

Immunohistochemical study of 22 cases of thymoma

DEBORAH LEE, D H WRIGHT

From the University Department of Pathology, Southampton General Hospital, Southampton

SUMMARY The histological and clinical features of 22 thymomas were reviewed. Immunohistochemical studies were performed using antibodies to cytokeratins (CAM 5.2 and S29) and to desmosomal protein. The heterogeneity of staining patterns seen in the epithelial cells supported the concept of separating thymomas into cortical, medullary, or mixed groups. Interdigitating cells were identified by antibody to S100 protein, and these varied in number between different tumours. Clustering of interdigitating reticulum cells occurred in association with foci of mature lymphocytes which were shown by staining of the leucocyte common antigen (CD45). The extent to which this occurred was used to assess the degree of medullary differentiation within the thymomas and this was correlated with the histological and clinical features. The lymphocyte population of six of the thymomas was studied using a range of antibodies to T and B cells; this showed the presence of B lymphocytes in thymomas.

Thymomas characteristically exhibit a degree of morphological and functional heterogeneity. Studies based on light microscopy and ultrastructure have failed to correlate histological features with the behaviour of the tumour.¹ With the development of immunohistochemical methods there have been several studies of the lymphoid component of thymomas.²⁻⁶ It has been shown that T cells may continue to differentiate within the microenvironment of the thymoma.^{3,7} Until now, it has been assumed that the lymphocytes in thymomas are of T lineage. The demonstration of B lymphocytes in normal thymus,⁸ however, has opened a new line of investigation into the microenvironment of thymomas. In this study we examined the lymphocytic component of six thymomas with a panel of antibodies to T and B cells.

Interdigitating reticulum cells constitute a normal component of the thymic medulla.⁹ They have been shown by S100 immunolabelling to be present in most thymomas.^{6,10} We found that clustering of interdigitating reticulum cells occurred in association with foci of mature lymphocytes which were shown by staining of the leucocyte common antigen (CD45). The extent to which this occurred was used to assess the degree of medullary differentiation within thymomas.

Recent studies of the epithelial component of thymomas^{2,3,6,7,11-13} have suggested that the tumour cells may have a cortical or medullary phenotype and that both types of cell may contribute to the tumour. Accordingly, it has been proposed that thymomas may

be classed as cortical, medullary, or mixed.^{14,15} The epithelial component in this series was examined using antibodies to cytokeratins (CAM 5.2 and S29) and to desmosomal protein. This paper presents an immunohistochemical study of 22 cases of thymoma with clinicopathological correlations.

Material and methods

Twenty five cases of thymoma were obtained from the files of the histopathology department, Southampton General Hospital, over 10 years. Subsequently, three of these were shown to be lymphomas on immunoperoxidase staining. The medical records in all cases were reviewed. The histology was re-examined and at least one representative paraffin wax block from each case was selected for immunohistochemical study. Assessment was performed using the following methods: peroxidase antiperoxidase (PAP) after trypsin digestion¹⁶; the unlabelled antibody enzyme method¹⁷; and avidin/biotin complex method.¹⁸ Antisera to S100 protein, low molecular weight cytokeratin (CAM 5.2), high molecular weight cytokeratin (S29) and the leucocyte common antigen (PD7-26) were applied as a routine panel to 4 μ m sections of all cases. In selected cases antiserum to desmosomal protein was used. Sections of normal thymus from two adults, an infant of 4 months, and a fetus of 20 weeks were examined, along with the thymomas.

A panel of lymphocyte markers was applied to normal thymus, thymus from a case of myasthenia gravis, and six of the thymomas. The antibodies were applied to paraffin wax sections using the modified

Table 1 *Antisera used for immunohistochemical staining of thymomas*

Antibody/specificity	Source of reference
S29	Histopathology Department, Southampton General Hospital
S100	Dakopatts
Antidesmosomal protein	M Vilela ¹⁹
CAM 5-2	Becton Dickinson ²⁰
<i>Lymphocyte markers:</i>	
MT1	T cells S Poppema ²¹
MB2	B cells S Poppema ²¹
4KB5	B cells K Gatter ²²
UCHL1	T cells P Beverley ²³
BERH ₁	Proliferating cells H Stein ²⁴
PD7/26 (CD45)	B + T cells Dakopatts
IgM	Immunoglobulin M Dakopatts
IgD	Immunoglobulin D Dakopatts
IgA	Immunoglobulin A Dakopatts
IgG	Immunoglobulin G Dakopatts

avidin/biotin complex immunoperoxidase method.¹⁸ Details of the antisera are listed in table 1. The negative controls for the rabbit polyclonal and mouse monoclonal antibodies were obtained by omitting the primary antibody. Sections of normal thymus were included in each run to serve as positive controls.

Results

PRESENTATION

Patients with thymomas comprised 13 men and nine women, ranging in age from 31 to 80 years. Most patients presented with the incidental finding of a mediastinal mass on chest x-ray picture following a minor illness. Cough and chest pain occurred in three cases and one of these was shown to have a widely invasive thymoma. Myasthenia gravis was present in seven cases. One patient presented with superior vena caval obstruction.

TREATMENT

The thymomas were resected in all cases with post-operative radiotherapy in three patients. At operation, 11 cases were described as well encapsulated and non-adherent. Fibrous adhesions to mediastinal structures were present in eight cases and were not considered to be neoplastic by the surgeon. Two cases were found to be invasive at operation. In both, tumour was firmly adherent to the superior vena cava, aorta, and pleura and complete resection was not possible.

FOLLOW UP

Of four patients who died, one had clinical evidence of disseminated tumour, one died of acute heart failure two days after extensive resection of an invasive tumour, and the other two patients died of causes unrelated to the thymoma. One patient was lost to

follow up. The remaining 17 cases were well at follow up with no evidence of tumour recurrence for periods ranging from two to 12 years.

PATHOLOGY

Resection specimens were received from all cases. Tumours which had fibrous adhesions were received in several fragments and assessment of the capsule was not reliable.

HISTOLOGY

The tumours were described initially according to the morphology of the epithelial cell component and to the relative proportions of epithelial cells and lymphocytes. Only two cases were considered to be predominantly lymphocytic in all sections examined. In at least six cases different sections of the same tumour varied in appearance from predominantly epithelial to predominantly lymphocytic, making any classification arbitrary. Ten thymomas were mainly epithelial with polygonal and oval cells arranged in sheets and clusters. Some of these tumours contained epithelial cells with more than one type of morphology. The most common was spindle cell areas in a tumour composed principally of polygonal cells with a poorly defined cytoplasmic membrane and vesicular nucleus. Four of the tumours were predominantly spindle cell type. Other features noted were the presence of prominent perivascular spaces (n = 9), microcysts and tubular structures (n = 4), Hassal's corpuscles (n = 3) and areas of necrosis (n = 3). The tumours were categorised as cortical, medullary, or mixed types according to the morphology of the epithelial cells as seen in the normal cortex and medulla, and as described by Marino and Muller-Hermelink.¹⁵ This resulted in 13 cortical thymomas, a mixed group of seven tumours with cortical cell type predominating in five cases, and two medullary type thymomas.

IMMUNOHISTOCHEMISTRY

The results for the panel of cytokeratin antibodies, antidesmosomal protein, S100 and PD7-26 are shown in table 2.

CAM 5-2, S29, antidesmosomal protein

CAM 5-2 has been shown to stain the epithelial cells of thymomas.^{19,25} In normal thymus Hassal's corpuscles and subcapsular epithelium stained strongly, with a weaker reaction in scattered epithelial cells of the medulla and cortex. The intensity of staining of the Hassal's corpuscles is stronger with S29 which also stains scattered epithelial cells in the medulla and subcapsular epithelium. Cortical epithelial cells stain very weakly with this antibody. Antibody to desmosomal protein shows an intense granular staining of

Table 2

Case No	Histological diagnosis	CAM 5-2	S29	Anti-desmosome	S100	PD7/26	Focal medullary differentiation	Comment
1	Cortical	+++	-		+++	+	Pronounced	
2	Cortical	++	+		+++	+	Pronounced	
3	Cortical	+	+HC	+	+++	+	Pronounced	
4	Cortical	+ / ++	+	++	++	++	Pronounced	
5	Mixed cortical predominating	+ / ++	+		+	+	Moderate	
6	Cortical	+++	-	+++	+++	++	Pronounced	Myasthenia gravis
7	Cortical	++	+HC	+++	++	+	Pronounced	Myasthenia gravis
8	Mixed cortical predominating	+	-		++	+	Pronounced	Myasthenia gravis
9	Mixed cortical predominating	+	-		+++	+	Pronounced	
10	Cortical	+++	++	+++	+++	++	Pronounced	Myasthenia gravis
11	Mixed cortical predominating	+	-	++	+	+	Minimal	
12	Cortical	+++	-		+	+	Moderate	
13	Medullary	-	-	+	+	+	Absent	
14	Cortical	++	+		+++	++	Pronounced	Myasthenia gravis
15	Cortical	+	+		+++	++	Pronounced	Myasthenia gravis
16	Medullary	-	-	+	+	+	Absent	
17	Cortical	++	-		+	+	Absent	Invasive
18	Mixed cortical predominating	+ / ++	+	+ / ++	+	+	Minimal	
19	Mixed medullary predominating	±	-		+	+	Minimal	
20	Cortical	++	++		+++	++	Pronounced	Myasthenia gravis
21	Cortical	+++	+		++	+	Absent	Invasive
22	Mixed medullary predominating	±	-		+	+	Minimal	

+ - + + + = Relative number of staining cells (S100 + PD7/26) or intensity of staining (cytokeratins + anti-desmos Ig).

membranes and cytoplasm in cells surrounding Hassal's corpuscles and in subcapsular epithelium. A weaker reaction is present in scattered epithelial cells of the cortex and medulla. Within the thymomas there was considerable variability in staining within individual tumours and between different tumours.

Strong staining of polygonal epithelial cells by CAM 5-2 was present in 13 cases (fig 1). As was noted in a previous study²⁵ staining was more intense at the periphery of lobules, within Hassal's corpuscles, and surrounding microcysts and tubular areas. S29 stained Hassal's corpuscles in thymomas strongly but was only weakly positive in other areas and negative in 11 cases. Antibody to desmosomal protein showed a range of staining intensities between tumours, similar to that shown by CAM 5-2. A notable finding was the almost completely negative reaction of the spindle cell thymomas with CAM 5-2. In two of these tumours where there were microcystic and tubular areas the cells stained strongly with CAM 5-2 (fig 2). Antibody to desmosomal proteins stained the spindle cells weakly and showed a stronger reaction in the tubular areas. Both the tumours that were invasive at operation were strongly positive for CAM 5-2 (fig 3), although staining was focal.



Fig 1 Cortical thymoma showing positive staining of epithelial cells by CAM 5-2.

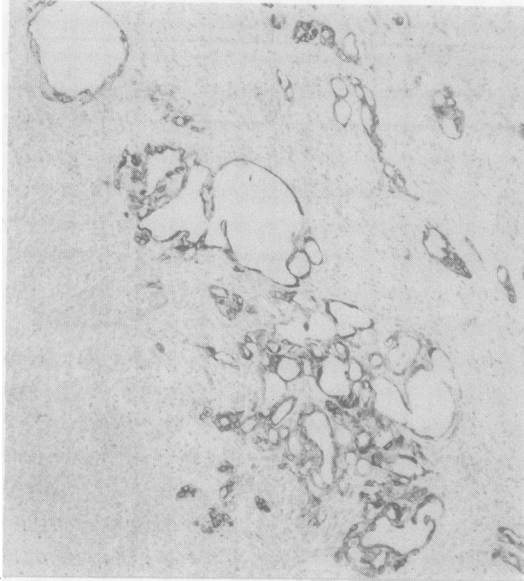


Fig 2 Microcystic and tubular areas in a medullary thymoma staining strongly with CAM 5-2 (note absence of staining in spindle cells).

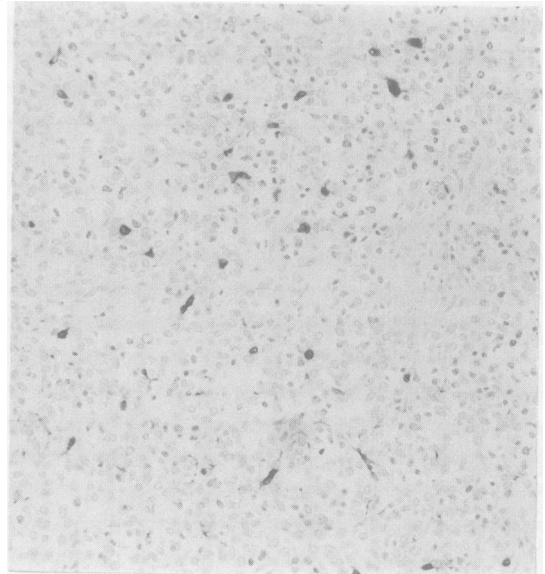


Fig 4 Normal thymic medulla showing staining of interdigitating reticulum cells with S100.

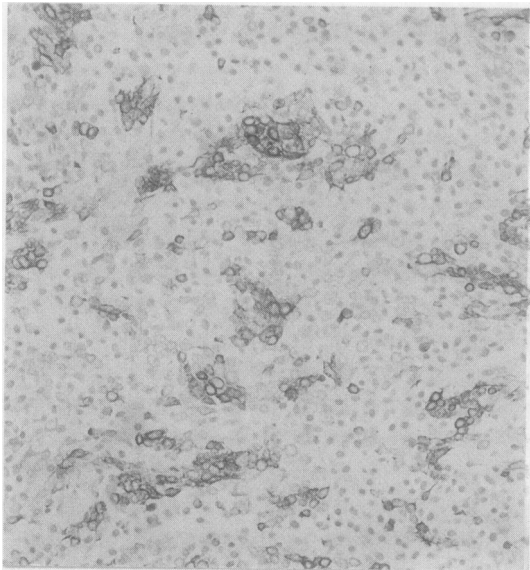


Fig 3 Malignant thymoma showing strong focal staining with CAM 5-2.

S100

In the normal thymus antisera to S100 stains interdigitating reticulum cells scattered throughout the medulla (fig 4). In the tumour sections the interdigitating reticulum cells showed areas of clustering, around

blood vessels, which has been previously reported,¹⁰ and also in relation to small lymphocytes. The extent to which this occurred varied in the different cases but was particularly prominent in 12. Of these 12, seven had associated myasthenia gravis. The cases of myasthenia gravis also seemed to have more numerous interdigitating reticulum cells. In the spindle cell tumours scattered interdigitating reticulum cells were present throughout the lesion but there was some clustering in tubular areas in two of the cases. The invasive tumours showed only a few scattered cells.

PD7-26 (CD45)

This antibody stains the small lymphocytes in the medulla of the normal thymus. Cortical lymphocytes consistently failed to stain with PD7-26. It has been noted elsewhere¹¹ that the F8-11-13 form of Dako leucocyte common antigen only recognises the high molecular weight form of the leucocyte common antigen, not normally expressed by cortical lymphocytes. PD7-26 seems to behave in the same way. The result is a useful marker for mature medullary thymocytes in paraffin wax sections. Many of the thymomas showed grouping of mature lymphocytes, highlighted by PD7-26. Seven cases showed prominent focal collections of positive lymphocytes and these correlated with the clusters of interdigitating reticulum cells. In the remaining thymomas the positive lymphocytes showed little grouping effect and in the spindle cell cases few lymphocytes were present.

Table 3

CAM 5-2	S29	Anti-desmosome Ig	S100	PD7/26 LCA	Focal medullary differentiation	No of Cases	Comment
+++	±	+++	+++	+/++	Pronounced	7	Myasthenia gravis
+	+	++	+++	+/++	Pronounced	5	Myasthenia gravis (in 2)
++	±	+++	+	+	Minimal	4	
±	-	++	+	+	Absent	4	Spindle cell tumours
+++	-	+++	+	+	Absent	2	Invasive tumours

Table 4 Lymphocyte markers

Antibody	Normal thymus	Myasthenic thymus	Thymomas					
			Case No					
			1	3	6†	8†	9	20†
MT1	++++	++++	+++	+++	++++	++++	+++	+++
MB2	+ medulla	+ + medulla + cortex	+	++	++	++	+	+
4KB5	+ + medulla	+ + + medulla + cortex	+	++*	++	++*	+	++*
UCHL1	++++	+++	+++	+++	++++	+++	+++	+++
BerH2	+ medulla	+	-	+	+	-	-	-
LCA	+ + + medulla	+ + + medulla + cortex	++	+++	+++	++++	+++	+++
IgM	+	++	-	+	+	+	-	+
IgG	-	+	-	-	-	+	-	+
IgD	+ + medulla + cortex	+	-	-	-	-	-	-
IgA	-	-	-	-	-	-	-	-

*Grouping in medullary areas and around blood vessels
 †Associated with myasthenia gravis
 + - + + + Relative number of lymphocytes staining

On the basis of these results the tumours were divided into five groups (table 3).

LYMPHOCYTE MARKERS

A panel of markers to T and B lymphocytes was applied to normal thymus, thymus from a case of myasthenia gravis, and six thymomas (table 4). As expected, most of the lymphocytes in the normal and myasthenic thymus stained with the T cell markers MT1 and UCHL1. There was, however, a population of B cells in the medulla shown by the monoclonal antibodies MB2 and 4KB5. As was noted in the study by Isaacson *et al*⁸ the lymphocytes tended to group around epithelial cells surrounding Hassal's corpuscles and to cluster at the cortico-medullary junction. The B cells in normal thymus stained for cytoplasmic and surface IgM and surface IgD, as did the lymphocytes in the myasthenic thymus, although there was also some surface staining for IgG in the latter.

The thymomas showed a range in the numbers of B cells staining. In three cases they were scattered throughout the thymoma in small numbers. In the other three tumours the B cells tended to cluster around blood vessels and showed some grouping in medullary areas (fig 5). Two of these thymomas were

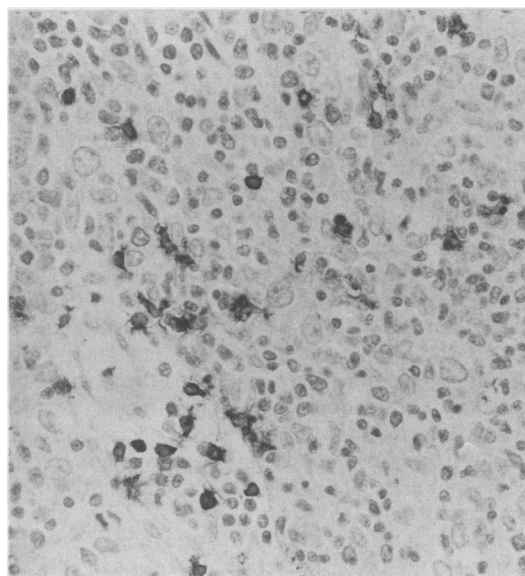


Fig 5 Thymoma showing clustering of B cells stained by 4KB5 in a medullary area.

associated with myasthenia gravis. In four cases surface IgM could be shown on the lymphocytes but the cells were negative for IgD in all the tumours. Two thymomas contained B cells showing surface staining for IgG and these were both associated with myasthenia gravis.

Discussion

Many studies have attempted to predict the behaviour of thymomas on the basis of their histological features.^{1,26,27} To this aim the tumours were placed in groups, the classification being based on the proportion of epithelial cells to lymphocytes and on the morphology of the epithelial cells. Three main categories are commonly used: predominantly lymphocytic, predominantly epithelial, and spindle cell. To some extent this classification is arbitrary as thymomas often show morphological heterogeneity within the same tumour. In a recent review Rosai suggests that it may be preferable to classify thymomas into lymphocyte-rich and lymphocyte-poor.²⁸ Classification based on the light microscopic features of normal thymic epithelial cells divide thymomas into cortical, medullary, or mixed.¹⁵ Studies of the phenotype of epithelial cells^{2,3,12,14} in the thymus and thymomas support this division which was the one adopted in this study.

The question of malignancy in thymomas is controversial, in particular, in relation to invasiveness. Fibrous adhesions to mediastinal structures and irregular sclerosis of the capsule makes assessment of capsular invasion very difficult. In this series two thymomas invaded mediastinal structures causing superior vena caval obstruction. Prognosis is reported to be related to epithelial predominance in thymomas,^{26,29} and the two malignant tumours studied in this series showed sheets of epithelial cells with very few lymphocytes present. Spindle cell tumours are, however, associated with a good prognosis. In a recent study malignant behaviour was noted to occur in thymomas of the cortical type of thymoma.¹⁵ Rosai and Levine suggest that thymic tumours, which are overtly malignant, should be termed "thymic carcinoma", while retaining the term "malignant" for tumours which are invasive but have otherwise typical features of thymoma. Metastasis from cases of thymoma is very rare. Many reports have included cases of lymphoma and carcinoids.³⁰ In a more recent review by Verley²⁷ only two cases in the study of 200 tumours disseminated.

It has been suggested that the presence of myasthenia gravis no longer influences prognosis in cases of thymoma.²⁷ In the present series the seven tumours associated with myasthenia gravis showed no evidence of invasion. They were all of the cortical type and

showed prominent areas of medullary differentiation. It was found that interdigitating reticulum cells were more numerous in these cases, and in two tumours clustering of B lymphocytes occurred. Studies of the microenvironment of the thymus in myasthenia⁹ have shown an expanded medulla due to epithelial hyperplasia and increased numbers of interdigitating reticulum cells as well as the presence of lymphoid follicles. The nature of the relation between the changes in the thymus and the evolution of myasthenia gravis remains unknown but it is of interest that in this series immunoglobulin-containing B cells were present in three thymomas associated with myasthenia gravis.

Cortical type thymomas showed variation in the intensity and distribution of staining of the epithelial cells with anti-cytokeratin antibodies and with antibody to desmosomal protein. The mixed tumours contained more than one morphological type of epithelial cells and these showed different intensities of staining with CAM5-2, S29, and anti-desmosomal antibody. The polygonal cells stained more strongly than the spindle cells. In largely spindle cell (medullary) tumours foci of stronger staining were identified where there were groups of polygonal cells or microcysts and tubules.

Cortical and medullary differentiation within thymomas was described by Rosai and Levine³⁰ and since then further studies have confirmed this division based on the phenotype of cortical and medullary lymphocytes³ and of epithelial cells. Cortical epithelial cells have been found to express HLA DR and LEU-7 (HNK1) antigens^{2,4,7,12} and medullary, but not cortical, epithelial cells have been shown to react with the monoclonal antibody RFD-4.⁷ Three recent studies have shown evidence of cortical or medullary differentiation in the epithelial component of thymomas using immunohistochemical methods.^{2,12,14} These involved larger numbers of cases and concluded that thymomas could be divided into those composed mainly of cortical epithelium, medullary epithelium, or a mixture of both. It is believed that the medullary type of thymoma corresponds to the spindle cell thymoma.¹⁵

Interdigitating cells were first identified in thymomas by S100 immunolabelling by Lauriola *et al.*,¹⁰ who described these cells as being most often found in spindle cell tumours and rarely in round or oval cell thymomas. Other workers have also suggested that cortical thymomas contain fewer interdigitating reticulum cells.^{14,31} This differs from the finding in the present series which, although showing scattered interdigitating reticulum cells in medullary tumours, has also shown numerous interdigitating reticulum cells in cortical and mixed tumours. In many cases pronounced clustering was seen in areas of medullary differentiation. No association has previously been made with interdigitating reticulum cells in thymomas

accompanied by myasthenia gravis. It was notable that the cortical and mixed thymomas associated with myasthenia gravis in this series contained numerous interdigitating reticulum cells. The two thymomas which were invasive showed very few interdigitating reticulum cells and lymphocytes and no evidence of medullary differentiation. This supports the study by Vanderkwast *et al.*,² who found that cortical thymoma in their series.

The areas of medullary differentiation in the thymomas were highlighted by the staining with PD7-26 of mature, small lymphocytes. While the number of lymphocytes staining was very variable from case to case, they did show clustering with interdigitating reticulum cells in many cortical and mixed thymomas. In the pure medullary tumours they were randomly distributed in small numbers. Markers to T and B lymphocytes showed that although the predominant cell type was of the T lineage there was a significant population of B cells present. These varied in number between tumours but showed some clustering in three of the six cases examined. While the B cells in the normal thymus express IgM and IgD, in only two tumours could IgM be identified, accompanied by IgG containing lymphocytes. It is of interest that both these thymomas were associated with myasthenia gravis.

A study of sclerosing B cell lymphomas of the thymus³² and B cells within normal thymus⁸ suggest that the cells have features of mucosa-associated B lymphocytes. This observation might be consistent with the derivation of the thymus from the endoderm of the third branchial pouch. It is well established that thymomas can reproduce the microenvironment required for T cell maturation and now further investigation of the B cell population may advance our understanding of the role that thymomas have in the development of myasthenia gravis and other disorders of immunity.

In conclusion, the reaction of the epithelial cells with anti-cytokeratin antibodies and anti-desmosomal antibody supports the concept of dividing thymomas into cortical, medullary, and mixed types. Cortical thymomas showed a stronger reaction with these antibodies than the medullary type. It is still not known whether the proliferation of different epithelial cell types reflects different phenotypic expression of one epithelial stem cell or the combined growth of different stem cells. Areas of medullary differentiation were shown in the cortical thymomas and were prominent in cases associated with myasthenia gravis. The presence of B cells in thymomas has been shown and an association with areas of medullary differentiation found. The two malignant tumours in this study showed no evidence of medullary differentiation.

We thank Karen Britten, Julie Williams, Ron Lee and Brian Mepham, for technical assistance, and Dr M J Vilela for generously providing the antidesmosomal antibody. Our thanks also go to Miss Julie T Foster for typing the manuscript.

References

- 1 Rosai J, Levine GD. Tumors of the thymus. *Atlas of tumor pathology*. 2nd series, fascicle 13. Washington, DC: Armed Forces Institute of Pathology, 1976.
- 2 Van der Kwast TH, Van Vliet E, Christen E, Van Ewijk W, Van der Heul RO. An immunohistologic study of the epithelial and lymphoid components of six thymomas. *Hum Pathol* 1985; **16**:1001-8.
- 3 Mokhtar N, Hsu SM, Lad RP, Haynes BF, Jaffe ES. Thymoma: lymphoid and epithelial components mirror the phenotype of the normal thymus. *Hum Pathol* 1984; **15**:378-84.
- 4 Chan WC, Zaatari GS, Tabei S, Bibb M, Brynes R. Thymoma: an immunohistochemical study. *Am J Clin Pathol* 1984; **82**:160-6.
- 5 Reddick RL, Jennette JC. Immunologic and ultrastructural characterisation of the small cell population in malignant thymoma. *Hum Pathol* 1983; **14**:377-80.
- 6 Kornstein MJ, Hoxie JA, Levinson AI, Brooks JJ. Immunohistology of human thymomas. *Arch Pathol Lab Med* 1985; **109**:460-3.
- 7 Chilosi M, Iannucci AM, Pizzolo G, Menestrina F, Donati L, Jannosy G. Immunohistochemical analysis of thymoma—evidence of medullary origin of epithelial cells. *Am J Surg Pathol* 1984; **8**:309-18.
- 8 Isaacson PG, Norton AJ, Addis BJ. The human thymus contains a novel population of B-lymphocytes. *Lancet* 1987; **ii**:1488-90.
- 9 Janosy G, Thomas JA, Bollum FJ, *et al.* The human thymic microenvironment: An immunohistologic study. *J Immunol* 1980; **125**:1:202-11.
- 10 Lauriola L, Michetti F, Stolfi VM, Tallini G, Cocchia D. Detection by S100 immunolabelling of interdigitating reticulum cells in human thymomas. *Virchows Arch (Cell Pathol)* 1984; **45**:187-95.
- 11 Salter D, Krajewski A. Metastatic thymoma: A case report and immunohistochemical analysis. *J Clin Pathol* 1986; **39**:275-8.
- 12 Kodama T, Watanabe S, Sato Y, Shimosata Y, Miyazawa N. An immunohistochemical study of thymic epithelial tumors. 1. Epithelial component. *Am J Surg Pathol* 1986; **10**:26-33.
- 13 Willcox N, Schlupe M, Ritter MA, Schuurman HJ, Newsom-Davis J, Christensson B. Myasthenic and non-myasthenic thymoma. An expansion of a minor cortical epithelial cell subset? *Am J Pathol* 1987; **127**:447-59.
- 14 Eimoto T, Teshima K, Shirakusa T, *et al.* Heterogeneity of epithelial cells and reactive components in thymomas: an ultrastructural and immunohistochemical study. *Ultrastruct Pathol* 1986; **10**:157-73.
- 15 Marino M, Müller-Hermelink HK. Thymoma and thymic carcinoma. Relation of thymoma epithelial cells to the cortical and medullary differentiation of thymus. *Virchows Arch (Pathol Anat)* 1985; **407**:119-49.
- 16 Mepham BL, Frater W, Mitchell BS. The use of proteolytic enzymes to improve immunoglobulin staining by the PAP technique. *Histochem J* 1979; **11**:345-58.
- 17 Sternberger LA, Hardy PH, Cuculis JJ, Meyer HG. The unlabelled antibody enzyme method of immunohistochemistry. *J Histochem Cytochem* 1970; **18**:315-33.
- 18 Hsu SM, Raine L, Farger H. Use of avidin-biotin peroxidase complex in immunoperoxidase techniques. A comparison between the ABC and unlabelled antibody (PAP) procedure. *J Histochem Cytochem* 1981; **29**:557-80.
- 19 Vilela MJ, Parrish EP, Wright DH, Garrod DR. Monoclonal antibody to desmosomal glycoprotein 1—A new epithelial marker for diagnostic pathology. *J Pathol* 1987; **153**:365-75.

- 20 Makin CA, Bobrow LG, Bodmer WF. Monoclonal antibody to cytokeratin for use in routine histopathology. *J Clin Pathol* 1984;37:975-83.
- 21 Poppema S, Hollema H, Vissier L, Vos H. Monoclonal antibodies (MT1, MT2, MB1, MB3, MB3) reactive with leucocyte subsets in paraffin embedded tissue. *Am J Pathol* 1987;127:418-29.
- 22 Pulford KAF, Falini B, Herget A, Gatter KC, Mason DY. 4KB5, a new monoclonal anti-B-cell antibody for routine diagnosis of lymphoid tissue biopsies. *Leucocyte typing III*. Oxford: Oxford University Press, 1987.
- 23 Smith SH, Brown MH, Rowe O, Callard RE, Beverley PEL. Functional subsets of human helper-inducer cells defined by a new monoclonal antibody UCHL1. *Immunol* 1986;58:63-70.
- 24 Stein H, Mason DY, Gerdes J, *et al*. The expression of the Hodgkin's disease associated antigen, Ki-1, in reactive and neoplastic lymphoid tissue: evidence that Reed-Sternberg cells and histiocytic malignancies are derived from activated lymphoid cells. *Blood* 1985;66:848-58.
- 25 Ring N, Addis BJ. Thymoma: an integrated clinicopathological and immunohistochemical study. *J Pathol* 1986;149:327-37.
- 26 Verley JM, Hollman KH. Thymoma: a comparative study of clinical stages, histologic features and survival in 200 cases. *Cancer* 1985;55:1074-86.
- 27 Osborne B, MacKay B, Battifora H. Thymoma: a clinicopathological study of 23 cases. *Pathol Annu* 1985;20:289-315.
- 28 Rosai J. The Pathology of thymic neoplasia. *Monographs in pathology* 1987:161-83.
- 29 Battifora H, Sun TT, Bahu RM, Rao S. The use of antikeratin antiserum as a diagnostic tool: thymoma versus lymphoma. *Hum Pathol* 1980;11:635-41.
- 30 Levine GD, Rosai J. Thymic hyperplasia and neoplasia: a review of current concepts. *Hum Pathol* 1978;9:495-512.
- 31 Müller-Hermelink HK, Marino M, Palestro G. Pathology of thymic epithelial tumors. The human thymus: histophysiology and pathology. *Current topics in pathology*. Springer Verlag: Berlin, 1986:242-51.
- 32 Addis BJ, Isaacson PG. Large cell lymphoma of the mediastinum: a B-cell tumour of probable thymic origin. *Histopathology* 1986; 10:379-90.

Requests for reprints to: Professor D H Wright, Level E, South Lab/Pathology Block, Southampton General Hospital, Tremona Road, Southampton SO9 4XY, England.