclonal antibody against a surface antigen of human thymic epithelial cell that is immunohistologically different from known epithelial cell surface antigens reported so far.

Key Words : *Monoclonal antibody, surface antigen, thymic epithelial cell, human*

INTRODUCTION

The thymus gland is composed of a reticular framework of epithelial cells influencing T-cell precursors to undergo differentiation into mature T lymphocytes. Thymic epithelial cells express class I and class II major histocompatibility complex antigens and are thought to be responsible for positive and negative selection of thymocytes(Sprent et al., 1988 ; Schwartz RH, 1989 ; Nikolic-Zugic J,1991). Furthermore, thymic epithelial cells contain a variety of well-defined thymic hormones, such as thymulin, thymopoietin and thymosin, all of which are known to induce certain stages of T-cell maturation. In spite of the central role of the thymic microenvironment in T-cell maturation, little is known regarding the differentiation processes of the thymic epithelium itself and the antigenically definable subsets of cells within the non-lymphoid component of the thymic microenvironment. Due to a paucity of reagent antibodies specific for defined cell surface molecules of thymic stromal cells, it has been difficult to place the in vitro activity of many of these thymic stromal cell lines in the physiological context of the thymic microenvironment. We have attempted to produce monoclonal antibody against the thymic stromal cells. In this study we describe a monoclonal antibody to a novel cell surface antigen of human thy-

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mic epithelial cell, which is different from known surface antigens in terms of immunohistological reactivity.

MATERIAL AND METHODS

Materials

Fresh tissues of various types of tumors and normal tissues surgically resected, and postnatal thymi were obtained from the Department of Pathology of Seoul National University Hospital.

Normal human thymi were collected from patients who had portions of their thymus removed during corrective cardiac surgery. Human tumor cell lines used for screening included H9, CCRF-CEM, K562, FaDu, A253, SK-N-MC and SNU-1.

Production of TE-1 mAb

After Balb/c mice were immunized with 1×10^7 human thymic epithelial cells at 2-week intervals for 2 months, the spleen cells were fused with 1×10^7 SP2/0-Ag 14 mouse myeloma cells using polyethylene glycol (PEG 4000). One resulting hybridoma clone, whose supernatant was reactive to human thymic epithelial cells, was named TE-1.

Immunofluorescence Method

Several tumor cell lines were freshly prepared and stained by indirect immunofluorescence method employing TE-1 mAb followed by FITC-conjugated goat anti-mouse and analyzed by a flow cytometer (FACScan, Becton-Dickinson, CA, U. S. A.).

Immunoperoxidase Method

Snap frozen sections of a variety of normal human tissues were treated with 3% (v/v) H_2O_2 in distilled water for 10 minutes at room temperature for blocking endogenous peroxidase. Nonspecific immunoglobulin binding was blocked with 3%(v/v) normal goat serum. Sections were incubated with TE-1, anti-cytokeratin mAb(MW ; 42KD, 55KD ; Dako) and anti-epithelial membrane antigen mAb-(Dako) in a moist chamber overnight at 4℃ respectively. After rinsing in phosphate buffered saline (PBS), sections were incubated for 30 minutes in affinity-purified biotinylated goat anti-mouse IgG, IgA and IgM, followed by 1 : 400 dilution of streptadividin horseradish peroxidase product and was visualized by 3.3'-diaminobenzidine(Sigma, 0.5 mg/mL in 50 mM tris-Hcl, pH 7.4). Counterstain was not performed and the reaction pattern was analyzed based on serial hemotoxylin-eosin stained sections.

Determination of Antibody Isotype

The isotype of TE-1 was determined by enzyme immunoassay using ScreenType™ (Boeringer Manheim, Biochemicals). Isotyping was performed with goat anti-murine isotype specific antisera (IgG1, IgG2a, IgG2b, IgG3, IgM, kappa, lambda) followed by peroxidase-labeled swine anti-goat IgG as the secondary antibody. With the addition of ABTS (2,2'zinobis[3-ethylbenzothiazoline -6-sulfonic acidic]) and hydrogen peroxide(H_2O_2) as substrates, positive samples turned out to be an intense blue green color.

RESULTS

Tissue distribution of the TE-1 antigen tested on fresh and paraffin-embedded sections of normal and neoplastic tissues.

In cryostat sections of thymic tissue, the whole thymic epithelial network was intensively stained with TE-1 and two anti-cytokeratin antibodies respectively while anti-EMA (epithelial membrane antigen) showed localized strong reactivity in Hassall's corpuscles. The antigen recognized by TE-1 was mostly confined to the cell surface membrane and arranged in reticular network with long processes between thymocytes that were not stained at all. The Hassall's corpuscle was nonspecifically positive which was positive in negative control also (Fig. 1). The cultured thymic epithelial cells were intensively labeled with TE-1 (Fig. 2). When paraffin embedded tissue sections of normal thymic tissue and thymoma were tested, those were negative for immunoreactivity to TE1 but positive for two anti-cytokeratin antibodies. The cryostat sections of thymoma, which showed histologic features of predominant epithelial cell component in hematoxylin-eosin sections, also exhibited intense immunoreactivity to TE-1 and two anti-cytokeratin antibodies. The TE-1 could not recognize normal epithelial cells of the uterine exocervix, skin and stomach, and neoplastic cells of squamous cell carcinoma and gastric adenocarcinoma, all of which were stained with two anti-cytokeratin and epithelial membrane antibodies. Lymphoid cells in the germinal center,

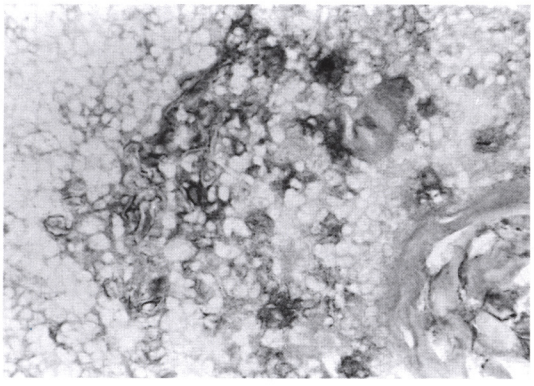

Fig. 1. Immunohistochemical staining of the human neonatal thymus. The medullary thymic epithelial cells were stained with TE-1. The antigen recognized by TE-1 was mostly confined to the cell surface membrane and arranged in reticular network with long processes between thymocytes that were not stained at all. The Hassall's corpuscle was nonspecifically positive. (ABC and DAB method, ×200)

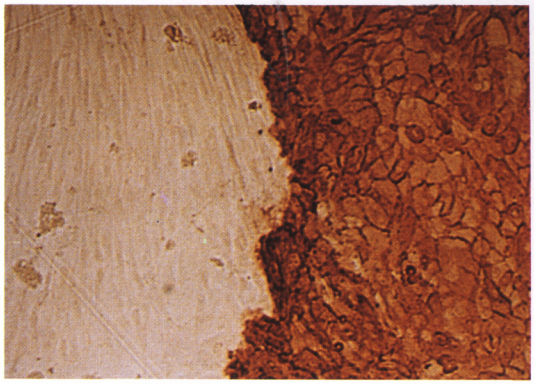

Fig. 2. Immunohistologic analysis of TE-1 antigen in cultured thymic epithelial cells. The cultured thymic epithelial cells were incubated with TE-1, then with biotinylated anti-mouse Ig, and streptavidin-peroxidase conjugate followed by freshly prepared 3.3'-diaminobenzidine solution. The thymic epithelial cells (right side) show intense staining in contrast with fibroblasts (left side) that are not stained at all (×40).

Table 1. The results of immunoperoxidase analysis of various normal tissues

Tissue	TE - 1	Cytokeratin (42KD)	Cytokeratin (55KD)	EMA
Thymus	+	+	+	+[a]
Uterine cervix	−	−	+	+
Stomach	−	+	+	+
Tonsil	−	−	−	−
Lymph node	−	−	−	−
Skin(anus)	−	+	+	+

a : Immunoreactivity was localized to Hassall's corpuscles

Table 2. The results of immunoperoxidase analysis of various neoplastic tissues

Diagnosis	TE - 1	Cytokeratin (42KD)	Cytokeratin (55KD)	EMA
Thymoma	+	+	+	−
SCC[a] (Uterine cervix)	−	+	+	+
Gastric adenocarcinoma	−	+	+	+
Colonic adenocarcinoma	−	+	+	+
Hepatocellular carcinoma	−	+	+	+

a : Squamous cell carcinoma(SCC)

mantle zone and interfollicles as well as in secondary lymphoid organs such as the lymph node and tonsil, were not stained at all. The results of the immunohistological study are summarized in Table 1 and 2.

Distribution of the TE-1 Antigen in Various Tumor Cell Lines Analyzed by Immunofluorescence

Among the tumor cell lines analyzed by a flow cytometer, the two tumor cell lines (FaDu, A253) of epidermoid origin showed some degree of im-

Table 3. The flow cytometric analysis of TE - 1 antigen in various tumor cell lines

Cell lines	Origin	Results(%)[a]
H9	T cell leukemia	16.1
CCRF - CEM	Acute lymphoblastic leukemia	12.7
K562	Chronic myelogenous leukemia	2.0
FaDu	Squamous cell carcinoma, pharynx	50.4
A253	Epidermoid carcinoma, submaxillary gland	50.8
SK - N - MC	Neuroblastoma	26.7
SNU - 1	Gastric adenocarcinoma	4.8

a : Percent of positive cells

munoreactivity to TE-1, while hematopoietic tumor cell lines (H9, CCRF-CEM, K562), neuroblastoma (SK-N-MC) and gastric adenocarcinoma(SNU-1) were negative for immunoreactivity.(Table 3)

Determination of Antibody Isotype

The subclass of TE-1 was determined by enzyme immunoassay, and it was shown to be of the IgG2a subclass.

DISCUSSION

The results of the present study demonstrated that the cell surface molecule identified by monoclonal antibody TE-1 was present on the reticular framework of thymic epithelial cells of normal thymus and neoplastic cells of thymoma in fresh frozen tissues. This suggests that TE-1 antigen is on the cell surface of thymic epithelial cell.

In an attempt to find the inductive microenvironmental signals that promote T cell differentiation and positioning, many investigators have raised a large number of mAb against the antigens in/on thymic epithelial cells, especially human(Mc Farland et al., 1984 ; De Maagd et al., 1985 ; Dispasquale et al., 1990) and mice(Hirokawa et al., 1986 ; Small et al., 1989 ; Godfrey et al., 1990). These mAbs showed phenotypic heterogeneity within a thymic microenvironment distinguishing three distinct epithelial compartments : subcapsular/medulla, cortex and Hassall's corpuscles. However, TE-1 could identify all thymic epithelial cells of both the cortex and medulla with patterns of even distribution.

We have tried to distinguish TE-1 antigen from known surface antigens of thymic epithelial cells, especially cytokeratin and epithelial membrane antigen. There is supportive evidence that TE-1 antigen seems to be different from cytokeratin and epithelial membrane antigen. First, the tissue distribution of TE-1 antigen is unique : on immunohistochemical analysis TE-1 is intensively reactive with fresh-frozen tissue of normal thymus and thymoma. This mAb could not recognize normal epithelial cells of the uterine exocervix, skin and stomach and neoplastic cells of squamous cell carcinoma, hepatocellular carcinoma and gastric adenocarcinoma, although some cells of epidermoid tumor cell lines showed reactivity to TE-1 monoclonal antibody on flow cytometric analysis. Second, TE-1 could not react with surface antigen of paraffin-embedded tissue that exhibited intense immunoreactivity to two anti-cytokeratin mAb.

Although a number of mAb reacting with thymic stromal cells have been described in animals and humans, based on patterns of reactivity with extrathymic tissues, it appears that TE-1 is detecting novel stromal cell surface molecules. De Maagd et al reported a monoclonal antibody named as IP1 that was raised against human thymic stromal cells and binds to all epithelial cells of both the cortex and medulla(De Maagd et al.,1985). In addition to the reactivity of IP1 to thymic epithelial cells, IP1 also bind to epithelium of tonsil, skin, gut and kidney, indicating that the antigen might be a general epithelial cell marker. MR 10 and 19 reported by Gaudecker et al recognized intracellular molecules within subcapsular, perivascular and some medullary epithelium(Gaudecker et al., 1989). However, TE 1 antigen was a cell surface molecule that was absent in tonsil, stomach and skin.

It is also clear, based on patterns of intrathymic distribution and extrathymic labeling , that TE-1 antigen is distinct from cell surface molecules related with intercellular adhesion such as ICAM-1, CD58, CD11a and CD18. The anti-CD54 and CD11a mAb recognize subcapsular thymocytes and epithelium as well as thymic accessory cells within the medul-

la, the distribution of which closely resembles that of MHC class II molecule (Harvey et al., 1990 ; Singer et al., 1990). Recently, some authors have reported several cell surface antigens of mouse thymic epithelial cell involved in thymocytes and thymic epithelial cell adhesion(Couture et al., 1990 ; Kinebuchi et al., 1991). Further functional and biochemical analysis of TE-1 antigen would have to follow.

In summary TE-1 is a monoclonal antibody to a surface antigen of human thymic epithelial cells that appears to be immunohistochemically different from known thymic epithelial antigens reported so far.

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