IMMUNOHISTOCHEMISTRY WITH KERATIN, VIMENTIN, DESMIN, AND α-SMOOTH MUSCLE ACTIN MONOCOLOCAL ANTIBODIES IN CANINE MAMMARY GLAND: NORMAL MAMMARY TISSUE

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SUMMARY
Normal canine mammary gland tissue was studied immunohistochemically with monoclonal antibodies (MoAbs) directed against various human keratin types, vimentin, desmin, and α-smooth muscle actin. Both ductal and alveolar luminal cells were immunoreactive with MoAbs recognizing respectively human keratins no. 7, 8, 18 and 19. In addition, some ductal luminal cells were labelled with a keratin 4 and a keratin 10 MoAb. Basal/myoepithelial cells were immunoreactive only with MoAbs directed against keratin 14, keratins 14 and 17, and α-smooth muscle actin. The vimentin MoAb merely labelled solitary loose intraluminal cells representing macro-phages or sloughed epithelial cells. These findings correspond largely to observations made in human breast tissue.

INTRODUCTION
The mammary gland of all mammals is a complex organ, continuously changing morphology throughout lifetime, mostly due to body growth and cyclic hormonal stimulation (42). This compound tubuloalveolar gland is composed of fibroconnective stromal tissue surrounding parenchyma and consists of a secretery and an excretory ductal component. The secretery component is formed by alveoli and the initial portion of the intralobular ducts (secretory tubules) (3). Both alveoli and ducts are lined by two cell layers, i.e., the luminal cells and basal or myoepithelial cells (42, 49). The latter are in direct contact with the basement membrane (42, 49). Luminal and basal/myoepithelial cells can be identified his-

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Table 1. Specificity, appropriate dilutions, source, and relevant references of the monoclonal antibodies used in this study.

<table>
<thead>
<tr>
<th>MoAb</th>
<th>Specificity</th>
<th>Dilution</th>
<th>Source</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCK 102</td>
<td>K 5+8</td>
<td>1:10</td>
<td>Euro-Diagnostics B.V.</td>
<td>7,35</td>
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<td>CAM 5.2</td>
<td>K 8</td>
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<tr>
<td>RGE 53</td>
<td>K 18</td>
<td>1:10</td>
<td>Euro-Diagnostics B.V.</td>
<td>36</td>
</tr>
<tr>
<td>RCK 105</td>
<td>K 7</td>
<td>1:10</td>
<td>Euro-Diagnostics B.V.</td>
<td>34.35</td>
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<tr>
<td>LP2K</td>
<td>K 19</td>
<td>1:10</td>
<td>Amersham Ltd.</td>
<td>20.29</td>
</tr>
<tr>
<td>6B10</td>
<td>K 4</td>
<td>1:5</td>
<td>Euro-Diagnostics B.V.</td>
<td>29</td>
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<tr>
<td>RKSE 60</td>
<td>K 10</td>
<td>1:5</td>
<td>Euro-Diagnostics B.V.</td>
<td>36</td>
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<tr>
<td>RCK 107</td>
<td>K 14</td>
<td>undiluted</td>
<td>F. Ramaekers</td>
<td>64</td>
</tr>
<tr>
<td>8.7</td>
<td>K 14+17</td>
<td>1:200</td>
<td>D. Ivanji</td>
<td>18</td>
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<td>Sm-1</td>
<td>α-smooth muscle actin</td>
<td>1:15,000</td>
<td>Enzo Diagnostics Inc.</td>
<td>44</td>
</tr>
<tr>
<td>RV 203</td>
<td>vimentin</td>
<td>1:5</td>
<td>Euro-Diagnostics B.V.</td>
<td>43</td>
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<tr>
<td>RD 301</td>
<td>desmin</td>
<td>undiluted</td>
<td>Euro-Diagnostics B.V.</td>
<td>7</td>
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</tbody>
</table>

MoAb = monoclonal antibody
K = human keratin type according to Moll et al. (23).

Table 2. Immunohistochemical labelling patterns of canine mammary epithelium after incubation with monoclonal antibodies against keratins, smooth muscle actin, vimentin, and desmin.

<table>
<thead>
<tr>
<th>Mammary gland tissue</th>
<th>Monoclonal Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue structures</td>
<td>RCK 102 (K 5+8)</td>
</tr>
<tr>
<td>Alveoli</td>
<td>luminal</td>
</tr>
<tr>
<td></td>
<td>basal/myoepithelial</td>
</tr>
<tr>
<td>Ducts</td>
<td>luminal</td>
</tr>
<tr>
<td></td>
<td>basal/myoepithelial</td>
</tr>
</tbody>
</table>

(= keratin types according to Moll et al. (23).

myoepithelial is the only scattered in lobar ducts were positive

Figure 1. Immunoperoxidase labelling pattern of canine mammary gland with 6B10, directed against human keratin type 4. Note that only a few luminal cells in a large duct are labelled. Frozen section, 400X.

In this study the immunohistochemistry of normal canine mammary gland tissue was established with oligo- or monospecific monoclonal antibodies against keratins and with monoclonal antibodies against vimentin, desmin, and α-smooth muscle actin. The results are discussed in the light of data on the mammary gland of other mammalian species, particularly humans.

MATERIALS AND METHODS
Specimens of normal canine mammary gland were obtained.
from five bitches of unknown hormonal cycle stage. The tissue specimens were frozen in liquid nitrogen precooled isopentane immediately after surgical excision or euthanasia of the animals. The specimens were stored at -70°C until use. The monoclonal antibodies (MoAbs) used for immunohistochemistry, their specificity, dilutions used, source, and relevant references are presented in Table 1.

Five-micron-thick frozen sections were fixed in cold acetone (-20°C) for 10 minutes and air dried for 5 minutes. Immunohistochemical labelling was performed using the indirect immunoperoxidase technique as described previously (60).

The labelling results are expressed semi-quantitatively as follows:

- +  = < 10% positive cells
- ++ = > 10% but < 50% positive cells
- +++ = > 50% but < 90% positive cells
- ++++ = > 90% positive cells

RESULTS

The ducts in the various specimens of the normal canine mammary gland were surrounded either by very small lobules of inconspicuous ductular or alveolar structures, or by distinct lobules of slightly distended alveoli.

The labelling patterns of the MoAbs are presented in Table 2. Although some differences in labelling intensity were observed, no significant differences in the labelling patterns were present between the specimens from the different dogs (n=5).

Ductal and alveolar structures showed almost identical labelling patterns, except for the keratin MoAbs 6B10 (K 4) and RKSE 60 (K 10). MoAb 6B10 labelled sporadic luminal cells in large, most likely lobar ducts (Figure 1). MoAb RKSE 60 also labelled a few ductal luminal cells in some specimens (Figure 2), whereas in other specimens a significant number, but still a minority, of these cells was labelled. No alveolar cells were labelled with these two latter MoAbs. All epithelial cell types in the canine female mammary gland were positive with the broad-spectrum antibody RCK 102 (K 5+8). Both in ducts and alveoli immunohistochemical differences were observed between luminal and basal/myoepithelial cells. Although the absence of labelling of basal/myoepithelial cells was sometimes difficult to assess, luminal cells were exclusively and almost homogeneously labelled with the keratin MoAbs CAM 5.2 (K 8) and RCK 105 (K 7). They were less homogeneously labelled with RGE 53 (K 18) and LP2K (K 19). In addition to showing immunoreactivity with RCK 102 (K 5+8), the basal/myoepithelial cells were only labelled with the keratin MoAbs RCK 107 (K 14) (Figure 3) and 8.7 (K 14+17) and the α-smooth muscle actin MoAb Sm-1. In large ducts and ductules these MoAbs revealed a continuous layer of distinctly labelled cells (Figure 4), whereas in the smallest ducts and alveoli a more discontinuous layer of elongated small cells was present (Figure 5). Although solitary intraluminal cells were labelled with the vimentin MoAb (RV 203), the lining epithelium showed no immunoreactivity with this antibody (Figure 6). The vimentin MoAb labelled fibroblasts and endothelial cells in the stromal tissue of the mammary gland. The desmin MoAb (RD 301) only labelled smooth muscle cells in vessel walls and did not show labelling of epithelial cells in the mammary gland tissue.

DISCUSSION

The various specimens of normal canine mammary tissue
luminal cells with a K 18 MoAb has been reported previously (58). Our findings are similar to findings in human breast tissue, where luminal cells have been shown to be labelled with MoAbs directed against K 7 (6,34,42,49,53,54), K 8 and K 18, either heterogeneous (6,34,40,41,53,54) or homogenous (4,15,28,34,42,49,55,64), and for K 19 (30), which occurs heterogeneously in small ducts and ductal lobular units (4,5,9) and homogenously in large ducts (5,42,49,53,54). In humans, luminal cells in large ducts have also been found to be K 14 immunoreactive (63). Although K 4 and K 10 have not been identified in human mammary gland ducts (25,26), the MoAbs directed against these (human) keratin types labelled some ductal luminal cells in the dog. In humans, labelling was not observed with these MoAbs in breast tissue (29,30,34). In the cat, as in our dogs, mammary luminal cells have also been shown to be immunoreactive with K 5+8, K 7, K 18 and K 19 MoAbs (18,22). Also in rat mammary tissue luminal cells are labelled with K 8, K 18 and K 19 MoAbs (21), although also the absence of labelling with K 8 and K 19 MoAbs has been documented (53). In the dog a minority of luminal cells was not labelled with the K 19 MoAb (LP2K), as has also been observed in humans (4,5). For the human system it has been postulated that these cells constitute a proliferative compartment, possibly stem cells (4,5).

Basally located (myoepithelial) cells in the canine mammary gland were selectively labelled with the keratin MoAbs RCK 107 (K 14), 8.7 (K 14+17) and the α-smooth muscle actin MoAb (Sm-1). In humans, a similar immunoreactivity pattern is present for K 14 (14,28,42,49,53,54,63), which is sometimes heterogeneously present (57), particularly in alveoli (64), and for K 17 (14), which is found homogenously in large ducts and heterogeneously in small ducts and alveoli (15,51). The basal cells in humans are also immunoreactive for K 5 (28,30,42,49), although K 5-negative basal cells have been reported (24). Feline basal mammary cells have been shown also to be reactive with K 5+8, K 14 and K 14+17 MoAbs (18,22). Mouse mammary gland basal cells have been shown to express K 14 immunoreactivity (46,53) and in similar cells in rat both K 5 and K 14 labelling has been reported (21,53). In humans, a fraction of basal cells in large ducts also contain K 7 (31,64), and K 19 (4,64). Similarly, rat basal cells are heterogeneously labelled with a K 19 MoAb (21).

The α-smooth muscle actin MoAb (Sm-1) is known to label myoepithelial cells specifically as the only epithelial cell (16,44,53) both in human breast tissue (4,14,41,50,53,57), and in mammary gland tissue of mouse (47) and rat (2,16). Canine myoepithelial cells were unequivocally labelled with this antibody. No labelling of canine myoepithelial cells was observed with the vimentin MoAb, similar to what has been found in humans (48,50), mice (12,47), rats (2,13,48), and cattle (12,13). However, vimentin labelling of these cells has been reported in different species, e.g., humans (11,14,15,28,40,41,49,57), rats (21,32) and dogs (17). These discrepancies may be due to differences in antibodies, fixation, and immunohistochemical methods used, or may be due to interspecies differences and variation in differentiation of the cells (28,49). In rats, myoepithelial cells have been recently found to be desmin immunoreactive (32), contrary to previous reports for rats (2,13), humans (11,38,52), mice (12,47), cattle (12,13), and our present findings. The vimentin-labelled solitary cells in ductal or alveolar lumina may represent macrophages or sloughed epithelial cells (3).
in which expression may possibly be induced by the loss of cell-to-cell contact (39).

In conclusion, we can state that luminal and basal/myoepithelial cells of the canine mammary gland can be differentiated immunohistochemically with monoclonal keratin MoAbs and α-smooth muscle actin antibodies. The canine epithelial lining of ducts and alveoli does not seem to express vimentin. All in all, the immunohistochemical labelling profiles with keratin antibodies correspond to findings in other species, indicating only minor differences exist in the pattern of keratin expression in breast epithelia amongst species.

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REFERENCES


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106

ERRATUM
Unfortunately, in the article ‘Potential of Trypanotolerance as a contribution to sustainable livestock production in tsetse affected Africa’ by R. W. Paling and R. H. Dwinger, The Veterinary Quarterly 1993; 14(2): 60-7, the first author’s foot-note has fallen off. The foot-note should be: R. W. Paling, Bureau Internationale Contacten, Faculty of Veterinary Medicine, Utrecht University, P.O. Box 80.165, 3508 TD Utrecht, the Netherlands.

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107