Cytokeratin immunostaining in normal dog major and minor salivary glands
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Abstract — Immunohistochemical staining, with a monoclonal antibody specific for cytokeratins 5, 6, 8, 17 and 19 of dog submandibular, sublingual, parotid, palatine, tongue and zygomatic salivary glands resulted in staining of myoepithelial cells, duct epithelial cells (including intercalated, striated and secretory ducts) and some acinar epithelial cells. Differences in acinar staining between glands reflected the predominance of serous or mucous cells. Acinar epithelial staining was confined to serous cells in sublingual and zygomatic glands and in some parotid glands; serous cells in other parotid glands, and serous demilunes in submandibular, tongue and palatine glands, were unstained. Mucous cells were not stained in any of the glands. © Inra/Elsevier, Paris

gland salivaire / dog / immunohistochemistry / cytokeratin
1. INTRODUCTION

Cytokeratins represent the epithelial class of intermediate-sized, cytoskeletal filaments [29]. In man, the presence of 19 different keratin polypeptides has been established [26]. A correspondingly large number of keratin-types has been identified biochemically in several animal species such as the mouse and cow [30], rabbit [25] and cat [19].

Expression and distribution of cytokeratins in normal salivary glands has been described in humans [14] and in dog parotid and palatine glands [37]. This study describes the expression and distribution patterns of cytokeratins in different normal dog major and minor salivary glands.

2. MATERIALS AND METHODS

2.1. Animals and tissues

Six normal salivary tissue samples from the palatine, parotid, sublingual, submandibular, tongue and zygomatic salivary glands were removed during the course of post-mortem examination from a total of 12 dogs [32]. In all cases, the cause of death or euthanasia was not related to salivary gland disease and the glands were macroscopically normal. Details of the samples examined, age, sex, breed and disease background of the dogs are described in Table 1.

The tissues were fixed in 10 % neutral buffered formol saline for 12 days at room temperature before being routinely processed and embedded in polywax at 60 °C. Sections of 3 μm were cut and mounted on 3-amino-propyltriethoxysilane (APES)-coated slides.

Table 1. Age, sex, breed and disease background of the dogs.

<table>
<thead>
<tr>
<th>Dog</th>
<th>Age (year)</th>
<th>Sex</th>
<th>Breed</th>
<th>Diagnosis confirmed at PM</th>
<th>SL</th>
<th>Pr</th>
<th>SM</th>
<th>Z</th>
<th>Pl</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>F(N)</td>
<td>JRT</td>
<td>perivascular cuffing</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>F</td>
<td>WSS</td>
<td>cirrhosis</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>F(N)</td>
<td>CKCS</td>
<td>retropharyngeal tumour</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>M</td>
<td>cross</td>
<td>disc prolapse</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>M(N)</td>
<td>FCR</td>
<td>lymphoma</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>F(N)</td>
<td>Whippet</td>
<td>inflammation with abscess</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>8.9</td>
<td>F</td>
<td>Shetland</td>
<td>inhalational pneumonia</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>F(N)</td>
<td>ESS</td>
<td>anaemia</td>
<td>~</td>
<td>-</td>
<td>~</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>M</td>
<td>cross</td>
<td>renal infarction</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>F(N)</td>
<td>cross</td>
<td>pyloric ulceration</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>11</td>
<td>F(N)</td>
<td>Keeshund</td>
<td>pneumonia</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>F</td>
<td>Great Dane</td>
<td>chondrosarcoma</td>
<td>~</td>
<td>~</td>
<td>~</td>
<td>~</td>
<td>~</td>
<td>+</td>
</tr>
</tbody>
</table>

a JRT: Jack Russel Terrier; WSS: Welsh Springer Spaniel; CKCS: Cavalier King Charles Spaniel; FCR: Flat Coat Retriever; ESS: English Springer Spaniel; b SL: sublingual; Pr: parotid; SM: submandibular; Z: zygomatic; Pl: palatine; T: tongue; N = neutered.
2.2. Cytokeratin immunohistochemistry

Trypsinised sections were incubated with a 1/100 dilution of monoclonal mouse anti-human cytokeratin antibody MNF116 (Dako, High Wycombe, UK). The antiserum has a restricted spectrum of specificity, for 40-60 kDa keratin polypeptides, and stains keratins nos 5, 6, 8, 17 and 19. Washed sections were treated with biotinylated rabbit anti-mouse antibody (Dako, High Wycombe, UK) at a dilution of 1/100 and then peroxidase-conjugated streptavidin (Dako, High Wycombe, UK) at a dilution of 1/500 for 30 min. Diaminobenzidine was used as a chromogen and Mayer's haematoxylin was used as counterstain. All steps were carried out in a moist chamber at room temperature.

Positive control tissue, for epithelial and smooth muscle staining, was normal human small intestine; normal mouse serum was substituted for antibody in negative controls.

3. RESULTS

There was staining of some epithelial cells and of myoepithelial cells. Differences between different glands related to their predominant serous or mucous character; the results are summarised in table II. There were no differences related to the age or sex of the dogs examined.

Staining, which was interpreted as being of myoepithelial cells, appeared as intense staining of crescent- or spindle-shaped cells bordering some acini and ducts; such staining was present in all glands and is illustrated in sublingual and submandibular glands in figures 1 and 2, respectively.

Serous acinar and demilunar cells were moderately stained in all sublingual and zygomatic glands; demilune cells in submandibular, tongue and palatine

Figure 1. Dog sublingual salivary gland; cytokeratin staining is present in the cytoplasm of serous acinar epithelial cells (s) and duct (d) epithelial cells; mucous acinar cells (m) are unstained. Myoepithelial cells are also stained (arrow). Streptavidin/biotin immunoperoxidase complex
**Table II.** Staining patterns of keratin monoclonal antibody in major and minor salivary glands in 12 dogs.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Submandibular</th>
<th>Sublingual</th>
<th>Parotid</th>
<th>Zygomatic</th>
<th>Palatine</th>
<th>Tongue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serous cells</td>
<td>–</td>
<td>+</td>
<td>−/+(^a)</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mucous cells</td>
<td>–</td>
<td>–</td>
<td>n/a</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Duct epithelial cells</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Myoepithelial cells</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Basal cells</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

\(^a\) Weak staining in two specimens only.

n/a: not applicable (cell type not present).

**Figure 2.** Dog submandibular salivary gland: cytokeratin staining is present in myoepithelial cells (arrows) and in the cytoplasm of duct epithelial cells (d); acinar cells are unstained. Streptavidin/biotin immunoperoxidase complex x 40.
glands were not stained. Positive staining of acinar serous cells appeared as moderately intense, finely granular staining of the entire cell cytoplasm; an example, in the sublingual gland, is shown in figure 1.

Weak staining of seromucous acinar epithelial cells was present in only two of the parotid gland samples; the other parotid glands were unstained. There was no staining of mucous acinar cells in any other gland; the counterstain revealed the outlines of the large, clear vacuolated mucous acinar cells which were sometimes bordered by portions of positively stained myoepithelial cells, as seen in the submandibular gland in figure 2.

Duct epithelial staining was also present in all glands. The entire cytoplasm of duct epithelial cells (including intercalated, striated and secretory ducts) was intensely stained. The unstained nuclei appeared as clear rounded spaces within the cells, as can be seen in the examples of sublingual gland (figure 1) and submandibular gland (figures 2 and 3). In all glands duct epithelial cell staining was particularly intense at the luminal surface; this is particularly evident in figure 3. In addition to staining of columnar duct epithelial cells, there was staining of polyhedral cells at the base of the epithelium. Such staining was interpreted as representing epithelial basal cells and was observed in some striated and excretory ducts of all the salivary glands, an example of basal cell staining is seen, in the submandibular gland, in figure 3.

Figure 3. Dog submandibular salivary gland: cytokeratin staining is present in basal epithelial cells (circle) in a excretory duct. Most other duct basal epithelial cells are also stained. Streptavidin/biotin immunoperoxidase complex x 60.
4. DISCUSSION

In the present study, the use of a restricted-spectrum antibody, which specifically detects a few members of the cytokeratin family, has allowed immunohistochemical characterisation of the distribution of cytokeratins in different cell types of dog major and minor salivary glands.

Similar patterns of cytokeratin labelling have been reported, in various organs, between man and several other animal species [27] and between different animal species [17]. Although the cross-reactivity with the antibody to anti-human cytokeratins indicates a high degree of molecular similarity between polypeptides of different species, it may be less than might be expected [24]. While the cytokeratin labelling pattern of some canine epithelial structures (such as epidermis and sweat glands) is similar to corresponding structures in man [31] differences exist in the distribution of other cytokeratin types (for example CK 19 in the rectum and basal anal gland cells) [23]. While the absence of staining may indicate an interspecies difference, it need not indicate the absence of the corresponding cytokeratin polypeptide [34] as this can be the result of selective masking of the corresponding epitope [4]. In the normal foetus, low molecular weight cytokeratins are replaced with cytokeratins of higher molecular weight as embryogenesis proceeds [36]. It has also been shown in dogs that foetal tissues are less reactive than their adult tissue counterparts suggesting that, as development proceeds, epithelial cells show greater cytokeratin expression [6]. In this report, there was no obvious difference in staining related to the age of the animals, all of which were more than 3 years old.

In the present study there was staining of dog salivary gland epithelial cells and of myoepithelial cells. A similar distribution of staining, of epithelial and myoepithelial cells, has been reported in various tissues and species. There were differences in cytokeratin expression of serous and mucous acinar cells; similar differences have been reported by other authors. Mucous acinar cells in human submandibular gland stained for CK7, 8, 18 and 14 [3], whereas CK19 and CK7, 8, 17 and 18 were absent from human parotid acini which are serous [5]; serous cells in canine palatal gland were CK7 positive while parotid acinar cells were negative [38]. While it is not possible to directly compare the results of the present study with those of Vos et al. [38], who used a range of monoclonal antisera specific for different individual cytokeratins, they are broadly consistent.

The pattern of cytokeratin staining of acinar cells differed from that of ductal and myoepithelial cells. Similar differences have been reported in other glandular tissues including mammary glands in dog [37], rat [21] and mouse [2] and human parotid salivary gland [9]. In the present study, there was intense staining of duct epithelial cells (including intercalated, striated and secretory ducts) in all the glands examined. Marked staining of duct epithelial cells has been observed in human major salivary glands with cytokeratins 7, 8, 18 and 19 [14], and dog palatal and parotid glands with cytokeratins 5 and 8 [38] while in rat salivary glands, duct epithelial cells were usually not strongly positive [1]. In the present study, epithelial cells of excretory ducts displayed marked polarity of their cytokeratin staining; the luminal side of ducts was stained more strongly than the basal zone. Cytological studies in salivary ductal epithelial cells also have shown that intermediate filament proteins were mainly at the luminal border, while mitochondria and succinate dehydrogenase activity were situated in the basal aspects [20].
In the present study, as in reports in humans [18] and dog parotid gland [37], a population of so-called basal or reserve cells was detected along the striated and excretory ducts of all the salivary glands; these basal cells are generally considered to be progenitor cells of the simple duct epithelium [40]. Some reports have identified cytokeratin positive cells located at the basal layer of excretory ducts in human salivary glands as myoepithelial cells [7, 16]; in the present study, no such myoepithelial cells were recognised; myoepithelial cells, as demonstrated by staining with antibodies to α-smooth muscle actin, were generally localised at the periphery of acini and not surrounding excretory ducts (Sozmen, unpublished results). In the present study, the staining by monoclonal antibody MNF116 of epithelial as well as myoepithelial cells may have made recognition of the myoepithelial cells difficult. Reports on the distribution of intermediate filament proteins in normal myoepithelial cells are conflicting. Some authors [8, 15] have found that myoepithelial cells contain a small amount of vimentin, whereas Krepler et al. [22] believed that staining for vimentin in normal myoepithelial cells was non-specific. Morinaga et al. [28] also reported myoepithelial cells to be negative for vimentin as well as cytokeratin 5 and 6; other authors have reported that certain anticytokeratin antibodies selectively detect myoepithelium. Such a feature has been described with CK14 in human salivary gland [11, 13] and breast tissue [10]. CK14 reacts with myoepithelial cells in salivary glands in humans [5, 12] and in mammary glands of dogs [39] and rats [35].

Immunohistochemical staining for a narrow band of cytokeratins has shown differences in staining between serous and mucous acinar cells and duct epithelial cells in dog major and minor salivary glands. The results provide a basis for comparison of possible changes in cytokeratin expression which might occur in salivary gland diseases, the most common of which are neoplasia, sialadenitis, sialoceles and salivary gland infarction [33].

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REFERENCES


