The Use of Keratin Antisera in the Characterization of a Feline Thymoma

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Summary
A cystic mass in the anterior mediastinum of a 9-year-old female European Shorthair cat was classified as a lymphocytic thymoma based on its histopathological features which were in accordance with those reported in the literature concerning feline thymomas. The application of a polyclonal keratin antiseraum and monoclonal keratin antisera RCK 102, RKSE 60 and RGE 53 resulted in staining of foetal feline thymic cells, oesophageal epithelial cells as well as numerous stellate tumour cells and Hassall's corpuscles. As a result, the epithelial origin of the neoplastic cells could be established and the classification of thymoma confirmed. The results indicate the value of keratin antisera in the differentiation of thymoma and non-epithelial tumours in the anterior mediastinum.

Introduction
The description “thymoma” formerly also included thymic lymphosarcoma (Mettler, 1975; Parker and Casey, 1976). However, the only common characteristic feature of thymoma and lymphosarcoma appeared to be the thymic localization. Thymoma is now considered a distinct tumour type, which has to be differentiated from thymic lymphosarcoma (Jarrett and Mackey, 1974; Moulton, 1978; Jubb, Kennedy and Palmer, 1985). Thymomas are defined as tumours in which both the lymphoid and epithelial elements of the normal thymus proliferate (Jarrett and Mackey, 1974) and in which the epithelial cells constitute an essential component (Moulton, 1978).

Thymomas are rare in domestic animals (Parker and Casey, 1976; Moulton, 1978) and are most frequently encountered in dogs, sheep, goats (Jubb et al., 1985) and cattle (Mackey, 1975). Feline thymoma is also a sporadically occurring neoplasm (Carpenter and Holzworth, 1982) and hence, in the literature, reports are mainly concerned with single cases of this tumour type (Mackey, 1975; Richards, 1977; Hauser and Mettler, 1984; Middleton, Ratcliffe and Xu, 1985; Martin, Evans, August and Franklin, 1986). Dyspnoea is the most frequent clinical sign (Carpenter and Holzworth, 1982) lasting a few days (Mettler, 1975; Martin et al., 1986), several weeks (Willard, Tvedten, Walshaw and Aronson, 1980) or even months (Richards, 1977). Less
frequently occurring signs are coughing (Mettler, 1975; Carpenter and Holzworth, 1982; Middleton et al., 1985), anorexia (Mettler, 1975; Middleton et al., 1985) and vomiting (Carpenter and Holzworth, 1982; Hauser and Mettler, 1984). At necropsy, thymomas are manifested mostly as circumscribed neoplasms confined to the cranial mediastinum (Mettler, 1975; Richards, 1977; Carpenter and Holzworth, 1982). However, tumour implantations have also been encountered on the pericardium (Parker and Casey, 1976; Martin et al., 1986), the mediastinum (Dubielzig and DeLaney, 1980; Carpenter and Holzworth, 1982), and the parietal and visceral pleura (Martin et al., 1986); metastases have been found in lungs (Hauser and Mettler, 1984) and kidneys (Middleton et al., 1985). Although apparently a guarded prognosis is indicated (Middleton et al., 1985), thymomas usually are benign neoplasms (Jarrett and Mackey, 1974; Moulton, 1978) which rarely metastasize (Martin et al., 1986).

In this paper, the pathomorphological and immunohistological findings with polyclonal and monoclonal keratin antisera in a feline thymoma are presented.

Materials and Methods

Tissues

A surgically excised tumour located in the cranial mediastinum of a 9-year-old female European Shorthair cat was sent for histological examination. Samples were fixed in 10 per cent buffered formalin. After paraffin wax embedding, 6-μm thick sections were cut and stained with haematoxylin and eosin (HE) and Weigert’s van Gieson stain. Also tumour tissue and thymic and oesophageal tissue were frozen in liquid nitrogen. The thymic and oesophageal tissue were derived from a 50-day-old feline foetus. Several keratin antisera were applied by an indirect immunoperoxidase technique on cryostat sections of both tumour tissue and foetal tissues.

Antisera

(1) A polyclonal keratin antiserum raised in a rabbit against human skin keratins (Euro-Diagnostics B.V., Apeldoorn, The Netherlands). This antiserum does not stain non-epithelial tissues and reacts with normal epithelial tissues and epithelial tumours.

(2) A monoclonal antiserum RCK 102, directed against keratins from a human lung cancer cell line (MR21). This antiserum stains virtually all epithelial tissues, but not non-epithelial tissues, and is directed against human keratins 5 and 8 (Moll, Franke, Schiller, Geiger and Krepler, 1982).

(3) A monoclonal antiserum RGE 53 directed against cytokeratin 18 originally derived from HeLa cells, which shows no reaction with squamous epithelium and non-epithelial tissues (Ramaekers, Huysmans, Moesker, Kant, Jap, Herman and Voojis, 1983a).

(4) A monoclonal antiserum RKSE 60, directed against keratin 10 derived from human callus and reacting specifically with keratinizing stratified squamous cells (Ramaekers, Puts, Moesker, Kant, Huysmans, Haag, Jap, Herman and Voojis, 1983b).

Indirect Immunoperoxidase Reaction

Frozen sections were fixed in acetone for 2 to 3 min, air dried and then incubated with the primary antiserum, at room temperature for 90 min, washed twice with PBS,
and incubated with peroxidase-conjugated antibodies for 30 min. Depending on the first antibody, either goat anti-rabbit peroxidase (1 in 50) or rabbit anti-mouse peroxidase (1 in 20) was used diluted in PBS, supplemented with 10 per cent normal goat or rabbit serum respectively. After the sections were washed twice in PBS, the antigens were visualized with a freshly prepared solution of 3,3'-diaminobenzidine tetrahydrochloride (DAB, Sigma) in 0.05 M Na acetate (pH 4.9) and 0.01 per cent H₂O₂. After rinsing in PBS, the sections were counterstained with haematoxylin.

Results

The tumour specimen was a grey white circumscribed mass of 2.1 × 0.6 × 0.9 cm containing a cyst of 1.0 × 0.3 cm. On histological examination the tumour was composed of a vascular, cellular mass predominantly of lymphoid cells with small hyperchromatic or moderately vesicular nuclei. The lymphoid cells often appeared to be arranged in follicular structures, in which no germinal centres could be observed (Fig. 1). In addition to the numerous lymphoid cells, some other cells could be observed with round or ovoid vesicular nuclei each with a prominent single nucleolus and with a moderate amount of eosinophilic cytoplasm and with indistinct cell membranes, dispersed through the tumour. These cells also seemed to line the cyst lumen. Only rarely these cells showed structures resembling Hassall’s corpuscles (Fig. 2). The moderate amount of fibrous tissue present in the tumour was not arranged in distinct septa, so no lobular structures could be distinguished. Local haemorrhage was present in the neoplasm.

The staining results of the keratin antisera on tumour tissue, foetal thymus and oesophagus are presented in Table 1. With the polyclonal antisera and the monoclonal antisera, RCK 102 and RGE 53 numerous positive stellate cells could be observed in the tumour with scattered negative-staining cells (Fig. 3). Neither the loosely arranged lymphoid cells, nor the cells arranged in follicular structures reacted with the antisera. With the RKSE 60 antisera only a few positive cell clusters could be seen, representing the structures resembling Hassall’s corpuscles.

Discussion

According to the cases described in literature, thymomas are encountered in aged cats, as in the case described above, at a mean age of 9 to 10 years, and a broad age range of 4 to 18 years. One case in a young cat of 1 1/2 years has been reported (Richards, 1977).

The tumour described here showed the characteristic features of thymoma, viz. an admixture of lymphoid cells and epithelial cells, locally arranged in Hassall’s corpuscle-like structures (Jarret and Mackey, 1974; Moulton, 1978; Jubb et al., 1985). This tumour is thus classified as lymphocytic thymoma as lymphoid cells were the predominant cells on histological examination (Jarret and Mackey, 1974; Moulton, 1978; Jubb et al., 1985). The presence of cysts is a fairly common feature of feline thymoma (Mettler, 1975; Parker and Casey, 1976; Carpenter and Holzworth, 1982). Follicular structures of lymphoid cells, as in our case, are apparently rare, as this feature has been reported in only one other case (Mettler, 1975).
Fig. 1. Dense aggregation of well- and moderately differentiated lymphoid cells with local arrangement of cells in a follicular structure. HE × 700.

Fig. 2. Arrangement of cells in Hassall's corpuscle-like structures (arrows). These cells show, in contrast to the surrounding lymphoid cells, vesicular nuclei and a moderate amount of cytoplasm. HE × 140.

Fig. 3. Stellate cells showing a positive staining reaction with the monoclonal keratin antiserum RCK 102 among non-reacting cells. Indirect immunoperoxidase method × 140.
Keratin in a Feline Thymoma

Table 1
Immunohistochemical staining of feline tissues by keratin antisera

<table>
<thead>
<tr>
<th></th>
<th>Polyclonal anti-keratin</th>
<th>RCK 102</th>
<th>RKSE 60</th>
<th>RGE 53</th>
</tr>
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<tbody>
<tr>
<td>Oesophagus</td>
<td>+</td>
<td>+</td>
<td>+*</td>
<td>-</td>
</tr>
<tr>
<td>Squamous epithelium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thymus</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Epithelial cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hassal's corpuscles</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Thymoma</td>
<td>+</td>
<td>+</td>
<td>+†</td>
<td>+</td>
</tr>
</tbody>
</table>

*With the exception of the basal layer, *only* Hassal's corpuscle-like cell clusters.

The monoclonal antibodies raised against keratins derived from human epithelial cells (Ramackers et al., 1983a,b) showed cross-reactivity with feline epithelial cells, if the positive staining reaction of Hassal's corpuscles and scattered stellate cells is taken into consideration, both in the foetal feline thymus and the thymoma, as well as the positive staining of epithelial cells in the oesophagus. Moreover, non-epithelial cells in these tissues did not react with the antisera. Keratins are intracellular filaments which are components of the intermediate filaments (IF) of epithelial cells. IF are a specific and distinct class of filaments in most vertebrate cells which constitutes, in association with microtubules and microfilaments, the cell cytoskeleton (Schliwa, 1986). Although sharing a similar ultrastructure, immunologically and biochemically five distinct IF types can be differentiated, each with a cell-type specific expression pattern (Osborn, 1985). Thus, for example, keratins are the IF characteristic of epithelial cells (Schliwa, 1986). As the IF type characteristic of the cell is generally retained upon malignant transformation, IF typing in man is a valuable method in determining whether a tumour is of epithelial, mesenchymal, muscle, glial or neuronal origin (Osborn, Altmannsberger, Debus and Weber, 1985). Thus, the epithelial origin of a surprisingly large number of tumour cells could be demonstrated by virtue of positive staining by the various monoclonal keratin antisera.

The presence of keratin-positive cells in feline thymoma is in accordance with observations of thymomas of man (Battifora, Sun, Bahu and Rao, 1980; Miettinen, Partanen, Lehto and Virtanen, 1983; Lee and Wright, 1988). Similar to our case, the number of epithelial cells in these human tumours, as detected immunohistochemically, appeared to be much more numerous than expected on routine histological tissue examination (Battifora et al., 1980; Miettinen et al., 1983). Also the presence of both low molecular weight cytokeratins (RCK 102, RKSE 60) and high molecular weight cytokeratins (RCK 102, RGE 53) in epithelial tumour cells is analogous to findings in human thymomas (Lee and Wright, 1988). The staining patterns of polyclonal keratin antiserum, RCK 102 and RKSE 60, are comparable with results in human tissues, since RCK 102 stains virtually all epithelial tissues (Broers, Carney, Klein Rot, Schaart, Lane, Vooijs and Ramackers, 1986) and RKSE 60 exclusively reacts with keratinizing stratified squamous cells (Ramackers et
al., 1983b). RGE 53 also reacted with feline thymic epithelial cells and with numerous cells of the feline thymoma, which is in contrast to the negative staining of human thymic epithelial cells (Ramaekers et al., 1983a). RGE 53 did not react with oesophageal epithelial cells in accordance with observations of the human oesophagus (Ramaekers et al., 1983a). Thus a species difference appears to exist in the reactivity of thymic epithelial cells with this monoclonal antisera. Moreover, feline thymic epithelium apparently expresses a keratin pattern different from oesophageal epithelium, although both originate embryologically from the endodermal tube (Noden and de Lahunta, 1985).

In conclusion, in cats, as in man, keratin antisera can be used to differentiate thymoma from lymphosarcoma. The application of these antisera could be an important diagnostic aid when only small biopsy samples are available. Clinically, this differentiation is of major importance since lymphosarcomas have a poor prognosis whereas thymomas can be treated surgically (Dubielzig and Delaney, 1980; Willard et al., 1980).

References


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