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Immunohistological evidences of cortical and medullary differentiation in thymoma*

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Summary. The phenotypical characteristics of human epithelial and lymphoid cells have been studied with immunohistochemical methods on frozen sections of 12 thymomas. On the basis of the cytohistological characteristics of thymoma epithelial cells (EC) the thymomas were divided in cortical, medullary and mixed types, according to recently developed light microscopical criteria. When tested with a series of monoclonal antibodies, thymoma EC were all stained by the antibody Ki-M3 (as in the thymus), but reacted with anti-HLA-DR, anti-HLA-A,B,C and with a new monoclonal antibody to cortical EC,21A6, to a lesser extent and with weaker, variable intensity in comparison with the normal thymus. Cortical type thymomas were most reactive and the medullary type almost negative. Thymomas, like normal thymus showed different immunoreactivity patterns with antibodies to prekeratins of different specificities. Cortical type thymomas and areas in mixed thymoma showed an EC staining with the antibody to non-squamous type keratin (35βH11) whereas medullary type thymomas and areas showed staining with antibodies to squamous-type keratin (34βE12-IV/82) in addition. Lymphoid cells with cortical (OKT6+, Leu 1 weakly +, Leu 2a +, Leu 3a +) or mature medullary (OKT6 –, Leu 1 strongly +, Leu 2a or Leu 3a +) phenotype were found to colonize tumours with diferent EC types. These immunohistochemical findings largely confirm our earlier cytological distinction of thymoma EC. In addition important differences have been observed in neoplastic cortical EC concerning the HLA-DR and 21A6 immunoreactivity that may be intimately related to the neoplastic process and paraneoplastic immune phenomena.

Key words: Thymoma – cortical/medullary differentiation – Immunohistology

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The antigenic phenotype of human thymoma epithelial cells (EC) and of the lymphoid component of thymoma have been recently investigated with immunohistochemical methods and compared with the findings in normal thymus (Mokhtar et al. 1984; Chilosi et al. 1984; Chan et al. 1984; Savino et al. 1984; Haynes 1984). Thymoma EC shared with their normal counterparts the expression of several antigens, although differences in extent and staining intensity were noticed with anti-HLA-DR, antikeratins and anti-p 19 antibodies (Mokhtar et al. 1984; Chilosi et al. 1984). The antigen defined by the anti-p 19, which in the normal thymus was found to be developmentally regulated (Haynes et al. 1983), was often lost, mainly in malignant cases (Savino et al. 1984). One of the cases showed evidence of a medullary EC phenotype (Chilosi et al. 1984). Similar conclusions were drawn by Hofmann et al. (1984), whereas our preliminary results showed EC with cortical as well as medullary features (Müller-Hermelink and Marino 1984). Immature thymocytes were the predominant lymphoid cells in thymoma (Mokhtar et al. 1984; Chan et al. 1984); some areas contained mature T lymphocytes (Chilosi et al. 1984). A cortico-medullary differentiation was observed with the use of lymphoid cell markers but could not be related to EC distribution (Mokhtar et al. 1984).

We recently described (Marino and Müller-Hermelink 1985; Müller-Hermelink et al. 1985) the cytohistological criteria for a distinction, in routine paraffin sections, of thymoma with cortical or medullary differentiation according to the characteristics of the proliferating EC type. Thymomas containing a mixture of both EC types were designated as mixed type. The morphological features of thymoma lymphocytes were related, in cortical and medullary thymoma, to those of cortical thymocytes and of medullary lymphocytes. An intermediate situation was found in thymoma of mixed type.

In the present immunohistochemical study of 12 thymomas, we found that different thymoma types may be distinguished according to the staining pattern with a set of monoclonal antibodies, that in normal thymus enabled us to distinguish several microenvironments in the cortical and medullary regions (Müller-Hermelink and Steinmann 1984; Müller-Hermelink et al. 1985). A newly developed monoclonal antibody, 21A6, a marker of normal cortical EC, reacted weakly with the cortical thymoma EC. In cortical and mixed type of thymomas, the phenotype of most lymphocytes was that of cortical thymocytes, whereas in medullary thymoma the lymphoid cells exhibited mostly the mature medullary lymphocyte phenotype. The observed antigenic patterns could be related to the previously described morphological features belonging to thymoma with cortical or medullary differentiation or of mixed type.

Materials and methods

Light microscopy. 5 μm thick paraffin sections from formalin-fixed specimens of 12 thymoma were stained with H&E, Giemsa, PAS and silver stains. Subdivisions of different thymoma types was performed as described elsewhere (Marino and Müller-Hermelink 1985; Müller-Hermelink et al. 1985).

Immunohistology. Fresh thymoma fragments were snap frozen in liquid nitrogen. Air-dried, 8 μm thick frozen sections were fixed in acetone (20′) and chloroform (10′) at room temperature. The incubation procedure of Sternberger et al. (1970) was used with minor modifications. The incubation steps were the following: I) purified monoclonal antibody (Table 1) for 30′; II) peroxidase-conjugated rabbit anti-mouse immunoglobulin (30′) (DAKO, Copenhagen, Denmark); III) sheep anti-rabbit antibody (30′) (own production); IV) rabbit peroxidase anti-peroxidase complex (30′) (DAKO, Copenhagen, Denmark). Sections were then incubated for 15′ with diaminobenzidine (DAB) (Walther, Kiel, FRG) and the peroxidase activity was demonstrated according to Graham and Karnovski (1966). After every incubation with antibody and with DAB, sections were washed with TRIS (Tris-hydroxymethyl-aminomethane) buffer 0.2 M (pH 7.2). Slides were mounted without or with counterstain with haemalum. For control of non-specific binding, primary antibody was replaced by phosphate-buffered saline (PBS).

The immunohistochemical reactivity patterns of thymoma EC and lymphoid cells were evaluated in parallel sections. In table III an evaluation of the amount of reactive cells was given as well as of the staining intensity in single cases and in comparison to normal thymus. The macrophage content was evaluated by comparying anti-HLA-DR reactivity with the staining pattern of anti-macrophage antibodies (Ki-M1 and Ki-M6).

The thymoma sections have been stained in parallel with other thymoma or with normal thymus, so that the staining intensity could be compared and evaluated.

Results

Light microscopy

The 12 thymomas were divided according to the described criteria (Marino and Müller-Hermelink 1985; Müller-Hermelink et al. 1985) as cortical type (1), mixed type with cortical predominance (2), mixed type common (3) and medullary type (4). In the tumours tested the basic character of the growth (cortical, medullary or mixed type) was observed in all the specimens available for light microscopy and immunohistology.

Immunohistology

The immunohistological reactivity pattern of EC and lymphoid cells in the *normal thymus* has been described elsewhere (Müller-Hermelink and Steinmann 1984; Müller-Hermelink et al. 1985) and Table 2 briefly summarizes the main findings for EC. The antibody Ki-M3 stains in the thymus all EC types, whereas HLA-DR and HLA-A, B, C antigenic determinants are mainly expressed by the cortical (subcortical epithelium) and medullary EC, the surface and perivascular EC being negative. All the prekeratins are present in the medullary, surface and perivascular epithelium, whereas cortical EC are stained only by the 35βH11 antibody to non-squamous epithelium keratin. The antibody 21A6 reacts only with cortical EC, and Leu 7, as reported by others (Chan et al. 1984), stains the surface epithelium. Anti-Ep 1 stains EC in cortex and medulla of the fetal thymus, whereas in the adult thymus cortical EC are usually negative. Hassall's corpuscles are variably stained by the antibodies to the different prekeratins and express HLA-A, B, C determinants as well.

With the antibodies tested, a surface or cytoplasmic staining pattern was observed in *thymoma* EC: monoclonal antibodies recognizing surface

Table 1. List of antibodies used in this study

Antibody	Clones or producer animal	mc[a]	Antigen or cells identified	Source
Anti-HLA-A,B,C	61D2	mc	Major serologically defined antigens HLA-A,B,C	Bethesda Research Laboratories, Neu-Isenburg, FRG
Anti-HLA-DR	7.2	mc	HLA-DR antigens (Ia-like; p 28, 33)	New England Nuclear, Dreieich, FRG
KiM3	Balb/c mouse	mc	Monocytes and subpopulations of macrophages; all thymic epithelial cells	Radzun and Parwaresch, Cell Immunol., 82, 174, 1983
21A6	Balb/c mouse	mc	Cortical epithelial cells of thymus; some non lymphoid cells in human tonsilla	Gift of Dr. Harpprecht and Dr. Westphal, Dept. of Immunol. University of Kiel
IV/82	Balb/c mouse	mc	Squamous epithelium keratin	Pathology Institute, Kiel, FRG
34βE12	Balb/c mouse	mc	57/66kd[b] keratins in squamous epith., ductal epithel., parabasal glandular cells	Gown and Vogel, J. Cell Biol 95, 414, 1982
35βH11	Balb/c mouse	mc	54kd[b] keratin(s) in most non-squamos epithelia	Gown and Vogel, J. Cell Biol. 95, 414, 1982
Anti-Ep-1	F_6B_6	mc	Ep-1; epithelial cells	Dr. M. Dardenne, Paris, France
Ki-M1	Balb/c mouse	mc	Monocytes and macrophages	Radzun and Parwaresch, Cell Immuno. 82, 174, 1983
Ki-M6	Balb/mouse	mc	Monocytes and macrophages	Dr. Radzun, Kiel, FRG
Anti-Leu1	L17F12	mc	All T cells (gp 67)	Becton-Dickinson, Oxnard, Calif., USA
Anti-Leu2a	SK1	mc	Suppressor/cytotoxic subset of T cells	Dr. R. Evans, New York, N.Y., USA
Anti-Leu3a	SK3	mc	Helper/inducer subset of T cells	Dr. R. Evans, New York, N.Y., USA
Anti-Leu4	SK7	mc	Sheep erythrocyte receptor-positive T cells	Becton-Dickinson, Oxnard, Calif., USA
Anti-Leu6	SK9	mc	Common thymocytes, IDCs and Langerhans cells of the skin	Dr. R. Evans, New York, N.Y., USA
OKT6	OKT6	mc	Human common thymocytes	Ortho Diagnostic System, Inc., Raritan, New Jersey, USA
OKT9	OKT9	mc	Proliferating cells (transferrin receptor)	Ortho Diagnostic System, Inc., Raritan, New Jersey, USA

Table 1. (continued)

Antibody	Clones or producer animal	mc[a]	Antigen or cells identified	Source
Anti-Leu7	HNK-1	mc	Human lymphocyte antigen (Mr 11 OK daltons) in Natural Killer cells	Becton-Dickinson, Oxnard, Calif., USA

[a] mc: monoclonal
[b] molecular weight of keratin polypeptide

Table 2. Immunohistochemical reactivity of non-lymphoid cells in human thymus[a]

Antibodies	Ki-M3	HLA-DR	HLA-ABC	21A6	IV/82	34βE12	35βH11	Ep-1	Leu7	Ki-M1	Ki-M6
A) *EC*											
Surface epithelium	+	−	−	−	+	+	+	+	+	−	−
Perivascular epithelium	+	−	−	−	+	+	+	+	−	−	−
Cortical epithelium	+	+	+	+	−	−	+	−	−	−	−
Medullary epithelium	+	+	+	−	+	+	+	+	−	−	−
Hassall's bodies	−	−	+	−	−[b]	+	−	−	−	−	−
B) *Other non-lymphoid cells*											
Interdigitating reticulum cells (IDC)	−	+	+	−	−	−	−	−	−	+	−
Macrophages	+/−	+	+	−	−	−	−	−	−	+	+

[a] Derived with minor modifications from Müller-Hermelink et al. 1985
[b] Only the peripheral part is stained

antigens were Ki-M3, 21A6, anti-HLA-DR, anti-HLA-A,B,C, anti-Leu 7 and anti Ep 1. A cytoplasmic staining pattern was shown by the different anti-prekeratins (von Gaudecker et al., unpublished results). All lymphoid cell markers were surface-reacting antibodies. With OKT 6 and anti-Leu 6, in some cases, large non-epithelial, non-lymphoid cells were also reactive, as described later. Table 3 shows the immunohistochemical reactivity patterns of 12 thymomas.

As a feature common to all thymoma types, EC in thymoma were found to be all positive with Ki-M3 (Figs. 2a, 3a, 4a). The morphology of EC and their cellular processes showed many similarities to normal thymus in lymphocyte-rich tumours, whereas in EC-rich thymoma the EC processes were usually densely packed and more difficult to evaluate. With Ki-M3, EC

Table 3. Immunohistochemical reactivity of epithelial and lymphoid cells in thymoma

Case number	1	2	3	4	5	6	7	8	9	10	11	12
Histological diagnosis	Cortical	Mixed with cortical predominance			Mixed common				Medullary			
Antibodies EC												
Ki-M3	all	all	all	n.t.	all	all	n.t.	all	all	all	all	all
HLA-DR[a]	+/–[b,c]	most[c]	most[c]	all[c]	–	some	some[c]	–	+/–	some	–	some
HLA-A,B,C	most	all	all	n.t.	n.t.	all[c]	some	–	+/– weak	n.t.	–	–
21A6	most weak	n.t.	n.t.	n.t.	–	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
IV/82	–	+/–	+/–	+/–	+/–	+/–	n.t.	+/–	all	most weak	most	all
34βE12	–	+/–	n.t.	n.t.	+/–	+/–	most	most	all	all	all	all
35βH11	all	most	n.t.	n.t.	all	most	most	all	n.t.	all	all	all
Anti-Ep1	all weak	most weak	n.t.	n.t.	+/– weak	Periv.[d] EC	+/– weak	+/–	n.t.	n.t.	most	n.t.
Leu7	some	n.t.	n.t.	n.t.	–	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
Lymphocytes												
OKT6/Leu6	all	all	all	all	all	all	all	all	few	few	few	–
Leu1	weak[e]	weak[f]	weak[f]	weak[f]	weak[e]	weak[e]	weak[e]	weak[e]	all[c]	n.t.	+/–	all
Leu2a	all	all	all	all	all	all	all	all	+/–	few	few	few
Leu3a	all	all	all	all	all	all	all	all	–/+	most	most	most
Leu4	all	n.t.	all	all	all	all	all	all	n.t.	n.t.	n.t.	n.t.
OKT9	many	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.

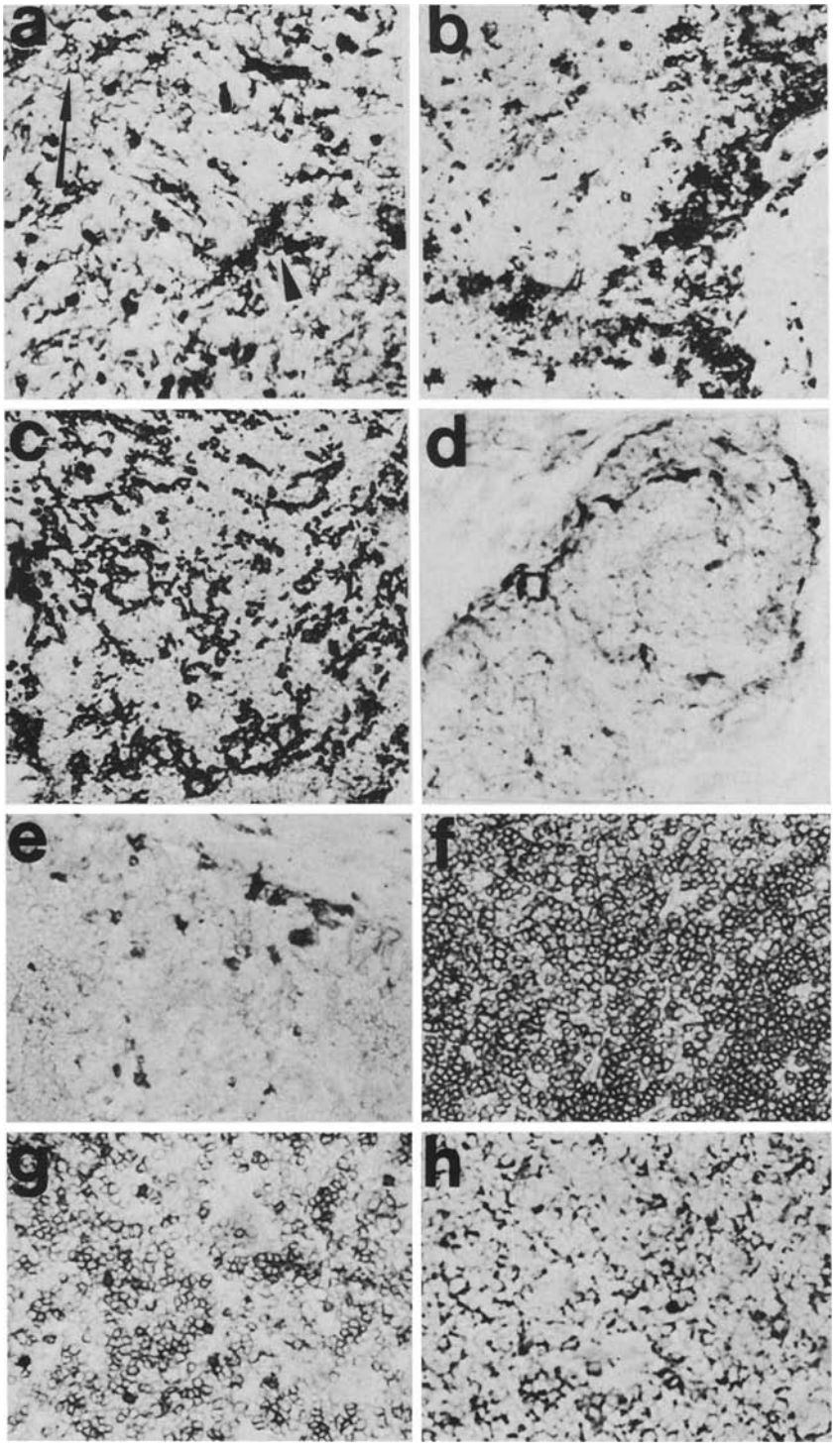
[a] With the exclusion of non-epithelial, non-lymphoid HLA-DR + cells
[b] +/– indicates that only part of the cells reacts
[c] variable intensity
[d] Periv.EC: perivascular EC+
[e] Few scattered strongly reactive lymphocytes were also present
[f] Strongly reactive lymphocytes were found mostly in areas of medullary differentiation or around vessels
[g] Numerous large non-epithelial, non-lymphoid cells were reactive

of cortical type appeared as large stellate EC with long conspicuous cellular processes, whereas EC of medullary type were seen as smaller spindle EC. Variations in immunoreactivity were seen in different thymoma types using a series of monoclonal antibodies.

Cortical type thymoma. In the cortical thymoma (case 1) (Fig. 1) the numerous EC reacted with the antibody Ki-M3 and groups and nests of large reactive stellate EC were very prominent on the background of lymphoid cells. As in the thymic cortex, the expression of Major Histocompatibility Complex (MHC) determinants was observed: the presence of class I (HLA-A,B,C) MHC antigens was not so strong and diffuse as in normal thymus, however, most EC reacted (Fig. 1a), whereas the HLA-DR expression was weak and restricted only to some EC or EC groups; variations in staining intensity were also observed (Fig. 1b). HLA-DR+ EC with the described morphological features of Thymic Nurse Cells (TNC) in thymoma (Marino and Müller-Hermelink 1985) were also observed. With the recently developed, surface-reacting antibody 21A6 thymoma EC were only very weakly reactive. Immunoreactivity to prekeratin was extensively and strongly positive in EC with the antibody to non-squamous type prekeratin (35βH11) (Fig. 1c) as observed in the normal cortex, whereas with antibodies to squamous epithelium prekeratins (IV/82 and 34βE12) most EC were unreactive (Fig. 1d). EC were weakly reactive with anti-Ep 1, an inconstant marker of thymic cortical EC in adult thymus but reactive in fetal thymuses. Anti-Leu 7, which in the normal thymus was observed to react with the surface epithelium (Chan et al. 1984) was reactive in cortical thymoma with some EC mainly at the lobule boundary (Fig. 1e).

The phenotype of lymphoid cells (OKT6/Leu 6+, Leu 4+, Leu 1 weakly+, Leu 2a+ and Leu 3a+) was similar to that of cortical thymocytes (Fig. 1f–g). Some HLA-A,B,C+ lymphocytes were observed in small foci of medullary differentiation (see also later). Few scattered Leu 7+ lymphoid cells were also found. With OKT 9, very numerous reactive lymphoid cells, slightly larger than lymphocytes, were also seen, scattered in the tumoral nodules; some macrophages were also reactive (Fig. 1h).

Mixed type thymoma with cortical predominance (cases 2–4) (Fig. 2). In this thymoma type EC were also extensively reactive with Ki-M3 (Fig. 2a) and anti-HLA-A,B,C (Fig. 2d). The HLA-DR instead was expressed on part of the EC present, and regional differences were found both in the extent of reactivity and staining intensity, some areas being unreactive (Fig. 2b). The positive EC were mostly stellate; some large EC seeming to include lymphoid cells in their cytoplasm (TNC) were observed. Areas containing numerous HLA-DR reactive large cells with the morphological aspect of Interdigitating Reticulum Cells (IDC) were seen and were related to areas of medullary differentiation (Fig. 2b). The staining with the 3 anti-keratin antibodies was positive in the case tested with all antibodies. However, variations were observed in the morphology of the reactive EC and in staining extent, as the anti-squamous epithelium keratin antibodies IV/82 and 34βE12 reacted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mostly with spindle-shaped EC (Fig. 2c). The most extended reactivity was observed with the antibody to non-squamous epithelium keratin 35βH11. By this reactivity a more extended network was formed of cells reacting with all types of prekeratins and those only reacting with non-squamous type keratin. This feature was considered as main immunohistochemical difference to the cortical type of thymoma, similar to the reactivity seen in the mixed type (Figs. 2c, 3c, d).

The vast majority of lymphoid cells was OKT6/Leu 6+, Leu 4+, Leu 2a+, Leu 3a+, Leu 1 weakly+ (Fig. 2e–h), with exclusion of those contained in areas of medullary differentiation and in perivascular location.

The areas of medullary differentiation appeared as foci mainly populated by mature lymphocytes. In some cases, Hassall's corpuscles were present. Here the lymphocytes were OKT6/Leu 6-, Leu 1 strongly+, Leu 4+, Leu 2a *or* Leu 3a+, HLA-A,B,C+, like medullary T lymphocytes (Fig. 2d–h).

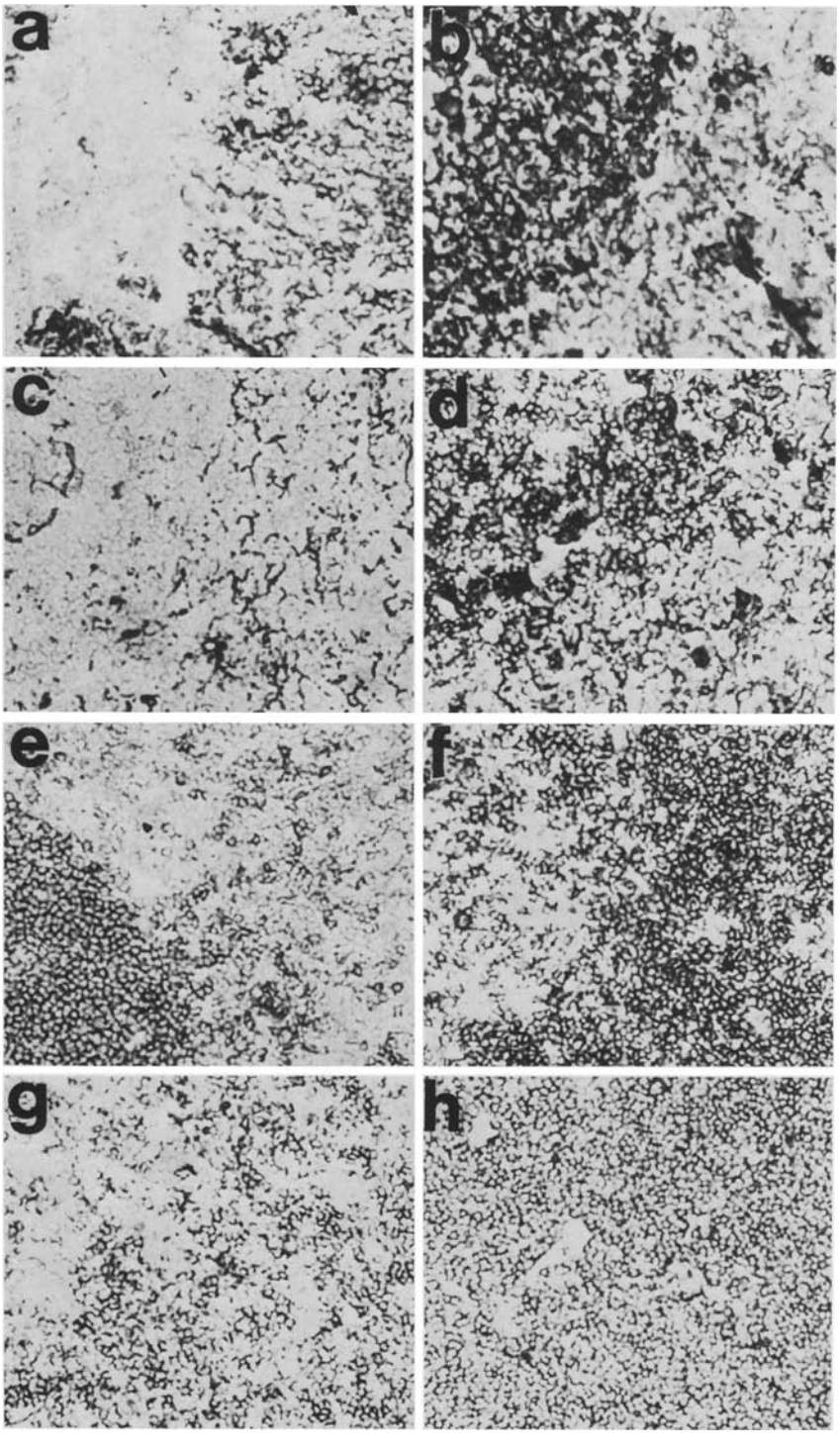
In *common mixed type thymoma* (cases 5–8) (Fig. 3) the Ki-M3 expression was observed (Fig. 3a), The HLA-A,B,C reactivity was found variably present in the tumours, ranging from a strong staining pattern by EC (case 6) to negative results (case 8). The HLA-DR determinants were present on few EC or EC groups, whereas in many parts of the tumours the epithelial network was negative and almost only macrophages reacted (Fig. 3b). The 3 keratin specificities were extensively and strongly expressed, however, mainly with the antibody IV/82 a more restricted reactivity was seen (compare Fig. 3c and d).

With the lymphocyte differentiation antibodies, most of the cells were OKT6/Leu 6+, Leu 4+, Leu 2a+, Leu 3a+, Leu 1 weakly+ (Fig. 3e–h).

Medullary type thymomas (cases 9–12) (Fig. 4) were EC-rich tumours, with scant lymphoid component. All EC reacted with Ki-M3 (Fig. 4a), whereas with anti-HLA-A,B,C very weak reactivity was found. The HLA-DR staining was almost negative. The few reactive cells were recognized as macrophages (reactive with Ki-M1 and Ki-M6) by comparison of distribution in parallel sections (Fig. 4b). However, case n. 9 showed groups of HLA-DR reactive cells of epithelial appearance. The 3 keratins were strongly and extensively expressed (Fig. 4c and d) with minor differences of the different antibody specificities.

A low number of OKT6/Leu 6+ thymocytes was observed (Fig. 4e) whereas most of the lymphocytes were Leu 1 strongly+, Leu 2a *or* Leu 3a+,

Fig. 1a–h. Immunohistological reactivity of cortical thymoma. **a** HLA-ABC: an extensive network of EC is seen, with long dendritic cellular processes (*arrow*). Many macrophages are also reactive (*arrow head*) (×150). **b** With anti-HLA-DR, less EC are stained and the long cellular processes are almost negative (×150). **c** 35βH11 antibody stains extensively and strongly the EC network (×150), **d** whereas with the IV/82 antibody to squamous type keratin few cells are stained mainly at the lobule boundary probably representing the surface epithelium (×240), **e** similar to the anti-Leu 7-reaction, where also few EC are positive at the lobule boundary. Few + lymphoid cells are aldo scattered around (×150). **f** most of lymphocytes are OKT6+ (×150), **g** whereas with anti-Leu 1 only a few strongly reactive lymphocytes are seen (×150). **h** OKT9 stains numerous scattered lymphoid cells of larger size. Some macrophages are also reactive (×240)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like mature medullary lymphocytes. In cases n. 10–12 a predominance of Leu 3a+ over Leu 2a+ cells was found by comparison of parallel sections (Fig. 4f–h). In one case (n. 9) numerous large IDC-like cells reacting both with anti-HLA-DR and OKT6 were found (Fig. 4e).

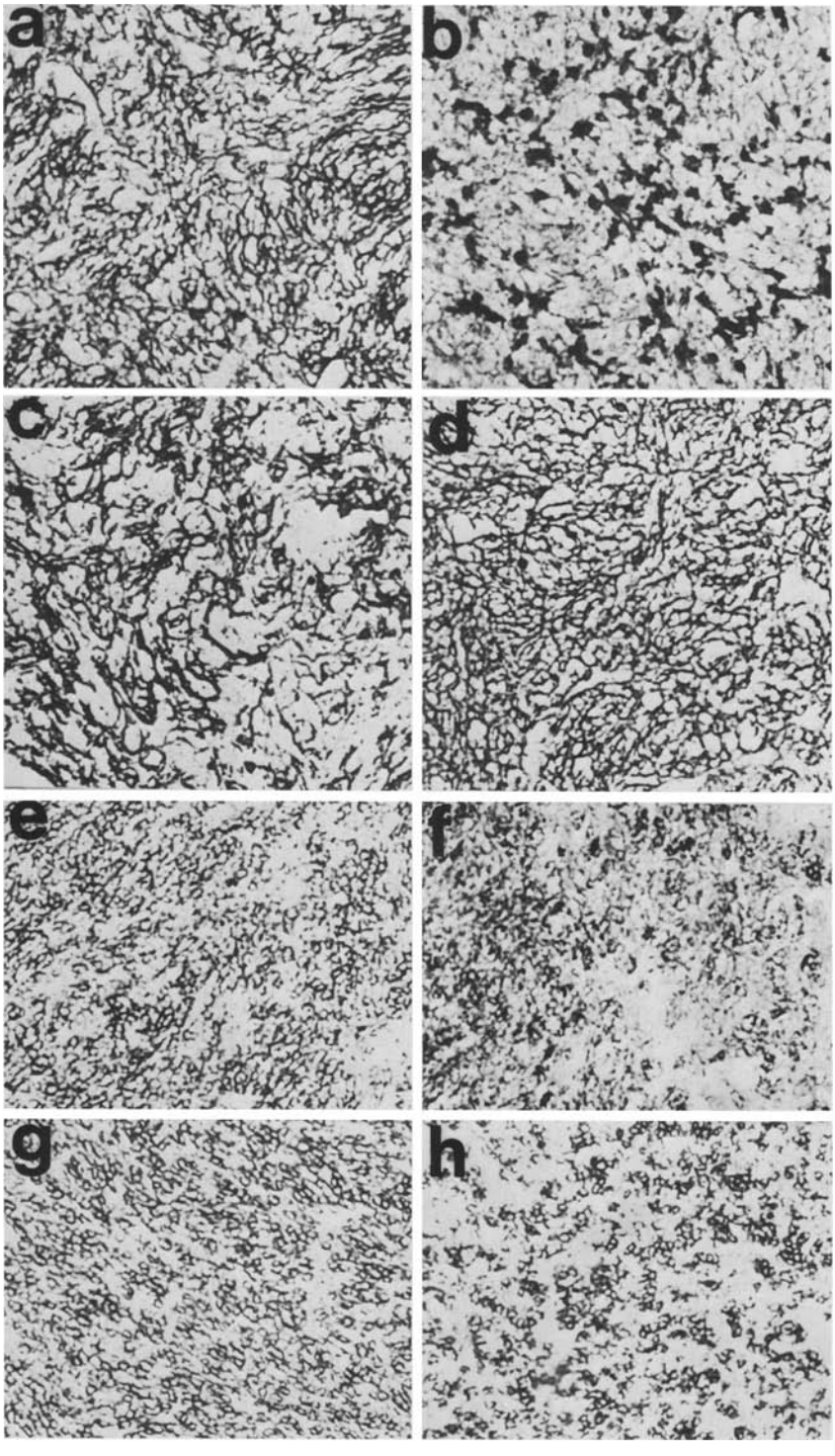
Discussion

The morphological variability of human thymoma EC has long been recognized (Castleman 1955; Lattes 1962; Rosai and Levine 1976) and a descriptive terminology has been used to designate the proliferating EC type in different thymoma, the spindle cell thymoma being the best characterized (Levine and Bensch 1972; Hofmann et al. 1985). Most thymomas show an EC component with round-oval nucleus, or a mixture of EC with round-oval or spindle nucleus. This range of morphological variation was mostly considered to be a spectrum of one EC type (Rosai and Levine 1976; Gray and Gutowski 1979).

In the normal thymus, evidence grows which indicates that the human thymic epithelium is also heterogeneous (for discussion the Ritter et al. 1981; Haynes 1984; Haynes et al. 1984; van de Wijngeart et al. 1984; von Gaudecker 1985; Müller-Hermelink and Steinmann 1984; Müller-Hermelink et al. 1985; Janossy et al. 1985; Wekerle and Müller-Hermelink 1985). Antigenic heterogeneity among topographically distinct thymic EC has been demonstrated with monoclonal antibodies, some of which were raised against human thymic tissue (Haynes et al. 1984; McFarland et al. 1984). On the basis of topographically determined antigenic pattern, a maturation sequence from the subcapsular cortex to Hassall's corpuscle EC has been proposed for the thymus as for epidermal keratinocytes from the basal layer to the stratum corneum (Haynes 1984). In our own immunoperoxidase studies, the occurrence of distinct phenotypes in cortical and medullary EC as well as in EC bordering perivascular spaces and cortical lobules and those EC forming Hassall's corpuscles was shown (Müller-Hermelink and Steinmann 1984; Müller-Hermelink et al. 1985).

At light microscopical level we found that differences in normal cortical and medullary thymic EC may constitute a basis for the distinction of human thymoma with cortical or medullary differentiation of EC (Marino and

Fig. 2a–h. Immunohistological reactivity of areas of medullary differentiation (a.m.d.) in mixed type thymoma with cortical predominance. **a** Ki-M3 stains all the EC present, but the a.m.d. is almost devoid of EC (×150). **b** With anti-HLA-DR, a network of variably reacting EC is seen on the right, but the a.m.d. shows the strong confluent pattern of HLA-DR staining seen in the medulla, mostly provided by IDC and macrophages (×150). **c** The squamous epithelium keratin IV/82 is stained in part of the surrounding EC, in the a.m.d. few EC react (×150). **d** The lymphocytes in the a.m.d. are also HLA-ABC+, whereas in the surrounding mostly EC are extensively stained as lymphoid cells are unreactive (×150). **e** Anti-Leu 1 stains weakly in the cortical type area, whereas all the lymphocytes in the a.m.d. are strongly reactive (×150). **f** and **g**) Anti-leu 3a (f) and anti Leu 2a (g) stain all the lymphocytes in the cortical type area, whereas in the a.m.d. only some cells are + (×150). **h** Outside the a.m.d. all the lymphocytes are OKT6+ (×150)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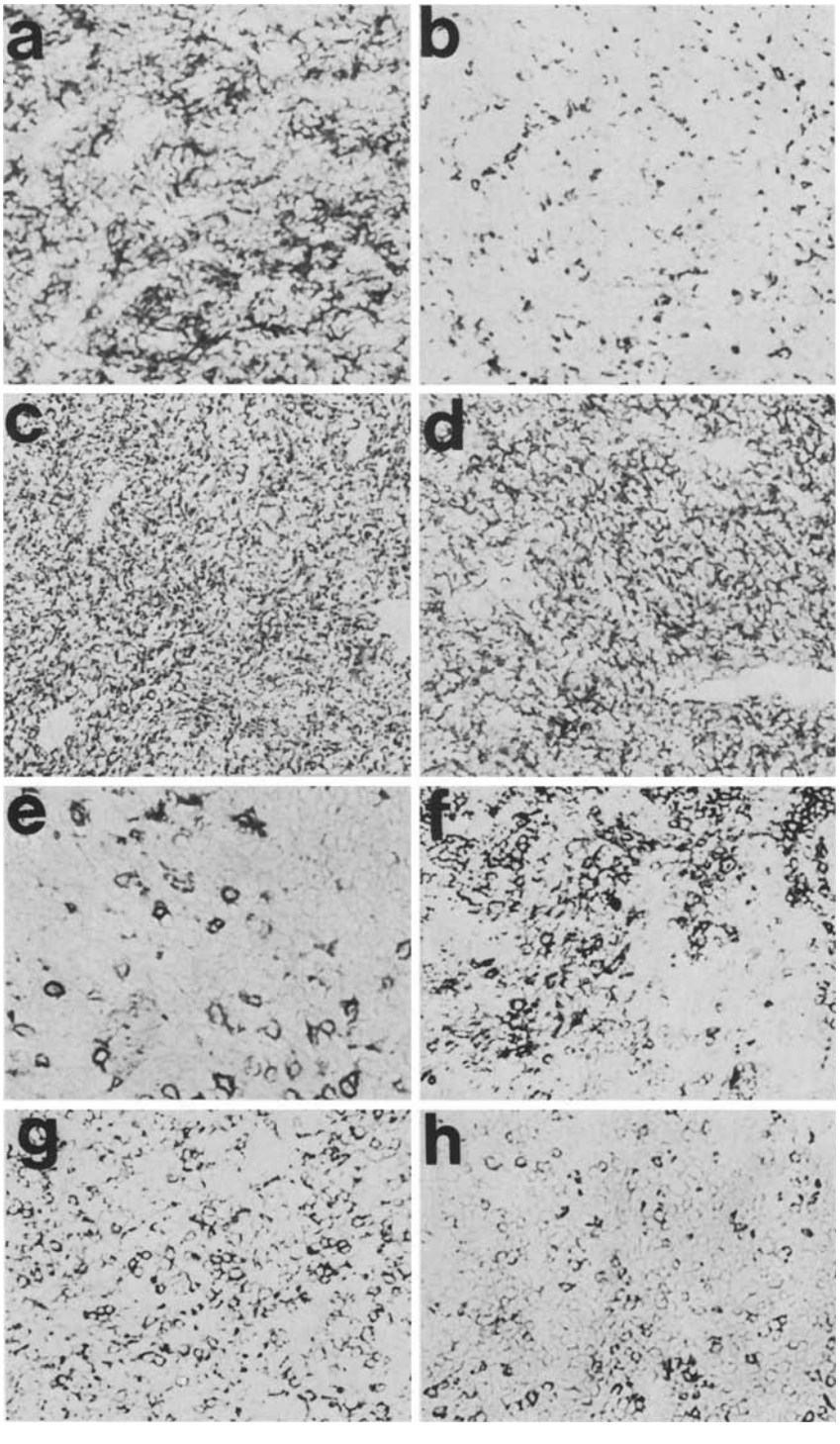
Müller-Hermelink 1985; Müller-Hermelink et al. 1985). Morphologically, thymoma EC with stellate outlines, large clear round-oval nucleus, conspicuous nucleolus and long prominent cellular processes were designated as of cortical type. Medullary type EC in thymoma, instead, are spindle-shaped, have a fusiform nucleus with coarser chromatin structure, inconspicuous nucleoli, scant eosinophilic cytoplasm, and thin cellular processes. Additional morphological microenvironmental features relating thymoma to the basic compartment of thymus, cortex and medulla, have been demonstrated.

The present immunohistological study showed that phenotypical characters of normal thymic EC are expressed by thymoma EC. Ki-M3, an antibody reacting with all normal thymic EC, was found to be a reliable marker of thymoma EC too, and extensively stained both stellate and spindle EC types. The HLA-DR staining, instead, showed that heterogeneous expression of class II MHC antigens exists, as a variable part of the overall EC population reacts weakly if at all. Similar findings were reported by other investigators (Mokhtar et al. 1984; Chilosi et al. 1984; Chan et al. 1984). The reduced HLA-DR staining in thymoma EC was referred to as heterogeneity in surface antigen expression (Chan et al. 1984). We observed the more extended HLA-DR expression on cortical EC-containing thymoma, whereas in the medullary and mixed, common types few if any EC were reactive. However, the HLA-DR staining intensity and extension on positive cases was always very variable, showing much less reactivity than normal thymic EC. The large non-epithelial, non-lymphoid HLA-DR+, OKT6+ cells in one medullary thymoma could correspond to those reported by Chilosi et al. (1984). Their morphology resembles IDC. In the normal thymus, IDC are HLA-DR+, OKT6-, but the acquisition of OKT6 positivity may be observed in pathological conditions (Wekerle and Müller-Hermelink 1985). Similar to HLA-DR expression, the reactivity to class I MHC antibodies (anti-HLA-A,B,C) was found to be preserved mainly in cortical EC-containing tumours and was usually more extended than the anti-HLA-DR reactivity.

The staining with anti-keratin of different specificities could be related to corresponding reactivities of normal thymus: 35βH11, the antibody to non squamous epithelium keratin, which reacts with all thymic EC (Müller-Hermelink and Steinmann 1984), stains all thymoma EC too, whereas the IV/82 and 34βE12 staining of squamous epithelium keratin was more restricted, being negative in the cortical thymoma, partially positive in mixed type thymoma and extensively and strongly positive in medullary thymoma.

Fig. 3a–h. Immunohistological reactivity of mixed type thymoma. **a** Ki-M3 stains extensively the EC (×150). **b** With anti-HLA-DR almost only macrophages are reactive whereas the epithelial network is negative (×150). **c** The anti-keratin IV/82 reacts with part of the EC (×240), **d** whereas with 35βH11 antibody to non-squamous epithelium keratin a more extended network is reactive (×150). **e** All lymphocytes are Leu 6+ (×150). **f** Only scattered cells are strongly stained by anti-Leu 1 (×150). **g** and **h** Most lymphocytes are Leu 3a+ (**g**) and (**h**) Leu 2a+(×150)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g
h

In the thymus, these antibodies react with medullary, surface and perivascular epithelium, but not with cortical EC.

Other investigators reported decreased or weak keratin staining of thymoma EC (Mokhtar et al. 1984); keratin was often referred as a medullary thymic EC marker (Chilosi et al. 1984). This may indicate squamous type of keratin. With antibody 21A6, reactive in the thymus only with cortical thymoma EC (Müller-Hermelink et al. 1985), the cortical thymoma EC were also very weakly stained.

The antibody Leu 7 was observed to stain only EC in the lobular periphery of the thymus (surface epithelium) (Chan et al. 1984). In thymoma, a high variability of staining pattern with Leu 7 was reported; perivascular and surface EC were often positive. The suggestion has been made that the target for Leu 7 is a specialized EC forming a boundary between the thymic parenchyma and the connective tissue, and that such cells are also present in thymoma, distributed in either an orderly or disorderly manner (Chan et al. 1984). In the cortical thymoma, Leu 7 stained scattered EC mainly at the lobule boundary. A much more extended reactivity with Leu 7 was observed in EC of a well-differentiated thymus carcinoma, which could correspond to the type 3 thymoma as recently reported by Verley and Hollmann (1985). In this tumour, Leu 7+ EC formed sheets or bordered cysts and perivascular spaces (unpublished observations). An undifferentiated EC bordering the thymic lobules (surface epithelium) could therefore be the proliferating EC type in this thymic carcinoma.

The thymoma lymphoid component was found to exhibite a definite phenotype corresponding to cortical or medullary lymphocytes, the thymocytic phenotype being the mainly represented in cortical EC-containing tumours, i.e. in cortical, in mixed type thymoma with cortical predominance and in the common type. In the cortical thymoma, we observed in addition a high number of large OKT9+ lymphoid cells. This may indicate a high content of immature thymocytes preceding the OKT6+ stage or/and that these cells are actively proliferating. The abundance of "lymphoblasts" in cortical thymoma was already been described (Marino and Müller-Hermelink 1985; Müller-Hermelink et al. 1985). In medullary thymoma, however, the scanty lymphoid component was found to be mostly of the mature, medullary phenotype, whereas only few cortical thymocytes were present. In 3 out of 4 medullary thymomas, a predominance of lymphocytes with helper type phenotype (Leu 1+, Leu 3a+, Leu 2a-) was observed, as is seen in the normal thymic medulla. Other authors have reported the thymocytic nature of most thymoma lymphocytes (Reddick and Jennette 1983; Woda et al. 1984; Mokhtar et al. 1984; Chilosi et al. 1984;

Fig. 4a–h Immunohistological reactivity of medullary thymoma. **a** Ki-M3 stains all the EC (× 150). **b** wheras anti-HLA-DR stains only macrophages (× 150). **c** and **d** Antibodies to keratin IV/82 (**c**) and 35βH11 (**d**) are both extensively and strongly expressed (× 150). **e** With OKT6 only few lymphocytes react; some large non-lymphoid, non-epithelial cells are also seen (IDC-like cells) (× 240). **f** Most of lymphocytes are strongly Leu 1+ (× 150). **g** The majority of lymphocytes are Leu 3a+ (× 150). **h** whereas only few are Leu 2a+ (× 150)

Table 4. Immunohistochemical reactivity of different thymoma types[a]

Histological diagnosis	Cortical	Mixed cortical predominance	Mixed common	Medullary
Epithelial markers				
Ki-M3	all	all	all	all
HLA-DR	numerous	numerous	+or−	+or−
HLA-ABC	most	most	+or−	+or−
IV/82	absent	+or−	+or−	most
34βE12	absent	+or−	+or−	all
35βH11	most	most	most	all
Lymphoid markers				
OKT6/Leu6	all	all	all	some
Leu1	weak/few strong	weak/few strong	weak/few strong	most strong
Leu2a	all	all	all	some
Leu3a	all	all	all	some or most
Leu4	all	all	all	n.t.

Leu7,21A6, anti-Ep 1, OKT9 could be tested only in some tumours and therefore were not included

Chan et al. 1984); however, the surface and functional variability of thymoma lymphocytes was also well documented (Lauriola et al. 1981; Musiani et al. 1982). The demonstration of in vitro reactivity of mature T-lymphocytes in thymoma was positively correlated with the percentage of mature T-lymphocyte present as determined by phenotypic analysis in suspension (Lauriola et al. 1983). Only a single thymoma with suppressor type lymphocyte phenotype studied in cell suspensions has been reported (Kuroda et al. 1984). Other papers (Mokhtar et al. 1984; Chilosi et al. 1984) describe foci and areas of thymoma containing lymphoid cells with medullary lymphocyte phenotype, surrounded by thymocyte-rich areas. Only in one case could the phenomenon be related to the medullary differentiation areas seen histologically (Mokhtar et al. 1984). We suggest that these areas could be related to medullary EC-rich areas.

We observed also, in mixed type thymoma, the foci of medullary differentiation described by Rosai and Levine (1976) and found that mature T-lymphocytes colonize these areas as well as perivascular areas. Foci of medullary differentiation were also shown to exhibite the confluent HLA-DR staining pattern of thymic medulla, whereas EC, in the cases examined, where almost absent. Therefore these foci were not identical with normal medulla.

The immunological phenotype of lymphoid cells in different thymoma types and areas is consistent with the morphological lymphoid features belonging to them (Marino and Müller-Hermelink 1985; Müller-Hermelink et al. 1985). The main immunohistochemical features of different thymoma types are summarized in Table 4.

Our immunohistochemical characterization of thymoma EC and lymphoid component allowed the recognition of distinct reactivity patterns

that can be related to the light microscopical findings in cortical or medullary differentiation of thymomas.

On the basis of immunohistological findings, several hypotheses of thymoma biology have been proposed. A frequent observation is the lack of structural organization of thymoma EC in comparison with normal thymus, and its distinct microenvironments. Cortico-medullary differentiation, as determined by lymphocyte differentiation markers, was seen only in some cases (Mokhtar et al. 1984). Thus the distinct areas in thymoma, although changed, may still operate as "cortex" and "medulla", as discussed by Chilosi et al. (1984). However, cortico-medullary differentiation could not be linked to EC distribution (Mokhtar et al. 1984).

EC were considered to induce or attract or maintain the predominant thymocytic phenotype of lymphocytes (Mokhtar et al. 1984; Chan et al. 1984) although a maturation process was observed slightly beyond the cortical thymic lymphocyte stage (Chan et al. 1984). Chilosi et al. (1984) reported mature T-lymphocytes in areas with scant lymphoid infiltration. An altered, peculiar or decreased differentiation of thymoma EC was proposed on the basis of variation in the EC antigenic reactivity in comparison with normal thymus (Mokhtar et al. 1984). From recent immunohistological data on thymus ontogeny (Haynes et al. 1983) dedifferentiation of thymoma EC towards a fetal phenotype was hypothesized (Haynes 1984). The protein p19 was reported to be lost in 12 malignant cases of 16 thymomas tested: its loss was referred as a function of dedifferentiation degree or, alternatively, as evidence of thymoma EC derivation from a negative clone (Savino et al. 1984).

In our immunohistological study we related the phenotypical characteristics of thymoma to different EC types, defined morphologically, to the phenotype of thymic EC with varying intrathymic location. Qualitative and quantitative antigenic differences in thymoma EC were found that could be related to the heterogeneity of normal thymic EC. Neoplastic thymoma EC show cortical or medullary characters, although their normal structural relationship and amount of HLA-DR and other cortical antigen expression is strongly altered, similar to the findings of Mokhtar et al. (1984), Haynes (1984) and Chan et al. (1984). This may be of importance for the deficient intrathymoma T-cell maturation and also for immunological paraneoplastic phenomena.

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