Immunohistochemistry with Keratin Monoclonal Antibodies in Canine Tissues: Urogenital Tract, Respiratory Tract, (Neuro-)Endocrine Tissues, Chorioid Plexus and Spinal Cord

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With 7 figures and 3 tables

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Summary

Twelve oligo- or monospecific monoclonal antibodies (MoAbs) directed against human keratin types were used in an immunohistochemical study of the canine male and female urogenital tract, the respiratory tract, the adrenal gland, the (para-)thyroid gland, the chorioid plexus and the spinal cord. The keratin MoAbs showed differences in staining patterns in the various epithelial tissues and the diverse epithelial cell types. The kidney was characterized by a complex keratin staining pattern and the canine urothelium showed regional differences in keratin staining. Also in the female genital tract different keratin staining patterns were observed. Testicular and adrenal gland cells did not react with any of the keratin MoAbs. The keratin staining patterns in the various canine tissues showed, in addition to similarities, also distinct differences when compared to the staining patterns in corresponding tissues of other species, e.g. of man. These staining dissimilarities indicate that the reactivity patterns of the keratin MoAbs with restricted keratin immunoreactivity can not be always extrapolated from one species to another. Nevertheless, MoAbs directed against human keratin proteins can apparently be used to differentiate between various types of canine epithelia or epithelial compartments.

Introduction

Keratins are intracellular intermediate filament proteins, occurring almost exclusive in epithelial cells (43). These proteins are part of the cytoskeleton of these cells (43) and in man constitute a heterogeneous group of proteins with different molecular weight and isoelectric point (12, 22, 45, 61). Thus, biochemically the presence of 20 different keratin polypeptides in human epithelial tissues has been established (45, 61). These keratins can be subdivided into acidic (type I) and neutral to basic (type II) proteins (12). The various epithelia in man are characterized by the presence of two to ten of these keratin-types (22,
Table 1. Staining patterns of keratin and smooth muscle actin monoclonal antibodies in the urinary tract of the dog

<table>
<thead>
<tr>
<th>Tissue/Cell type</th>
<th>RCK 105 (K7)</th>
<th>RCK 105 (K5, others)</th>
<th>RCK 102 (K5,8)</th>
<th>CAM 5.2 (K8)</th>
<th>RGE 53 (K18)</th>
<th>DE-K18 (K18)</th>
<th>LP2K (K19)</th>
<th>6B10 (K4)</th>
<th>DE-K10 (K10)</th>
<th>RKSE 60 (K10)</th>
<th>RCK 107 (K14)</th>
<th>B.7 (K14, 17)</th>
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<tr>
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</table>

( ) = keratin types according to Moll et al. (45); - = absence of staining; + = positive staining; (±) = faint positive staining; +h = heterogeneous staining; ± = scattered positive staining in a minority of cells.

* = only umbrella cells positive.

*1 = negative staining of umbrella cells.

*2 = umbrella cells and scattered other suprabasal cells.
45), whereby acidic keratins are paired with specific basic keratins (12, 43). As a result the various epithelia have characteristic keratin patterns (22, 45, 52).

Several polyclonal and monoclonal antibodies (MoAbs) have been raised against human keratins (6, 43, 49, 52, 56, 69, 71). The MoAbs can be separated into broad spectrum antibodies, directed against several keratins and reactive with many types of epithelia, antibodies with restricted specificity, i.e., directed against determinants common to a small number of keratins, and finally polypeptide specific antibodies, reactive only with a single keratin polypeptide (43, 49, 71). The application of these antibodies, particularly of keratin-type specific MoAbs, has evolved to be of great value to elucidate the expression patterns of the different keratins in various epithelial tissues, cell types and epithelial tumours (6, 12, 16, 31, 49, 54, 69). Many of these antibodies cross-react with animal epithelial tissues (4, 11, 14, 26, 51, 56, 69, 71), although incidentally cross-reactivity is restricted or lacking (62, 71).

As in man also in several animal species different keratin polypeptide patterns have been characterized by two-dimensional gel electrophoresis in epithelial tissues of, for instance cow (9, 47, 61), mouse (19, 47, 61), rainbow trout (39, 40), miniance pig (62) and rabbit (17, 23). Immunohistochemically also differences in staining pattern in different epithelial tissues and cell types have been reported within animal species, e.g., rat (4, 26, 30, 56), mouse (19), monkey (69), miniature pig (62), cat (32), horse (27), cow (1, 9, 30), pike (68), rainbow trout (39), rabbit (17, 23) and guinea pig (54). In the dog epithelial tissues can be differentiated immunohistochemically using broad spectrum polyclonal (73) or broadly reactive monoclonal keratin antibodies (11, 15, 57).

In the present paper the staining patterns obtained by a panel of mono- or oligo-specific MoAbs directed against human keratin-types are reported in the canine male and female urogenital tract, respiratory tract, adrenal gland, thyroid and parathyroid, choroid plexus and spinal cord. The staining patterns in these canine tissues are discussed particularly in relation to the reaction patterns in corresponding human tissues.

Material and Methods

Immediately after euthanasia specimens were obtained of the following tissues and organs: kidney, urinary bladder, urethra, prostate, epididymis, testis, ovary, uterus, cervix, vagina, vulva, trachea, lung, thyroid, parathyroid, adrenal gland, choroid plexus and spinal cord. As to the female genital tract tissues, the oestrus cycle was not known. The tissue specimens were frozen in liquid nitrogen precooled isopentane and stored at -70°C until processing for immunohistochemistry. In this study monoclonal antibodies (MoAbs) were used directed against human keratin-types (K). The MoAbs included: RCK 103 (K.7), RCK 102 (K.5 + others), RCK 102 (K.5 + 8), CAM 5.2 (K.8), RGE 53 (K.18), DE-K18 (K.18), LP2K (K.19), 6310 (K.4), DE-K10 (K.10), RGE 60 (K.10), RCK 107 (K.14) and 8.7 (K.14 + 17). The vast majority of these MoAbs can only be applied on frozen material. Only RCK 102, CAM 5.2 and DE-K10 are known to react with formalin fixed, paraffin embedded tissues.

The specificity, the dilution, the source and relevant references of these MoAbs and the procedure of immunohistochemical staining were published previously (74).

Results

All keratin MoAbs used in this study and directed against the various human keratin types showed cross-reactivity with canine epithelial cells. Additionally, RCK 103 (K.5 + others) and DE-K10 (K.10) also stained nervous tissue. DE-K18 (K.18) and DE-K10 were found to react faintly with smooth muscle cells and endothelial cells.

The staining patterns of the MoAbs in the various epithelial tissues and cell types of the kidney, urinary bladder and urethra are presented in Table 1. In the renal cortex, the parietal cells and podocytes in the glomeruli and the proximal tubules were characterized by a restricted keratin staining pattern, i.e., reacting mostly sporadically with DE-K18 (K.18), DE-K10 (K.10) and CAM 5.2 (K.8) and 8.7 (K.14 + 17) respectively. In contrast a broad spectrum of keratin immunoreactivity was seen in the distal tubules. In the cortico medullary rays tubular structures with either flattened or cuboidal epithelium could
Fig. 1. Immunoperoxidase staining of the kidney, cortical region. Staining of tubules lined by flattened epithelium with 6B10, directed against human keratin-type 6. Frozen section, 300×

observed morphologically. The tubules lined by flattened epithelial cells reacted with many keratin MoAbs and even showed staining with 6B10 (8, 4) (Fig. 1), whereas the tubules lined by cuboidal epithelial cells did not stain with any of the keratin MoAbs used. In the renal medulla the tubular structures lined by flattened epithelium did not react with 6B10

Fig. 2. Immunoperoxidase staining of the kidney, pyelum. Staining of the lining epithelium by CAM 5.2, directed against human keratin-type 8. Note the absence of staining of the basal cells in the pelvic epithelium (arrows). Frozen section, 200×
Fig. 3. Immunoperoxidase staining of the kidney, pyelum. Staining of the epithelium lining the papilla and the umbrella cells of the pelvic epithelium with RGE 53, directed against human keratin-type 18. Frozen section, 200×

(K 4), RKSE 60 (K 10) and 8.7 (K 14 + 17), in contrast to what we observed in morphologically similar structures in the cortico-medullary rays. In the ductal structures of the medulla RCK 105 (K 7) and RCK 103 (K 5 + others) proved to convert from no staining in

Fig. 4A. Immunoperoxidase staining of the kidney, pyelum. Absence of staining of pelvic epithelium with 6B10, directed against human keratin-type 4. Frozen section, 125×
Fig. 4B. Immunoperoxidase staining of the urinary bladder. Heterogeneous staining of the basal urothelial cells with 6B10, directed against human keratin-type 4. Frozen section, 125 x

Collecting ducts to homogeneous staining of the papillary epithelium. In the papillary ducts an increasing number of positive cells was seen towards the pyelum. The epithelial lining of the renal papilla and the renal pelvis showed some differences in keratin staining of the basally located cells as these cells in the papilla in contrast to the pelvis were stained by

Fig. 4C. Immunoperoxidase staining of the urethra. Staining of the urothelial cells with 6B10, directed against human keratin-type 4. Frozen section, 125 x
<table>
<thead>
<tr>
<th>Tissue/Cell type</th>
<th>Monoclonal Antibodies</th>
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<td></td>
<td>RCK 105</td>
</tr>
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<td></td>
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<td>Sertoli cells</td>
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<td>epididymus</td>
<td>basal cells</td>
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<td>luminal cells</td>
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<td>uterus</td>
<td>acinar epith.</td>
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<td>ovaries</td>
<td>ductal epith.</td>
</tr>
<tr>
<td>uterus</td>
<td>surface epith.</td>
</tr>
<tr>
<td>ovaries</td>
<td>follicular</td>
</tr>
<tr>
<td>epith.</td>
<td>superficial epith.</td>
</tr>
<tr>
<td>ovaries</td>
<td>glandular epith.</td>
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<tr>
<td>cervix</td>
<td>basal cells</td>
</tr>
<tr>
<td>ovaries</td>
<td>suprabasal cells</td>
</tr>
<tr>
<td>vagina</td>
<td>basal cells</td>
</tr>
<tr>
<td>ovaries</td>
<td>suprabasal cells</td>
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<tr>
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<tr>
<td>ovaries</td>
<td>cells</td>
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<td>ovaries</td>
<td>suprabasal cells</td>
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() : keratin types according to Moll et al. (45); epith. = epithelium; - = absence of staining; + = positive staining; (+) = faint positive staining; + = heterogeneous staining; ± = scattered positive staining in a minority of cells.
CAM 5.2 (K.8), RGE 53 (K.18) (Figs. 2 and 3), RCK 107 (K.14) and 8.7 (K.14 + 17), whereas on the other hand in the pelvis such cells were stained by LP2K (K.19).

Although in pyelum and urinary bladder RGE 53 (K.18) staining was restricted to the umbrella cells, in the urethra also scattered other solitary, suprabasal cells were stained. The transitional epithelium of the pelvis was entirely negative for 6B10 (K.4) (Fig. 4 a). However, the transitional bladder epithelium was stained with this MoAb particularly in basally located cells (Fig. 4 b), whereas urethral epithelium in addition showed diffuse suprabasal staining with 6B10 (Fig. 4 c). Although almost all epithelial cells in the bladder mucosa were labelled by LP2K (K.19), in the urethra merely superficial cells (umbrella cells) and other scattered suprabasal cells were stained.

The staining patterns of the MoAbs in the various epithelial cell types of the male and female genital tract are presented in Table 2. No keratin staining was observed in the testis. In the epididymis and prostate elaborate keratin staining was observed. In the epididymis the presence of basal cells was evident by their selective staining with RCK 103 (K.5 + others), RCK 107 (K.14) and 8.7 (K.14 + 17) (Fig. 5). With respect to these MoAbs it was surprising to see homogeneous staining of the prostatic acinar epithelium for RCK 103 and RCK 107, whereas 8.7 immunoreactivity was lacking (Fig. 6), and that in these acinar structures no basal cells could be distinguished immunohistochemically. Whereas prostatic ducts stained homogeneously with 6B10 (K.4), only a solitary positive cell was found in the acini. Prostatic duct cells stained heterogeneously with RKSE 60 (K.10) while acinar cells were negative with this MoAb.

In the canine female genital tract, the ovarian surface epithelium was stained by several keratin MoAbs. Superficially located epithelial structures within the ovary showed a keratin staining pattern similar to the surface epithelium, although less intensively. The rete ovarii was stained by DE-K10 (K.10) and only a number of rete cells was stained by RCK 102 (K.5 + 8), CAM 5.2 (K.8), DE-K18 (K.18) and 8.7 (K.14 + 17). The ovarian follicular epithelium was only positive with DE-K10 (K.10). In the uterus the surface epithelium could not be discriminated from glandular epithelium immunohistochemically, although in contrast to surface epithelial cells a proportion of the glandular epithelial cells

Fig. 5. Immunoperoxidase staining of the epididymis. Staining of basal cells with 8.7, directed against human keratin-types 14 and 17. Frozen section, 300 x
did not react with RCK 105 (K 7) and RCK 103 (K 5 + others). In comparison to cervical
epithelium, the vaginal epithelium showed a more diffuse staining pattern with LP2K
(K 19), 6B10 (K 4) and RKSE 60 (K 10), whereas the staining of suprabasal cells with RGE
53 (K 18) and RCK 107 (K 14) was less pronounced. The mucosal part of the vulva showed
a staining pattern most consistent with the epidermal outer part. However, the mucosal

Fig. 6 A. Immunoperoxidase staining of the prostate. Absence of staining of the prostatic cells with
8.7, directed against human keratin-types 14 and 17. Frozen section, 300 x

Fig. 6 B. Immunoperoxidase staining of the prostate with RCK 107, directed against human keratin
type 14. Frozen section, 300 x
Table 3. Staining patterns of keratin and smooth muscle actin monoclonal antibodies in the respiratory tract, (neuro-)endocrine tissues, choroid plexus and the spinal cord of the dog

<table>
<thead>
<tr>
<th>Tissue/Cell type</th>
<th>Monoclonal Antibodies</th>
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<tr>
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<tr>
<td>other cells</td>
<td>+</td>
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<td>broncholar epithelium alveolar epithelium</td>
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<tr>
<td>brain choroid plexus</td>
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<tr>
<td>spinal cord ependyma</td>
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(±) keratin types according to Moel et al. (45); - = absence of staining; + = positive staining; (±) = faint positive staining; ± = heterogeneous staining; ± = scattered positive staining in a minority of cells.
Fig. 7. Immunoperoxidase staining of the choroid plexus. Staining of solitary cells with RCK 102, directed against human keratin-types 5 and 8. Frozen section, 300 x.

part was 6B10 (K.4) positive and RKSE 60 (K.10) negative and showed only a restricted number of DE-K10 (K.10) positive suprabasal cells.

The staining patterns of the various epithelial tissues and cell types in the respiratory tract, the thyroid, the parathyroid, the adrenal gland, the choroid plexus and in the spinal cord are presented in Table 3. In the respiratory system basal cells could be identified in the trachea by the absence of staining with RGE 53 (K.18), DE-K10 (K.10) and 8.7 (K.14 + 17) and their immunoreactivity with RCK 107 (K.14). The bronchiolar epithelial cells showed a staining pattern similar to non-basal tracheal cells. The alveolar epithelium reacted with several MoAbs. Due to the relatively poor morphology of frozen sections no differentiation could be made between alveolar type I and type II cells. The thyroid showed a broad keratin staining pattern. As none of the MoAbs seemed to react exclusively with the parafollicular cells and morphologically these cells could not be differentiated in the frozen sections from solid groups of follicular cells, the staining pattern of the parafollicular cells could not be established. The parathyroid had a restricted keratin staining pattern. The epithelial cells of both adrenal cortex and medulla did not stain with any of the MoAbs used. In the central nervous system a varying number of cells lining the choroid plexus reacted with some of the keratin MoAbs (Fig. 7), whereas the ependymal cells lining the canal of the spinal cord only showed faint and heterogeneous immunoreactivity with CAM 5.2 (K.8). This MoAb also stained astrocytic cells in the spinal cord.

Discussion

The panel of MoAbs, each specific for one or some human keratin proteins, were used to differentiate immunohistochemically various canine tissues. Since a keratin catalogue of the canine epithelia is not available at the moment, the Moll-nomenclature used in this paper refers to the human keratin numbers. It has to be realized that the MoAbs at the best recognize the canine counterparts of the human keratin-types. Although MoAb cross-reactivity resulting in similar staining patterns in corresponding tissues of different species indicates a high and very localized degree of molecular similarity (38), this does not
explicitly implicate keratin similarity (39), as has been demonstrated in miniature pig oral epithelium (62).

Within the following paragraphs the immunohistochemical findings in the canine tissues are discussed and compared with the reviewed literature data about the distribution patterns of individual keratins in corresponding tissues of human beings and some other species.

**Urinary tract**

*Kidney.* The canine kidney was characterized by various keratin labelling patterns in the different parts of the nephron, also demonstrated in rat (4), bovine (1, 4, 30) and human kidneys (30). In the canine glomeruli only immunoreactivity with one of the K18 MoAbs (DE-K.18) and one of the K10 (DE-K.10) MoAbs of both podocytes and parietal cells was noticed. Parietal cells have been shown to be heterogeneous or homogeneously stained by epitidal keratins both in man (54, 66), rat (4, 66) and cow (4). Immunohistochemically in man the presence of K7, 8, 18 and 19 has been reported in parietal cells (20, 46, 54). The tubular and ductal structures in the canine kidney show a complex keratin staining pattern with distinct differences as compared to man, in which a clear pattern seems to exist with positive staining for K7, 8, 18 and 19 (6, 7, 20, 46, 54), although a heterogeneous distribution pattern of the K7 and K19 immunoreactivity was demonstrated (62). Selective staining was seen in the loops of Henle and ducts for K7, 8 and 19 (20, 46, 54). Biochemically in human cortical tissue K8 and K18 have been detected and minor amounts of K19, which type was also isolated from loops of Henle and collecting ducts (1). In the dog however, the proximal tubules only reacted with CAM 5.2 (K8) and 8.7 (K14 + 17). Both proximal and distal tubules did not react with the K19 MoAb (LP2K), whereas in rat proximal tubules scattered LP2K positive cells have been observed (26). The tubules with cuboidal cells in the cortico-medullary rays, possibly representing the first segment of the loop of Henle viz. the straight portion of the proximal tubule (S), did not stain with any of the MoAbs used. The tubules lined by flattened epithelium in both cortico-medullary rays and renal medulla, possibly representing the thin segments of the loop of Henle (S) exhibited a broad keratin staining pattern, even broader than in man. A striking feature were the differences in the staining patterns of the tubules lined by flattened epithelium in the cortico-medullary rays on the one hand and those in the medulla on the other hand. This phenomenon could possibly indicate regional differences in keratin staining of the epithelial cells in the different parts of the loop of Henle. In man, differences in keratin immunohistochemistry in the two types of thin limbs of the loop of Henle have been established (4). In the dog the ducts had a broad keratin staining pattern. Contrary to the dog, in the collecting ducts of rats scattered LP2K positive cells were seen (26). A remarkable feature was the occurrence of increasing positivity of the ductal epithelium towards the papilla for many keratin MoAbs. Although different structures in the canine kidney were stained by the K14 MoAb (RCK 107), this keratin-type was not noticed immunohistochemically in human kidney (52). The keratin staining patterns in the canine kidney apparently show differences compared to findings in human kidney.

*Urothelia.* The canine urothelia showed next to similarities also minor variances in keratin staining. The papillary epithelium did not show differences in staining of basal and suprabasal cells, whereas in the other structures covered by urothelium these cell types could be discriminated with some MoAbs, e.g. RGE 53 (K18). In human urothelium keratin-types 4, 5, 7, 8, 13, 14, 17, 18, 19 and 20 have been reported immunohistochemically (6, 7, 44, 46, 48, 52, 54, 58, 59, 78) as well as biochemically (1, 44, 45). In the dog one of the K18 MoAbs (RGE 53) merely stained the umbrella cells whereas the other K18 MoAb (DE-K.18) stained all cells. The selective staining of umbrella cells by some K18 MoAbs has also been observed in man (1, 53, 54, 58, 59) and other species (53, 56), as well as the staining of all urothelial cells by other K18 MoAbs (1, 54, 58, 59). Canine urothelial cells showed staining with both K10 MoAbs. In man, K10 MoAbs have been reported not to stain urothelium (31, 58), and only to be expressed in the squamous epithelium of the
urogenital tract (58). However, a very small number of human urinary bladder epithelial cells was positive with RKSE 60 (54) and a number of suprabasal urethral cells was labelled by a MoAb directed against K 10 and 11 (1). The K 4 MoAb (6B10) heterogeneously stained the basal urothelial cells and scattered suprabasal cells in the canine urinary bladder, whereas the same MoAb did not stain basal cells in human urinary bladder and homogeneously stained suprabasal cells (49). Another report, however, described sporadic labelling in man of individual basal urothelial cells or suprabasal cells in contact with the basal lamina (44) and some umbrella cells (59) with this MoAb. Similar as in the canine urethra, human urethral epithelium was homogeneously positive for K 4 (59). In man, urinary bladder epithelium is negative for K 14, but urethral epithelium appeared to be positive in basal cells (52). Canine bladder and urethral epithelium showed also a distinct staining difference with respect to the K 19 MoAb, as the former was almost homogeneously stained whereas in the urethra merely superficial positive cells were observed with solitary cells dispersed throughout the epithelium. Homogeneous staining of urinary bladder epithelium for K 19 has also been observed in man (6, 58, 59) and rat (56). Human urethral epithelium was also homogeneously positive for K 19 (58, 59), although K 19 expression decreased in the fossa navicularis and was entirely absent in the glans penis (59) or present only in basal cells and several suprabasal cells in this region (58). Similar to man, the epithelial lining of the pelvis, urinary bladder and urethra shows regional differences in keratin immunohistochemistry. However, in the dog as compared to man distinct differences are seen.

**Male genital tract**

**Testis.** The canine testis was characterized by the absence of keratin staining of germative cells, Sertoli and Leydig cells, similar to the situation in pig (72) and man (1, 6, 21, 54, 67, 72). Also biochemically no keratin polypeptides could be detected in human testis (1). Germative cells and Sertoli cells are thought to represent epitheloid differentiations of mesenchymal origin rather than genuine epithelial cells (21). Nevertheless, in human foetal, prepubertal and senile, and pathologically altered atrophic testes these cells could be labelled by broad spectrum keratin MoAbs and for K 8 and K 18 (20, 67). With respect to Leydig cells in some human testis was sporadic staining by a broad spectrum keratin MoAb was observed (18). In the human rate testes biochemically keratin-types 7, 18 and 19 were detected and additionally K 5 in the ductus efferens (1). Also in pig and canine testes these structures were labelled by an antibody directed against human skin keratins (72).

**Epididymis.** Canine epididymis showed extensive staining with the various keratin MoAbs and basal cells could be immunohistochemically differentiated from luminal cells. In man, biochemically and immunohistochemically keratin-types 7, 8, 18 and 19 were detected (1, 37) and biochemically also K 5 (1). In man, basal cells appeared to stain specifically for K 17 (37). K 19 immunoreactivity was not found in the canine epididymis whereas heterogeneous immunolocalization for K 19 has been observed in the human epididymis (5).

**Prostate.** Immunohistochemically basal and luminal prostatic cells can be discerned in man (20, 49, 52, 54, 78), rat (3, 70), calf (76) and goat (75), whereas in the canine prostate no immunohistochemical differences were observed in the acinic cells. In man, biochemically prostatic epithelium has been shown to contain keratin-types 5, 7, 8, 15, 17, 18, 19 (1, 63). Immunohistochemically also K 14 has been demonstrated in basal cells (52, 78) and a limited number of acinic cells contain K 4, K 13 (49) and K 20 (48). Although MoAbs directed against K 14 in the human prostate exclusively reacted with basal cells, the K 14 MoAb used in this study (RCK 107), which in other canine tissues also particularly demonstrates basal and myoepithelial cells (74), reacted with all canine prostate acinic cells. In man, K 7 is found sporadically in both luminal and basal cells (54), whereas in the dog all acinic cells are immunoreactive for the K 7 MoAb. Canine prostatic duct cells appeared to be stained heterogeneously by RKSE 60 (K 10) which MoAb also heterogeneously stained.
canine urethral epithelium. In contrast, human prostatic duct epithelium does not react with 6810 (49) or RSE6 (54). The canine male genital tract shows next to similarities also evident differences in keratin immunoreactivity compared to man.

**Female genital tract**

**Ovary.** The surface epithelium in accordance with findings in other species, i.e. rat, mouse and pig, was labelled by the K18 MoAb (14). In man, this surface epithelium contains keratin-types 7, 8, 18 and 19 (6, 13, 14, 47, 50). The superficially located structures with a keratin staining pattern identical to the surface epithelium were interpreted either as surface epithelium invaginations or premature primary follicles. Contrary to man the canine surface epithelium was not labelled by the K19 MoAb. In contrast to the dog in which follicular cells stained with DE-K10 (K10), in rat, mouse and pig no keratins could be detected in the follicles neither immunohistochemically nor biochemically (14). In man, follicular granulosa cells showed the presence of K8 and K18 with a gradual reduction of these keratins during follicular maturation (14, 50). Also hamster granulosa cells have been shown to be labelled by a "panepithelial" keratin MoAb (51). These species differences may reflect differences in follicle formation (14) or hormonal status.

**Uterus.** The canine endometrium and endometrial glands showed a keratin staining profile characterized by staining with the K7, 8, 18 MoAbs and RCK 103 (K 5 + others), RCK 102 (K 5 + 8) and DE-K10 (K 10). In man, the presence of keratin-types 7, 8, 18 and 19 has been established in the uterus, both biochemically (13, 47) and immunohistochemically (6, 9, 25, 47, 54). Although in man, as in the dog, staining of the endometrium with RCK 102 has been observed (54), only a weak and focal endometrial staining in human uterus has been reported with this MoAb in formalin fixed tissue samples (25). In man, endometrial cells did not stain for K 10 with RSE6 (54), which is concordant with the reaction in canine endometrium with this MoAb in spite of the staining of the canine endometrium with the other K 10 MoAb (DE-K10).

**Cervix and vagina.** Cervical and vaginal epithelium reacted with all keratin MoAbs used and showed an almost identical staining pattern which was different from the staining pattern in the endometrium. None of the keratin MoAbs specifically stained basal cells. Also in man, broad keratin reactivity of ectocervical and vaginal epithelium has been established. The presence of keratin-types 1, 2, 4, 5, 6, 8, 10, 11, 13, 14, 15, 16, 17, 18 and 19 has been demonstrated by biochemistry (13, 45, 47), immunohistochemistry (6, 25, 31, 52, 54, 64, 65), and mRNA hybridization (44). In man, CAM 5.2 (K8), K14 MoAbs and some K18 MoAbs specifically stained basal cells in ectocervix and/or vagina (64, 65, 78) and K14 MoAbs in additional parabasal cells (65). K19 was found homogeneously distributed in basal cells of these human epithelia (6, 64), but additionally also in scattered suprabasal cells (6, 64). In the dog, however, CAM 5.2 and both K14 MoAbs stained basal cells as well as suprabasal cells, whereas the K19 MoAb only stained the suprabasal cells. In man, K 4 and K 10 were only found in the suprabasal ectocervical and vaginal epithelial cell layers (31, 54, 64), whereas in the dog cells in all cell layers were labelled by MoAbs directed against these keratin types.

**Vulva.** The canine vulvar epithelium only showed a restricted keratin staining and basal cells were specifically stained by the K14 MoAb.

So, the canine female genital tract is characterized, as in man, cow and mouse (47), by different keratin staining patterns in the different regions. However, the staining patterns in the dog differ from those in the human female tract.

**Respiratory tract**

Like in man and cow (9, 10), also the canine bronchial/tracheal epithelium differs from alveolar epithelium with respect to keratin immunoreactivity. In man, biochemically various keratin-types have been isolated from tracheal and bronchial epithelium, i.e. K 5, 8, 13, 15, 17, 19 and minor amounts of K 6, 7, 14 and 18 (9, 45). In human alveolar cells only
K7, 8, 18 and 19 have been detected (7, 9), and in rat alveolar cells K18 and K19 immunoreactivity has been reported (77). In the canine trachea, basal cells could be recognized by their immunoreactivity with the K14 MoAb (RCK 107) and the absence of labelling with some other keratin MoAbs. In human bronchial epithelium basal cells contain keratin-types 5, 14 and 17 (29, 78) and not 8, 18 and 4 (9, 10, 49, 54). However, faint heterogenous staining of basal cells for K8 and K18 in addition to staining of the luminal cells has been reported (10, 54), and heterogeneous reaction for K4 (10). Also in the dog K18 immunoreactivity could be found in basal tracheal cells. Although K7 was not found in human tracheal basal cells (10, 54), in the dog some basal cells were stained by the K7 MoAb. LP2K (K19) stained all canine tracheal cells but the staining intensity diminished with decreasing diameter of the airways resulting in faint staining of bronchiolar cells, and absence of staining of alveolar cells. In man, K19 has been demonstrated in tracheal, bronchial and alveolar epithelial cells (9, 10, 45).

(Neuro-)endocrine tissues

Thyroid and parathyroid. Although canine thyroid and parathyroid cells were shown to be unreactive with a polyclonal antibody directed against canine footpads epidermis (33), these cells appeared to be stained by various MoAbs directed against specific human keratin-types. As in the dog, human thyroid follicular cells were homogeneously positive for K7 (16, 54), K8 (16, 60), K18 (16, 28, 54, 60) and heterogeneously for K19 (6, 16, 60). In man, the presence of K7, 8 and 18 has been confirmed biochemically (16, 29). Canine cells in addition reacted with one of the MoAbs against K10, whereas they did not stain with the other K10 MoAb (RRSE 60) which is accordant to findings in man with this K10 MoAb (28, 54). Although in the dog MoAb 8.7 (K14 + 17) showed a positive reaction, human follicular cells were not stained by a MoAb directed against K13, 14 and 17 (16) and a K14 MoAb (52, 78). Canine parathyroid cells showed immunoreactivity with the K8 and K18 MoAbs. In man, the presence of K8, 18 and 19 has been documented both immunohistochemically and biochemically (42).

Adrenal gland. In man, cortical cells have been shown to be positive for K8 and K18 and locally for K19 (24). Canine adrenal gland cells did not react with any of the keratin MoAbs. Canine fetal adrenal gland tissue also did not react with several broad spectrum keratin MoAbs (11).

Chorioid plexus and ependyma

Contrary to cells lining the canine choroid plexus, ependymal cells were only labelled by the K8 MoAb. Although human ependymal cells have been found to be keratin negative (35, 41), also K8 and K18 immunoreactivity has been reported (35). Chorioid plexus cells have demonstrated keratin positivity by broad spectrum MoAbs (16, 35, 41) and a K18 specific MoAb (35). Biochemically K8, 18 and 19 could be detected in these cells (41). In adult rats and mice ependymal cells have been shown to stain with a MoAb directed against K8, 18 and 19 (41). In the dog, the absence of staining by a polyclonal broad spectrum keratin antibody in formalin fixed choroid plexus and ependyma has been reported previously (8).

The various MoAbs directed against human keratin-types showed distinct differences in their immuno localization in the various canine tissues, and between canine tissues and the corresponding human tissues. Some MoAbs induced an almost identical staining pattern, for instance RCK 102, as in human tissues (54), stained almost all epithelial tissues in the dog. Other MoAbs induced quite different staining patterns, for instance LP2K, is often negative in canine cells, whereas on the other hand the corresponding human cells are immunoreactive with this MoAb. In the dog, DE-K10 (K10) resulted in staining of a large number of different epithelial cell types, whereas in man only a very limited reactivity with this MoAb was seen (31). Additionally this MoAb also stained canine non-epithelial cells.
The other K 10 MoAb, RKSE 60, showed a completely different staining pattern. Therefore, in our opinion, it has to be assumed that DE-K10 in canine epithelia recognizes other keratins. This K 10 MoAb has to be regarded as non-specific. Also RGE 53 and DE-K18 (both K 18), as well as RCK 107 (K 14) and 8.7 (K 14 + 17) showed slightly different staining reactions in the dog. Similar discordant observations in staining patterns revealed by MoAbs directed against identical keratins have also been made in man (54, 55, 65) and are thought to be due to selective epitope masking (1, 55, 59, 65) or differences in epitope affinities (65). Differences in keratin staining pattern were observed in the basal cells between the various canine epithelial tissues.

In conclusion, MoAbs directed against human keratin-types can be used to differentiate immunohistochemically between various canine epithelial tissues and cell types. This underscores the reported broad species cross-reactivity of keratin antibodies (2, 71). However, the reaction patterns are not entirely correspondent to the differentiation patterns in human tissues as revealed by identical keratin MoAbs or MoAbs with the same keratin type specificity. These MoAbs apparently can result in more or less pronounced staining differences in corresponding tissues and cells of man and dog. Therefore, staining results have to be carefully and critically evaluated, as even different MoAbs directed against identical human keratin-types can induce different staining reactions. Although it has been reported 1) that the tissue diversity and specificity of the keratin patterns is much more pronounced than species differences (22), 2) that the keratin tissue distribution patterns appear to be relatively conserved during mammalian evolution (69), and 3) that the keratin polypeptide pattern from the same tissue are similar in different species (61), our results emphasize that immunohistochemical staining patterns of keratin MoAbs with restricted keratin immunoreactivity can often not be extrapolated from one species to another. Therefore, the immunoreactivity of such MoAbs has to be evaluated extensively in normal tissues, before they can be applied to pathologically altered (inclusively neoplastic) tissues of specific animal species.

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