Immunohistological Analysis of Thymic Tumors with PE-35 Monoclonal Antibody Reactive with Medullary Thymic Epithelium

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ABSTRACT

PE-35 mouse monoclonal antibody (MoAb) (IgG1) detecting an epithelial antigen with a molecular weight of 35,000 was characterized serologically. Immunoperoxidase staining and double immunoenzymatic staining showed that PE-35 antigen is predominantly on nonlymphoid cells in the medulla of thymus. By immunoelectron microscopy, thymic epithelial cells in the medulla were positive with PE-35 MoAb, but macrophages, interdigitating reticulum cells, and thymocytes were negative with this MoAb, which demonstrated that PE-35 is a valuable marker for medullary epithelium.

Using PE-35 and other MoAbs detecting thymic epithelial antigens (TE-3A, RFD-4, TE-4, and HLA-DR), 25 thymomas were studied, together with 6 other tumors of thymic origin. Among 25 thymomas, all 6 cases of epithelial type and 8 of 14 mixed lymphoepithelial type were positive with PE-35 MoAb, but only one of 5 lymphocytic type was positive. PE-35 antigen has a tendency to be expressed in the cases retaining medullary type thymocytes, with the phenotype of cluster of differentiation (CD) 11/CD3*/CD6*, and also in the area of medullary differentiation. TE-3A, RFD-4, and TE-4 MoAbs reacted with most thymoma cases regardless of the types. HLA-DR was, however, expressed on a part of thymomas and the phenotype combined with that of PE-35 was as follows: PE-35*/HLA-DR*, 8 cases; PE-35*/HLA-DR*, 8 cases; PE-35*/HLA-DR*, 8 cases; PE-35*/HLA-DR*, one case. The results suggested that thymoma may originate from different subsets and/or different stages of thymic epithelium.

INTRODUCTION

Thymomas are defined as neoplasms arising from thymic epithelial cells associated with nonneoplastic lymphoid cells in various proportions. These neoplastic epithelial cells, sharing morphological features with their normal counterparts, exhibit a degree of heterogeneity (1). The association of thymoma with immunoregulatory syndromes such as myasthenia gravis is also well known (2). The biological basis of the diversity or heterogeneity displayed by different thymomas is, however, poorly understood.

In normal thymus, supporting mesh work, mainly composed of epithelial cells, plays an essential role in T-lymphocyte differentiation (3). This effect of the thymus is considered to be mediated by production of thymic hormones as well as by direct cell-to-cell interactions with epithelial cells and also with other nonlymphoid cells (4). Three different epithelial cell types are distinguished on the basis of their localization: subcapsular, cortical, and medullary (2). Thymic nurse cells have also been described in thymic midcortex in vivo (5), but their existence as a separate entity has not been well recognized (6). At the ultrastructural level, these epithelial cells have distinctive features. IDC's and macrophages are also present as nonepithelial components in the normal thymus (2).

The recent progress in cell phenotyping with monoclonal antibodies has expanded the information for both the epithelial and lymphoid elements of the normal human thymus. The cortical epithelium strongly expresses HLA-DR antigen, but the medullary epithelium does not seem to express large amounts of HLA-DR. TE-3A is also present predominantly in cortical epithelium (7), and Leu7 (HNK-1) MoAb stains epithelium in the outer cortex (8). In contrast, RFD-4 (9), TE-4 (10), A2B5 (11), and MR10 (12) are expressed in both subcapsular and medullary epithelium, similar to thymosin- 

MATERIALS AND METHODS

Tissues. Thirty-one thymic tumors were collected from various hospitals in Japan, and immunohistological analysis was carried out with the MoAbs listed in Table 1. Twenty-five cases of thymoma in this study were classified as 6 epithelial, 14 mixed lymphoepithelial, and 5 lymphocytic type. The rest were two thymic carcinoma, three carcinoid, and one small cell carcinoma. Histological classification is based on the report by Bernatz et al. (15) with a slight modification.

Three normal thymuses of children (2 to 4 years old) were collected at the time of corrective cardiovascular surgery. Thymic tumors and thymus specimens were divided in two: one part was processed for routine histological examination, and the other was snap-frozen in O-chlorotoluene compound (Ames, Elkhart, IN) and stored at −70°C for immunoperoxidase and double immunoenzymatic staining. Immunoelectron microscopy was also carried out for selected specimens.

Antibodies. The MoAbs detecting thymic epithelial antigens except PE-35 were obtained through the third workshop on Human Leukocyte Differentiation Antigens. TE-3A, RFD-4, and TE-4 MoAbs were originally produced by Mcfarland et al. (7), Bofill et al. (9), and Haynes et al. (10), respectively, and previously reported by the original investigators. PE-35 MoAb (IgG1) was raised against small cell lung cancer and detects panepithelial antigen with a molecular weight of 35,000, as described previously (14). CO17-1A and GA73.3 MoAbs showing a

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: IDC, interdigitating reticulum cell; MoAb, monoclonal antibody; CD, cluster of differentiation.
similar reactivity to PE-35 MoAb as well as goat anti-idiotypic antisera produced against these two MoAbs were kindly provided by Dr. D. Herlyn of Wistar Institute, Philadelphia, PA, who reported previously that these MoAbs detect the different epitopes on the same antigen molecule (16). NE-25 MoAb (IgG1) defining neural and/or (neuro)-endocrine differentiation antigen with molecular weight of 25,000/150,000 was also used (14). Rabbit anti-SI00 serum reactive with IDC was kindly provided by Dr. H. Hidaka of Meiji University School of Medicine, Tsu, Japan (17). Rabbit anti-keratin serum (A575) was purchased from Dakopatts (Copenhagen, Denmark). MoAbs detecting T-cell antigens, CD1(OKT6; Orthomune, Raritan, NJ), CD2(Leu5; Becton Dickinson, Mountain View, CA), and CD3(OKT3; Ortho- mune), as well as anti-HLA-DR MoAb (L243; Becton Dickinson), were also purchased commercially. Tp120 (CD6) was generated in our laboratories as described (18).

**Immunoperoxidase Staining for Light and Electron Microscopy.** Six-μm frozen sections of thymuses and thymomas were fixed in acetone, air-dried, washed, and then stained by immunoperoxidase staining techniques. For light microscopic observation with MoAbs, avidin-biotin-peroxidase complex method was used with Vectastain avidin-biotin-peroxidase complex kit (Vector, Burlingame, CA) as described (19). In the case of rabbit antisera, peroxidase-labeled goat anti-rabbit IgG (Dakopatts) was used as a second antibody and further reacted for peroxidase activity with diaminobenzidine-tetrahydrochloride as described (19).

Double immunoenzymatic staining procedure was performed after periodate-lysine-paraformaldehyde fixation as described (20). The immunoperoxidase staining was carried out first with anti-SI00 antisera using the indirect peroxidase technique, and then immunocolloidal gold-phosphatase monoclonal anti-alkaline phosphatase (APAAP) method was conducted with an immunocolloidal gold-phosphatase monoclonal anti-alkaline phosphatase kit (Zymed, San Francisco, CA) for staining with PE-35 MoAb.

For electron microscopic observation for normal thymuses with PE-35 MoAb, a two-step immunoperoxidase method was used after periodate-lysine-paraformaldehyde fixation. Peroxidase-labeled F(ab')2 rabbit anti-mouse IgG + A + M (Zymed) was used as a second antibody and further reacted for peroxidase activity with diaminobenzidine-tetrahydrochloride and tetraoxide. After washing in distilled water, they were dehydrated and embedded in Epon mixture as described (19).

**Biochemical and Epitope Analyses of PE-35 Antigen and Idiotype Analysis of PE-35 MoAb.** The SCLC-SA cell line, derived from small cell lung cancer, was labeled with 125I using Iodogen (Pierce Chemical, Rockford, IL) and solubilized with 0.5% Nonidet P-40. The labeled cell lysates were immunodepleted with either PE-35 or GA73.3 MoAb, and then immunoprecipitated with either GA73.3 or PE-35 MoAb, respectively, as described (21). Immunoprecipitates were analyzed by sodium dodecyl sulfate-polycrylamide gel electrophoresis in reduced conditions.

For analysis of the epitope detected by PE-35 MoAb, the SCLC-SA line was preincubated on ice with either CO17-1A or GA73.3 MoAb (100 μg/ml) followed by incubation with biotinylated PE-35 MoAb (10 μg/ml) and avidin-fluorescein isothiocyanate (20 μg/ml) (Vector) (22). The fluorescence profile was analyzed by cytofluorometry (FACS analyzer I; Becton Dickinson).

The idiotype of PE-35 MoAb was also studied by testing against the anti-idiotypic antisera generated against either CO17-1A or GA73.3 MoAb by enzyme-linked immunosorbent assay (22).

**RESULTS**

Reactivity of PE-35 and Other MoAbs Detecting Thymic Epithelial Antigens Tested against Normal Thymuses. Four MoAbs, PE-35, TE-3A, RFD-4 and TE-4, detecting thymic epithelium were tested against normal thymuses by the immunoperoxidase staining method, and the results are summarized in Table 1, together with those with additional two MoAbs and one polyclonal anti-keratin antibody. PE-35 MoAb reacted apparently with nonlymphoid cells in the medulla, but it did not react with any other components of thymus (Fig. 1A). By immunoelectron microscopy, surface membrane of epithelial cells were stained with PE-35 MoAb, whereas macrophages, IDC, and lymphocytes were negative (Fig. 2). Double immunoenzymatic staining with PE-35 MoAb and anti-SI00 antisera showed that epithelial cells were positive with PE-35 MoAb, while IDC were SI00 positive (data not shown). The staining pattern of normal thymus with PE-35 MoAb was quite similar to that observed with either CO17-1A or GA73.3 MoAb produced by Herlyn et al. (16) (data not shown). We showed here that PE-35 MoAb was reactive with medullary epithelial cells in the thymus, although we originally reported that PE-35 antigen was present on most epithelial cells of various other tissues (14).

In contrast to PE-35, TE-3A MoAb (7) predominantly stained epithelial cells in the cortex (Fig. 1B). Both RFD-4 (9) and TE-4 (10) MoAbs reacted with epithelial cells in the medulla and the subcapsular cortex, but not with those in the cortex (Fig. 1, C and D). All these MoAbs seemed to stain Hassall's corpuscles, but no definitive results were obtained because of the inevitable background staining. Anti-HLA-DR MoAb (L243) showed preferential staining of the cortical epithelial cells. Rabbit anti-keratin antisera (A575) strongly stained most thymic epithelial cells as well as Hassall's corpuscle.

**Biochemical and Epitope Analyses of PE-35 Antigen and Idiotype Analysis of PE-35 MoAb.** Recent reports by three independent laboratories suggested that the biochemical and immunohistological nature of CO17-1A and GA73.3 epithelial antigens was similar to that of PE-35 antigen (16, 22, 23). Thus, sequential immunoprecipitation was performed to compare the molecules recognized. As shown in Fig. 3, the antigen detected by PE-35 MoAb was specifically depleted by preincubation with GA73.3 MoAb, but not with NE-25 (a negative control). Similar results were also obtained in the reverse combination of the MoAbs. These results, together with those by Herlyn et al. (16), demonstrated that these three MoAbs, PE-35, GA73.3, and CO17-1A, were all directed against the same Mr 35,000 glycoprotein molecule.

To investigate the epitopes recognized by three MoAbs, cross-blocking experiments were performed (Fig. 4). As shown in Fig. 4C, preincubation of PE-35* SCLC-SA cells with CO17-1A MoAb did not alter the binding of biotinylated PE-35 MoAb to the target cells. In contrast, GA73.3 MoAb exerted the distinct blocking effect, showing that PE-35 and GA73.3 MoAbs detect a quite similar to or identical epitope on Mr 35,000 antigen, but CO17-1A MoAb recognizes a different one.
Fig. 1. Immunoperoxidase staining of a normal thymus with PE-35 and three other MoAbs, TE-3A, RFD-4 and TE-4, by the avidin-biotin-peroxidase complex method. A, PE-35 MoAb reacts with epithelial cells in the medulla including a Hassall's corpuscle at the middle left, but it does not react with any other components of the thymus (see Fig. 2). B, TE-3A MoAb predominantly stains epithelial cells in the cortex. C, RFD-4 MoAb reacts with epithelial cells in the medulla and subcapsular cortex. D, TE-4 MoAb is also reactive with epithelial cells in the medulla and subcapsular cortex. × 180.

In addition, the reactivity of PE-35 MoAb to the goat anti-idiotype antisera directed against either GA73.3 or CO17-1A MoAb was tested. PE-35 MoAb did not bind with both anti-idiotype antisera, showing that PE-35 MoAb has a different idiotype from CO17-1A or GA73.3 MoAb (data not shown).

Fig. 2. Immunelectron micrography of an area of the normal thymus medulla stained with PE-35 MoAb. Surface membrane of epithelial cells (E) with lighter nuclei and elongated cytoplasmic processes (arrows) are strongly stained. IDC possessing an irregularly shaped, lighter nucleus, macrophage (M) having many phagosomes in the cytoplasm and lymphocytes (L) with dense nuclei are not stained. Bar, 3 μm.
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with various MoAbs and the results were summarized in Table 2 and Fig. 5. Twenty-five cases of thymomas were histologically evaluated and classified into three groups: 6 cases of epithelial type, 14 cases of mixed lymphoepithelial type, and 5 cases of lymphocytic type. Foci of medullary differentiation, which were described by Rosai and Levine (24), were observed in 8 cases among mixed lymphoepithelial (5 of 14) and lymphocytic types (3 of 5). Myasthenia gravis was observed in five cases, while pure RBC aplasia was seen in five cases. The remaining tumor types were two carcinomas, three carcinoids, and one small cell carcinoma.

PE-35 MoAb reactive with normal medullary epithelial cells showed a positive reaction with many of the thymomas (15 of 25); all of the epithelial type (6 of 6; Fig. 5A), and more than one-half of the mixed lymphoepithelial type thymomas (8 of 14) were positive, while only one of 5 lymphocytic type cases was partially stained with this MoAb. This antibody also stained foci of medullary differentiation in four of eight cases (Fig. 5C). TE-3A MoAb reactive with cortical epithelial cells in the normal thymus stained the majority of thymomas with various histological types (21 of 25). RFD-4 and TE-4 MoAbs, reactive with normal epithelial cells in subcapsular cortex and medulla, stained most thymoma cases (23 of 25) regardless of the type of thymoma. All the thymomas were strongly stained with anti-keratin antiserum.

Anti-HLA-DR MoAb showed a positive reaction with a half of epithelial and mixed lymphoepithelial types of thymomas (12 of 20), and most cases of lymphocytic type showed diffuse strong staining (4 of 5; Fig. 5F). Eight cases among 25 thymomas were positive with PE-35 and HLA-DR MoAbs (PE-35+/HLA-DR+). However, PE-35+/HLA-DR− and PE-35-/HLA-DR+ phenotypes were observed in eight cases each. The remaining phenotypes of one case were both negative (PE-35−/HLA-DR−).

All thymic carcinomas were stained by MoAbs detecting thymic epithelial antigens and anti-HLA-DR as well as by antikeratin antiserum. On the other hand, all neuroendocrine thymic tumors consisting of three carcinoids and one small cell carcinoma were stained with PE-35 MoAb as well as with NE-25 MoAb, which is known to be reactive with the cells of...
neuroendocrine system (14), whereas other thymic epithelial antigens were generally negative. None of the thymomas and thymic carcinomas showed a positive reaction with NE-25 MoAb.

Reactivity of MoAbs Detecting T-Cell Antigens Tested against Lymphocytes in Thymic Tumors. The phenotype of lymphocytes in the thymic tumors was also analyzed. In the thymomas of epithelial type, a small number of the lymphocytes retained exhibited mainly the phenotype of medullary thymocytes, CD1+/CD2+/CD3-/CD6+. In two cases of mixed lymphoepithelial type (Cases 8 and 9), lymphocytes with medullary phenotype and those with cortical phenotype were observed in separate areas of the same specimens. Thus, the results of each area of thymomas are stated separately. However, in the text these two cases are generally described as PE-35+/HLA-DR+.

In two cases of mixed lymphoepithelial type (Cases 8 and 9), lymphocytes with cortical phenotype and those with medullary phenotype were found to coexist in the separate area in the same specimens. The thymoma cells with CD1+/CD6+ lymphocytes were strongly positive with PE-35 MoAb, while the epithelium containing CD1+/CD6− thymocytes was negative. All 9 thymomas retaining medullary type lymphocytes (including Cases 8 and 9 described above) were positive with PE-35 MoAb, whereas 12 of 18 thymomas with cortical phenotype lymphocytes (including Cases 8 and 9) failed to express PE-35 antigen. Among the remaining six cases with cortical type lymphocytes, only two were diffusely positive with PE-35, while the remaining four cases had the foci of medullary differentiation reactive with PE-35 MoAb. (These four cases are described as partially positive in Table 2). The lymphocytes outside of the area were CD1 positive (Fig. 5D), while the number of lymphocytes in those areas were generally small and had a tendency to show medullary phenotype (Fig. 5E). In contrast to PE-35, other thymic epithelial antigens studied showed no clear correlation to the phenotypes of the lymphocytes retained. Expression of HLA-DR antigen on thymoma cells was obtained in 12 of 18 cases (including Cases 8 and 9) with cortical type lymphocytes, while it was seen in 4 of 9 cases (including Cases 8 and 9) with medullary type lymphocytes.

In two cases of thymic carcinoma, both phenotypes of lymphocytes were retained, whereas lymphocytes were almost absent in three thymic carcinoids and one thymic small cell carcinoma.

**DISCUSSION**

The present study demonstrated the following two important points: (a) PE-35 MoAb is a valuable marker for medullary epithelial cells of the thymus and is directed against the same molecule as the one recognized either by CO17-1A or GA73.3 MoAb; (b) expression of PE-35 antigen in thymomas has a tendency to correlate with the histological type and the phenotype of the lymphocytes retained.

Several investigators have shown the antigenic differences between medullary and cortical epithelial cells using MoAbs to thymic epithelial antigens (7, 9–12). Among the MoAbs reported, ER-TR5 MoAb which was raised against mouse thymic stroma showed a similar specificity to PE-35, reacting with medullary epithelial cells, but it cannot be compared precisely with PE-35 MoAb, because the nature of the antigen is not well characterized (25). The present study showed that TE-3A MoAb was reactive with epithelial cells in the cortex, while both RFD-4 and TE-4 MoAbs were positive with those in the subcapsular cortex and medulla, in accordance with the reports by the original investigators (7, 9, 10). A recent report by Laster et al. (13) suggested that some of the thymic epithelial antigens detected with MoAbs are the subtype of keratin, which is a cytoplasmic antigen with molecular weight of 40,000 to 80,000, but PE-35 antigen is clearly different from keratin because of its presence on cell surface and different molecular weight (35,000). Sequential immunoprecipitation and cross-blocking tests together indicated that PE-35 antigen is identical to the one recognized by CO17-1A or GA73.3 MoAb and that the epitope recognized with PE-35 MoAb is quite similar or identical to the one with GA73.3 MoAb but different from the one with CO17-1A MoAb. The idiotype of PE-35 MoAb is, however, different from CO17-1A or GA73.3 MoAb. The present analysis of PE-35 MoAb demonstrated clearly that this MoAb is valuable for distinguishing epithelial cells in the medulla from those in the cortex or the subcapsular cortex and also from other nonlymphoid cells in the thymus, although MoAbs detecting the same antigen molecule were already reported by Herlyn et al. (16) and also by other investigators (22, 23) as described above.

Several reports of immunohistological analysis of thymomas

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**Table 2** Immunohistological analysis of 31 thymic tumors of various types by the avidin-biotin-peroxidase complex method

<table>
<thead>
<tr>
<th>Histology</th>
<th>Epithelial (6 cases)</th>
<th>Mixed-lymphoepithelial (14 cases)</th>
<th>Lymphocytic (5 cases)</th>
<th>Thymic carcinoma (2 cases)</th>
<th>Carcinoid (3 cases)</th>
<th>Small cell carcinoma (1 case)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenotype of lymphocytes*</td>
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<td>Med/Cor</td>
<td>Cor</td>
<td>Med</td>
<td>Cor</td>
<td>Absent</td>
</tr>
<tr>
<td>No. of cases</td>
<td>6(0, 2)</td>
<td>1(0, 0)</td>
<td>2(0, 0)</td>
<td>1(0, 0)</td>
<td>1(0, 0)</td>
<td>0(0, 0)</td>
</tr>
<tr>
<td>Phenotype of tumor cells</td>
<td>Med</td>
<td>Med</td>
<td>Cor</td>
<td>Med</td>
<td>Cor</td>
<td>Absent</td>
</tr>
<tr>
<td>with MoAb</td>
<td>PE-35</td>
<td>6*</td>
<td>1*</td>
<td>2*</td>
<td>2*</td>
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<td>0</td>
<td>2(1, 1)</td>
<td>10</td>
<td>5</td>
<td>1(0, 1)</td>
</tr>
<tr>
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<td>0(1)</td>
<td>2/2*</td>
<td>10</td>
<td>5</td>
<td>1(1)</td>
</tr>
<tr>
<td>TE-4</td>
<td>6*</td>
<td>0(1)</td>
<td>2/2*</td>
<td>11</td>
<td>4</td>
<td>1(0)</td>
</tr>
<tr>
<td>Keratin*</td>
<td>6</td>
<td>1</td>
<td>2/2</td>
<td>11</td>
<td>5</td>
<td>0(1)</td>
</tr>
<tr>
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<td>0/0*</td>
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<td>4</td>
<td>1(1)</td>
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<td>0/0</td>
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<td>0(0)</td>
</tr>
</tbody>
</table>

*Numbers in parentheses, number of cases accompanied by myasthenia gravis or pure RBC aplasia, respectively.

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Fig. 5. Immunoperoxidase staining of various types of thymomas by the avidin-biotin-peroxidase complex method. A. and B, epithelial type thymoma (Case 1) is stained with PE-35 or Tp 120 MoAb. Epithelial cells are diffusely stained with PE-35 MoAb (A). A few lymphocytes are scattered and stained predominantly with Tp 120 MoAb (CD6) (B). x 180. C to E, mixed lymphoepithelial type thymoma with foci of medullary differentiation (Case 19) stained with PE-35, OKT6 (CD1) or Tp 120 MoAb. Epithelial cells in the focus of medullary differentiation are PE-35 positive (C). OKT6" cortical type lymphocytes are distributed predominantly outside of the foci (D). In contrast, the lymphocytes in the foci are positive with Tp 120 MoAb (E). x 135. F, and G, lymphocytic type thymoma (Case 24) is stained with HLA-DR or OKT6 MoAb. Epithelial cells are strongly stained with anti-HLA-DR, showing a fine reticular pattern (F). The majority of lymphocytes are stained predominantly with OKT6 MoAb (G). x 180.
have appeared, but a relatively small number of the cases were studied (8, 26–30). In the present study, 25 thymomas were analyzed with the MoAbs detecting thymic epithelial antigens as well as with those against T-cell antigens, demonstrating a good correlation between histological type of thymomas and phenotype of the lymphocytes retained. Thymomas of epithelial type retained a small number of the medullary type lymphocytes, while those of mixed lymphoepithelial and lymphocytic type retained a small number of the medullary type lymphocytes and cortical phenotype. Similar observations were also reported previously by Mokhtar et al. (27) and Sato et al. (30) by studying six and nine cases, respectively. Expression of PE-35 antigen seemed to be in accordance with the medullary phenotype of lymphoid components in thymoma, i.e. all 9 thymomas retaining medullary lymphocytes (including Cases 8 and 9) were positive with PE-35, whereas only 2 of 18 cases with cortical type lymphocytes (including Cases 8 and 9) were diffusely stained. These latter cases might be due to the abnormal expression of PE-35 antigen, because such illegitimate or anomalous expression of certain markers is well recognized in various tumors of hematopoietic origin (31).

In addition, PE-35 antigen was expressed on the foci of medullary differentiation in four of eight cases. In contrast to PE-35, it is noted that the other thymic epithelial antigens studied showed no apparent concordance with the lymphocyte phenotype in thymoma, in spite of their restricted distribution in normal thymus. On the other hand, the expression of HLA-DR in thymomas, which is mainly expressed on cortical epithelium and IDC in the normal thymus (2), has a tendency to correlate with the cases retaining predominantly the cortical type lymphocytes. Accordingly, 16 cases were positive with either PE-35 or HLA-DR MoAb, while 8 cases were positive with both MoAbs. Müller-Hermelink et al. (1) also reported that the constant expression of HLA-DR antigen was found predominantly in thymomas classified as the cortical type. The present findings altogether suggested that epithelial cells still retain a physiological function to a certain extent and may interact with the lymphocytes in the tumors and could induce inappropriate T-cell maturation, which may result in immunoregulatory disorders such as myasthenia gravis and pure RBC aplasia. The present study, however, revealed no clear correlation between the phenotype of epithelial cells and the biological behavior of the tumors.

Two cases of thymic carcinoma were positive with MoAbs detecting thymic epithelial antigens; one case with medullary type lymphocytes was PE-35+ and another one with cortical type lymphocytes was partially positive. Although the number of the cases is very small, the results were similar to those with epithelial type thymomas. In contrast, thymic neuroendocrine tumors such as carcinoid and small cell carcinoma reacted with both PE-35 and NE-25 MoAbs, suggesting that NE-25 MoAb is useful for immunohistological diagnosis of neuroendocrine tumors of the thymus as well as of small cell lung cancer as we reported previously (14). These results also suggested that the origin of these neuroendocrine tumors are different from those of thymomas and thymic carcinomas.

In conclusion, it was shown that PE-35 is a valuable marker for medullary epithelial cells of the thymus and that thymomas may originate from different subsets and/or differentiation stages of thymic epithelium.

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