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Lectin histochemistry of the temporal gland of the African elephant (*Loxodonta africana)*

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Summary. The study demonstrates the free sugar spectrum of the secretion of the tubuloaveolar temporal gland of the African elephant (*Loxodonta africana*), using lectin histochemistry. In the elephant, the spectrum contained, besides strongly varying amounts of α-D-mannose, very remarkable reactions for α-D-galactose and to a certain extent also for α-D-N-acetyl-galactosamine or α/ß-D-N-acetylglucosamine. This is in contrast to the free sugar spectrum of the secretion of the mammalian apocrine tubular skin glands. Considering also that the production of any water binding mucus seems to be negligible, the variations of the free sugar contents found probably originate from another important task of the secretory cells of the temporal gland. This means that our findings corroborate the view of highly active acquired immunity by an intensive processing and presenting of lipid antigens by dendritic cells (APC), in particular of the CD1 family.

Key words: Elephant, *Loxodonta africana*, Temporal gland, Free sugars, Lectin histochemistry

Introduction

The apocrine tubuloalveolar temporal gland is a large organ found exclusively in elephants. It is located midway between the eye and the ear, lateral of the temporal muscle, medial and dorsal of the zygomatic arch and caudal of the orbital ligament. The dorsal region of the gland is surrounded by hypodermal fat tissue, whereas the ventral part of the gland is embedded in a strong layer of fibrous connective tissue. The secretory cells exhibit organelles that are typically adapted to the production of steroids, such as, for example, mitochondria of the tubule type. The intra- and interlobular ducts show a dense cover of myoepithelial cells, and the main duct is extended forming a sinus (Schneider, 1956; Estes and Buss, 1976; Weissengruber et al., 2000; Rajaram and Krishnamurty, 2003). Sometimes rather large sebaceous glands can be found near to the temporal gland, as related to a strong hair follicle. However, this gland type is small compared to the temporal gland and has no direct connection with the latter.

The function of the temporal gland system is clearly related to the musth, an annual period of heightened sexual activity of male and female elephants, which is combined with intensified aggressiveness and highly elevated testosterone concentrations particularly in the males. In this connection, the animals secrete copiously odoriferous messages from their temporal gland, responding earlier and to lower androgen levels than urine dribbling. Concentrations of testosterone in temporal gland secretions are elevated cyclically at times when typical musth behaviours are observed as predominantly related to social behaviour intrinsic to the elephant herd (Rasmussen et al., 1996; Rasmussen and Schulte, 1998; Ganswindt et al., 2005a,b). The temporal gland secretions release variable amounts of volatile substances, some of which may be chemical signals. The composition of the exudates is dominated by increased acetone and other ketones indicative of lipid metabolic alterations. In the Asian elephant these include large quantities of nonmethane hydrocarbons, especially 2-butanone and isoprene (Rasmussen and Perrin, 1999), but also farnesol-related sesquiterpenes as frequently occurring secondary metabolites and aroma compounds in the African elephant (Goodwin et al., 1999).

A recent study has demonstrated that the active

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temporal gland of elephants, additionally, elaborates large quantities of antimicrobial substances, such as lysozyme and cationic antimicrobial peptides (ß-defensins 2- and -3, cathelicidin) (Meyer, 2007). Most of these substances have been demonstrated likewise as components of glandular secretions of apocrine tubular skin glands in other mammals (e.g., Meyer et al., 2003; Stoeckelhuber et al., 2004; Yasui et al., 2005). This aspect may be based on the necessity to protect the enormous inner glandular surface area of the temporal gland against attacks of microbes invading from the epidermis (Kloos et al., 1976; Noble, 1993; Hadaway, 2003). Thus, microbial colonization may be controlled, as, for example, hypothesized for the large tubuloalveolar mammary gland and its defensins (Tunzi et al., 2000). Such proliferation control of bacteria and fungi is particularly of importance considering that the secretion of the temporal gland cannot initiate its typical scent effects before it is disintegrated by microbial activities in the excretory duct system and on the epidermal surface.

In view of this basic function, the present study is primarily related to the phenomenon that high concentrations of free sugars on the skin surface to a considerable extent impede attacks of commensal skin micro-inhabitants (bacteria and fungi) against the integrity of the first important and protecting skin structure, the epidermis, as shown in different terrestrial and aquatic mammals (Meyer et al., 2000, 2001, 2007). In this way, glycans support innate immunity activities against epithelial/mucosal or glandular pathogens (e.g., Perrier et al., 2006), featuring a basic biological mechanism with only low energy requirements, which operates before adaptive immunity is activated (for review see Meyer et al., 2007). However, considering the very specific composition of the temporal gland secretions and their functions, variations in the spectrum of free sugars present cannot be excluded when this massive tubuloalveolar gland is compared to the small apocrine tubular glands found as part of the hair follicle complex of mammals (Meyer, 2009).

Material and methods

Specimens from central and peripheral parts of the temporal gland of one adult male and one adult female of the African elephant (*Loxodonta africana*) were available from the collection of the Institute of Anatomy, University of Veterinary Medicine Hannover Foundation, Germany, and the Department of Macroscopic Anatomy, University of Veterinary Medicine Vienna, Austria. The samples had been fixed in 4% formalin, were then dehydrated in a graded series of ethanol, and embedded via xylene in paraffin wax (Paraplast plus, Tyco Health Care). 8 μm paraffin sections were deparaffinized in Histoclear (Shandon), hydrated through descending concentrations of ethanol and stained with hematoxylin – eosin (HE), or safranin - 0.1% toluidine blue (Csemniczky and Cziegler, 1962), for a structural overview. The latter approach also gives information indirectly about protein contents in tissues (Meyer and Zschemisch, 1999).

In general, the paraffin tissue processing performed is appropriate for lectin binding to tissue sections (Rittman and Mackenzie, 1983; Allison, 1987; Alroy et al., 1988). However, firstly, acid and neutral complex glycoconjugates were demonstrated for basic information using the following staining procedures: periodic acid-Schiff (PAS, Schiff`s reagent according to Barger and de Lamater, from Culling, 1974) (Spicer et al., 1967), including a control for the demonstration of glycogen by digestion with 1% α-amylase (from *Bacillus subtilis*, Sigma; pH 6.0, at 37°C for 1 h) (Culling, 1974), alcian blue 8GX (AB), Sigma; (pH 1.0) (Lev and Spicer, 1964), and AB (pH 2.5) (Pearse, 1985).

For the demonstration of terminal sugars, secondly, specific lectin histochemistry was employed, using 11 biotinylated lectins. All lectins were purchased from E.Y. Labs./Medac and Sigma and applied in concentrations of 10-20 μg/ml in 0.1 M PBS (pH 7.2) for 30 min at 20°C or 2 h at 4°C. The lectin spectrum was as follows: concanavalin A (Con A), wheat germ agglutinin (WGA), *Griffonia simplicifolia* agglutinin-I and -II (GSA-I and -II), *Dolichos biflorus* agglutinin (DBA), soy bean agglutinin (SBA), *Maclura pomifera* agglutinin (MPA), Peanut agglutinin (PNA), *Ulex europeaeus* agglutinin-I (UEA-I), *Sambucus nigra* agglutinin (SNA), *Maackia amurensis* agglutinin (MAA) (for lectin specificity see e.g., Yamada and Shimizu, 1977; Pearse, 1985; Alroy et al., 1988; Spicer and Schulte, 1992; Danguy et al., 1998). The reactions were visualized by peroxidase (PO) conjugated streptavidin, using the DAB-based BioGenex Super Sensitive Universal Immunostaining Kit (BioGenex Labs.). In this combination, the system is up to 25 times more sensitive than the traditional biotin-streptavidin methods, with better preservation of tissue reactivity and integrity.

Lectin controls: a) addition of the following saccharides at a final concentration of 0.01 M to the respective lectin solutions: α-methyl-D-mannose for Con A, N-acetyl-D-galactosamine for SBA and DBA, ß-D-galactose for BPA and PNA, ß-D-galactose for MPA and GSA-I, α-L-fucose for UEA-I, N-acetyl-D-glucosamine for WGA and GSA-II, NANA α(2,6)-N-acetyl-D-glucosamine for SNA, and NANA α(2,3)-galactose for MAA; b) substitution of unconjugated lectins for lectin-conjugates; c) exposure of sections to PO and DAB systems without lectins. To detect endogenous peroxidase activity in tissue, certain control sections were reacted with DAB only. In addition, control sections were preincubated in 0.3% hydrogen peroxide in methanol for 30 min to block intrinsic peroxidase activity; d) regarding that sections were stained with SNA and MAA, sialidase digestion (neuraminidase, from *Arthrobacter ureafaciens*; Boehringer, Germany) [0.5 mg/l in 0.1 M acetate buffer, pH 5.3, containing 0.04 M $CaCl_2$ at 37°C for 12 hrs (Spicer et al., 1967) was performed.

Results

The typical histological basics of the large temporal gland (diameter 200-180 mm, thickness 30 – 50 mm) (Fig. 1) became visible using hematoxylin – eosin staining, whereby the rather weak overall tinging of the glandular tubule system after the application of the safranin - toluidine blue procedure, nevertheless, indicated that the secretory cells did not contain any remarkable amounts of proteins. Regarding the general glycoconjugate histochemical results obtained, the latter observation was indirectly confirmed by the weak reaction after the AB (pH 2.5) - PAS staining (Fig. 2a), featuring only small amounts of neutral glycoproteins or acid mucus related complex glycoconjugates. For both findings no differences were recognized comparing the central and peripheral parts of the gland, or the male and the female animal studied. PAS staining combined with α-amylase digestion exhibited no or only very low amounts of glycogen as present in the glands.

Based on lectin histochemistry, the free sugar spectrum found was composed as summarized in Table 1. Remarkable or medium to strong reaction intensities in the secretory cells and the luminal secretion were observed for α-D-N-acetylgalactosamine (DBA, SBA) (Fig. 2b), α-D-galactose (GSA-I) (Fig. 2c), and to a certain extent for α/ß-D-N-acetylglucosamine (GSA-II) (Fig. 2d, right part); in all cases, the staining was somewhat stronger in the male animal. Sometimes lectin reactions of rather weak but also of medium intensity became visible in the cells and the luminal secretion for ß-D-galactose (PNA) (Fig. 2e), -L-fucose (UEA-I) (Fig. 2f), and for NANA α-(2,3)-galactose (MAA, sialic acids); the latter observations were very distinct in the female for UEA-I and in the male for MAA. Varying but distinct reaction intensities of the secretory cells could be observed, in particular, for α-D-mannose (Con A) in the male animal studied. The excretory duct cells and the myoepithelial cells revealed more or less negative to weak or medium staining intensities (e.g., Fig. 2d, left part). For all the lectin histochemical reactions obtained no differences were visible between the central and peripheral parts of the temporal gland.

Discussion

The results of this study demonstrate that the free sugar spectrum of the large elephant temporal gland has not quite the same quality as shown for the smaller apocrine tubular skin glands of different mammalian groups. In the elephant, the spectrum contained, besides varying reaction intensities for α-D-mannose, very remarkable and constant reactions for α-D-galactose, but also for α-D-N-acetylgalactosamine and α/ß-D-N-acetylglucosamine. Variations of lectin reactions intensities observed between the female and the male

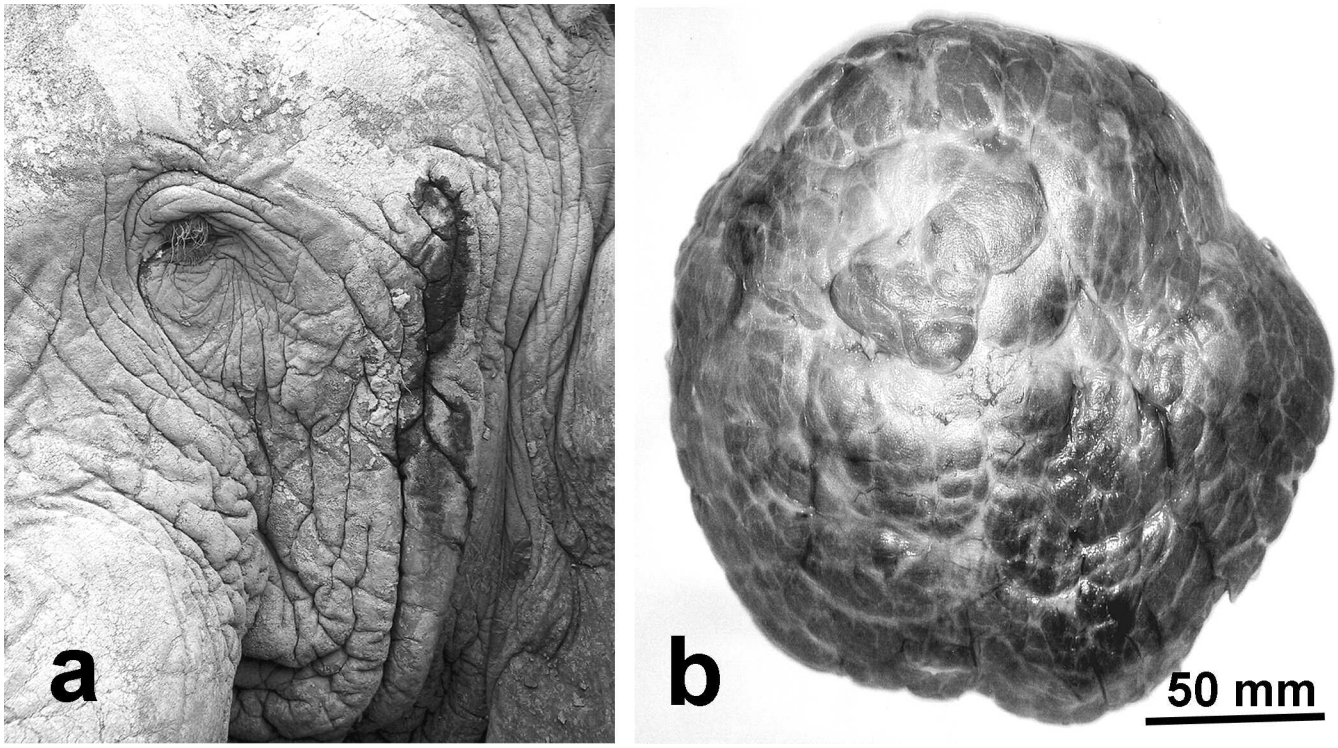

Fig. 1. General aspects of the temporal gland of the African elephant (*Loxodonta africana*). **a.** Secretion (black) is released from the slit-like orifice of the left gland (photo courtesy of P. Granli, Savanna Elephant Voices Project, www.ElephantVoices.org). **b.** Medial view of the large temporal gland.

animal studied are of interest, but for a relevant interpretation of this finding more analyses are necessary.

The rather low amounts of α-L-fucose, ß-D-galactose and sialic acid residues (only NANA α2-3 Gal) indicate that the production of O-linked high molecular weight glycoproteins (Campbell, 1999) are negligible in the elephant temporal gland system. Such types of glycoconjugates, which have the capability of water binding, may be specifically involved by its sialic acid component in the high viscocity of mucus lining epithelia (Schauer, 2004). In the secretory cells of the mammalian apocrine tubular skin glands, the spectrum of remarkable free sugar amounts features, besides α-D-mannose, specifically ß-D-galactose (Meyer and Tsukise, 1989; Meyer et al., 1987, 1993, 2000, 2001, 2007; Tsukise and Meyer, 1982, 1983; Yasui et al., 2006). The positive reactions for α-D-mannose in both gland types confirm a regularly proceeding glycogen metabolism, though of varying intensity.

The reason for the difference in composition of the free sugar spectrum of the specific elephant gland and the normal apocrine tubular skin gland type may be included in the fact that the temporal gland predominantly releases high amounts of volatile substances, originating from the desintegration of steroid hormones (testosterone, oestrogen) (Goodwin et al., 1999; Rasmussen and Perrin, 1999; Yon et al., 2008). The major pathways of metabolization of testosterone, for example, are related with the conjugation to testosterone glucuronide or testosterone sulphate (Möhle et al., 2002). In elephants, testosterone sulphate seems to be the major conjugated metabolite (Yon et al., 2010). Thus, no free sugars moieties are available as a result or as semifinished products during steroid hormone decomposition. The variations found in the spectrum of free sugars probably originate from another important task of this gland. Such a function is epithelial defense or to cope with the constant danger of microbial invasion, bearing in mind that the voluminous temporal gland system of elephants with its enormous inner surface, can easily be attacked by microbes. Moreover, the mechanical barrier properties of the epithelium, which normally contribute to the protection from microbes, are usually weakened by extraordinarily high secretion activities (e.g., Meyer et al., 2001; Ganz, 2002; Pavelka and Roth, 2005).

Independent of successfully demonstrated substances of innate immunity, i.e., continuously produced lysozyme and antimicrobial peptides in the temporal gland (Meyer, 2007), the benefits of the acquired immune system against the threat of microbial infection are of even greater importance. In this context, the CD1 family of antigen presenting glycoproteins mediates T-cell responses through the presentation of self and foreign lipids, glycolipids, lipopeptides, or amphipathic small molecules to T-cell receptors (for review see Wu et al., 2008). Among the several

Table 1. Lectin histochemical reaction intensities in the temporal gland of the African elephant (Loxodonta africana).

Lectins	Secretory cells	Luminal secretion	Myoepithelial cells.	Excret.duct cells	Inhibitory sugar
PO-Con A-DAB	3	3	0-1	1-2	α-D-Man; α-D-Glc
	3-4	*3-4*	*0-1*	*1*	
PO-WGA-DAB	2	1-2	0	1	ß-D-GlcNAc> Neu NAc
	1-2	*1*	*0*	*0*	
PO-GSA-II-DAB	2-4	2-3	0-1	2-3	α-/ß-D-GlcNAc
	2-4	*2-4*	*0-1*	*2-3*	
PO-DBA-DAB	2-4	2-4	0-1	1-2	α-D-GalNAc
	2-3	*2-3*	*0-1*	*1-2*	
PO-SBA-DAB	2-3	2	0	0-1	α-D-GalNAc> α-D-Gal
	3-4	*3-4*	*0-1*	*2-3*	
PO-MPA-DAB	2-3	2-3	0	1	α-D-Gal; α-D-GalNAc
	0-1	*1*	*0*	*0*	
PO-GSA-I-DAB	2-4	2-4	1	1	α-D-Gal; α-D-GalNAc
	2-4	*3-4*	*1*	*1*	
PO-PNA-DAB	1-3	1	0	1	ß-D-Gal; ß-D-GalNAc
	2-3	*2*	*0*	*1*	
PO-UEA-I-DAB	1-4	1-3	0-1	1-2	α-L-Fuc
	2-3	*1-3*	*0-1*	*1-2*	
PO-SNA-DAB	0-1	0-1	0	0	NANA α(2,6)-GalNac
	0-2	*0-1*	*0*	*0*	
PO-MAA-DAB	1-2	1	0	0	NANA α(2,3)-Gal
	2-4	*2-3*	*0*	*0*	

Reaction intensities: 0 = negative, 1 = very weak, 2 = weak, 3 = medium, 4 = strong; results in italics for the male animal.

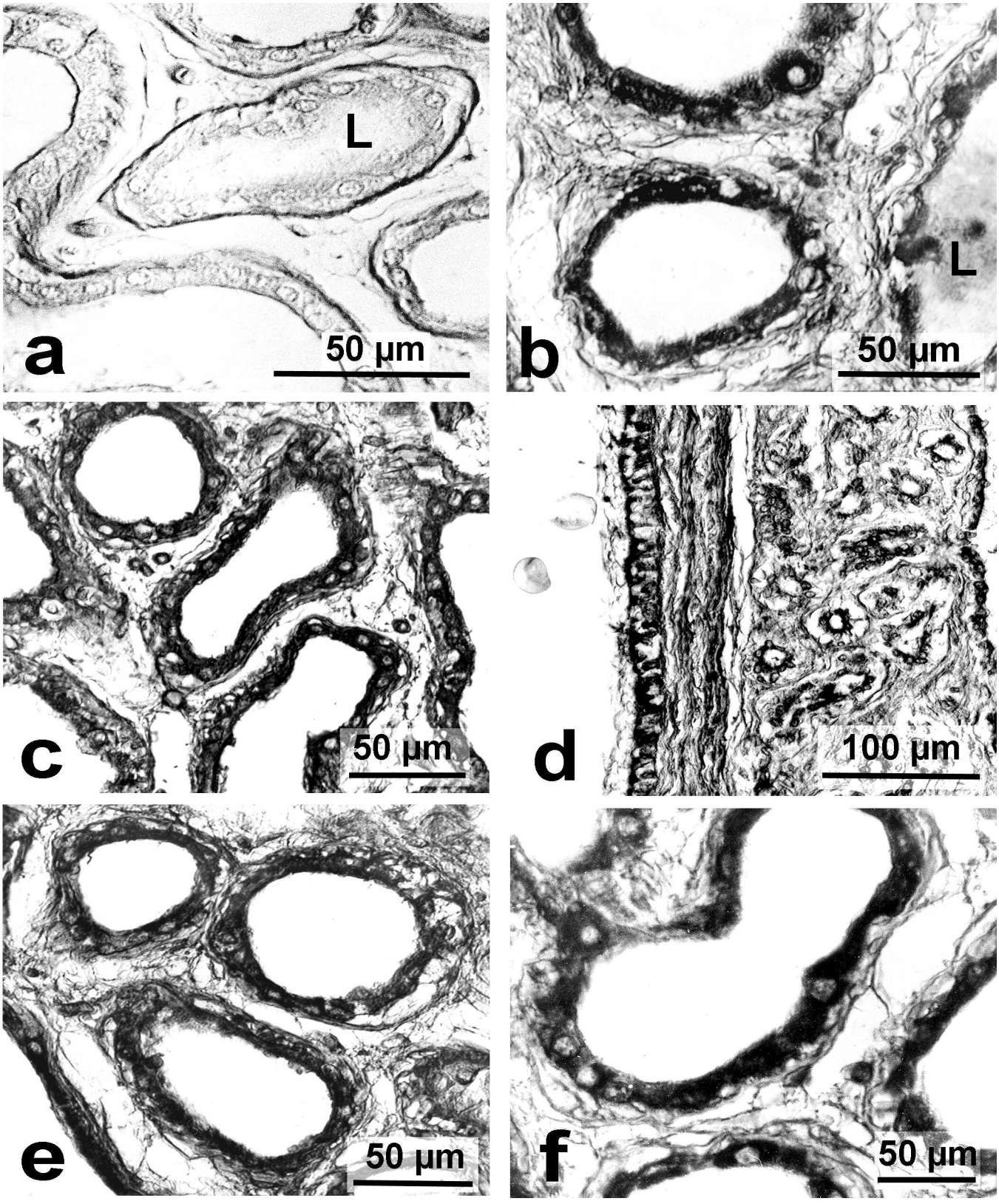

Fig. 2. Demonstration of complex glycoconjugates. **a.** AB (pH 2.5) - PAS staining, male, and different free sugars (lectin histochemistry with DAB visualization). **b.** α-D-N-acetylgalactosamine (DBA), male. **c.** α-D-galactose (GSA-I), male. **d.** α/ß-D-N-acetylglucosamine (GSA-II), male. **e.** ß-D-galactose (PNA), female. **f.** α-L-fucose (UEA-I), female, in the secretory epithelium and the excretory duct epithelium (**d**, left part) of the temporal gland of the African elephant (*Loxodonta africana*). L: luminal secretion.

(glyco)lipids identified to cause T-cell stimulation in complex with CD1, α-galactosyl ceramide is one of the best known and broadly distributed CD1d-presented antigens (e.g., Nicol et al., 2000; Gonzalez-Aseguinolaza et al., 2002). Generally, dendritic cells move from sites of antigen uptake to cellular interactions, and they are not only enriched in lymphoid organs but also in environmental contact sites such as epithelia and their lamina propria (Cutler and Jowani, 2006; Merad and Manz, 2009; Meyer et al., 2010). So, our findings of strong reactions for the related free sugars indirectly corroborate the view of highly active acquired immunity by an intensive processing and presenting of lipid antigens by dendritic cells (APC) in the temporal gland of elephants.

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References

Allison R.T. (1987). The effects of various fixatives on subsequent lectin binding to tissue sections. Histochem. J. 19, 65-74.

Alroy J., Ucci A.A. and Pereira M.E.A. (1988). Lectin histochemistry: an update. In: Advances in Immunohistochemistry (Neoplasms Diagnosis). DeLellis R.A. (ed.). Raven Press. New York. pp 93-13.

Campbell B.J. (1999). Biochemical and functional aspects of mucus and mucin-type glycoproteins. In: Bioadhesive drug delivery systems, drugs and the pharmaceutical sciences. Vol. 98. Mathiowitz E., Chickering D.E. and Lehr C.M. (eds.). Marcel Dekker, New York, pp 85-130.

Csemniczky F. and Cziegler S. (1962). Eine schnelle Färbemethode für frische Gefrier- (Kryostat) und Paraffinschnitte. Mikroskopie 17, 241-245.

Culling C.F.A. (1974). Handbook of histopathological and histochemical techniques. 3rd. Butterworth. London.

Cutler C.W. and Jowani R. (2006). Dendritic cells at the oral mucosal interface. J. Dent. Res. 85, 678-689.

Danguy A., Decaestecker C., Genten F., Salmon I. and Kiss R. (1998). Applications of lectins and neoglycoconjugates in histology and pathology. Acta anat. 161, 206-218.

Estes J.A. and Buss I.O. (1976). Microanatomical structure and development of the African elephant`s temporal gland. Mammalia 40, 429-436.

Ganswindt A., Heistermann M. and Hodges J.K. (2005a). Physical, physiological, and behavioral correlates of musth in captive African elephants (*Loxodonta africana*). Physiol. Biochem. Zool. 78, 505-514.

Ganswindt A., Rasmussen H.B., Heistermann M. and Hodges J.K. (2005b). The sexually active status of free-ranging male African elephants (*Loxodonta africana*): defining musth and non-musth using endocrinology, physical signals, and behavior. Horm. Behav. 47, 83-91.

Ganz T. (2002). Epithelia: Not just physical barriers. Proc. Natl. Acad. Sci. USA 99, 3357-3358.

Gonzalez-Aseguinolaza G., Van Kaer L., Bergmann C.C., Wilson J.M., Schmieg J., Kronenberg M., Nakayama T., Taniguchi M., Koezuka Y. and Tsuji M. (2002). Natural killer T cell ligand α-galactosyl-ceramide enhances protective immunity induced by malaria vaccines. J. Exp. Med. 195, 617–624.

Goodwin T.E., Rasmussen E.L., Guinn A.C., McKelvey S.S., Gunawardena R., Riddle S.W. and Riddle H.S. (1999). African elephant sesquiterpenes. J. Nat. Prod. 62, 1570-1572.

Hadaway L.C. (2003). Skin flora and infection. J. Infus. Nurs. 26, 44-48.

Kloos W.E., Zimmerman R.J. and Smith R.F. (1976). Preliminary studies on the characterization and distribution of *Staphylococcus* and *Micrococcus* species on animal skin. Appl. Environm. Microbiol. 31, 53-59.

Lev R. and Spicer S.S. (1964). Specific staining of sulphate groups with Alcian blue at low pH. J. Histochem. Cytochem. 12, 309.

Merad M. and Manz M.G. (2009). Dendritic cell homeostasis. Blood 113, 3418-3427.

Meyer W. (2007). Demonstration of lysozyme and antimicrobial peptides in the temporal gland of the African elephant (*Loxodonta africana*). Mammal. Biol. 72, 251-255.

Meyer W. (2009). Hair follicles in domesticated mammals with comparison to laboratory animals and humans. In: Hair loss disorders in domestic animals. Mecklenburg L., Linek M. and Tobin D.J. (eds). Blackwell Publ. Ames. pp 43-62.

Meyer W. and Tsukise A. (1989). Histochemistry of complex carbohydrates in the scrotal skin of the monkey, *Macaca cyclopis* (Swinhoe). Z. Säugetierkd. 54, 9-21.

Meyer W. and Zschemisch N.H. (1999). Remarks on the usefulness of toluidine blue staining for RNA cytophotometry in plastic embedded tissues. Cell. Mol. Biol. 45, 379-382.

Meyer W., Tsukise A. and Neurand K. (1987). Histochemical observations on the apocrine glands of the scrotal skin of the cat and dog. Morphol. Jb. 133, 163-173.

Meyer W., Saglam M., Tanyolaç A. and Schwarz R. (1993). Carbohydrate histochemistry of skin glands in the Turkish Angora goat. Eur. J. Morphol. 31, 157-167.

Meyer W., Bollhorn M. and Stede M. (2000). Aspects of general antimicrobial properties of skin secretions in the common seal *Phoca vitulina*. Dis. Aquatic Org. 41, 77-79.

Meyer W., Neurand K. and Tanyolac A. (2001). General anti-microbial properties of the integument in fleece producing sheep and goats. Small Ruminant Res. 41, 181-190.

Meyer W., Tsukise A., Neurand K. and Hirabayashi Y. (2001). Cytological and lectin histochemical characterization of secretion production and secretion composition in the tubular glands of the canine anal sacs. Cells Tissue Organs. 168, 203-219.

Meyer W., Seegers U., Herrmann J. and Schnapper A. (2003). Further aspects of the general antimicrobial properties of pinniped skin secretions. Dis. Aquatic Org. 53, 177-179.

Meyer W., Seegers U., Schnapper A., Neuhaus H., Himstedt W. and Toepfer-Petersen E. (2007). Possible antimicrobial defense by free sugars on the epidermal surface of aquatic vertebrates. Aquat. Biol. 1, 167-175.

Meyer W., Hornickel I. and Schönnagel B. (2010). A note on Langerhans cells in the oesophagus epithelium of domesticated mammals. Anat. Histol. Embryol. 39, 160-166.

Möhle U., Heistermann M., Palme R. and Hodges J.K. (2002). Characterization of urinary and fecal metabolites of testosterone and their measurement for assessing gonadal endocrine function in male

nonhuman primates. Gen. Comp. Endocrinol. 129,135-145.

Nicol A., Nieda M., Koezuka Y., Porcelli S., Suzuki K., Tadokoro K., Durrant S. and Juji T. (2000). Human invariant Vα24+ natural killer T cells activated by α-galactosylceramide (KRN7000) have cytotoxic antitumour activity through mechanisms distinct from T cells and natural killer cells. Immunology 99, 229–234.

Noble W.C. (1993). The skin microflora and microbial skin disease. Cambridge Univ Press. Cambridge, New York, Oakleigh.

Pavelka M. and Roth J. (2005). Functional ultrastructure. An atlas of tissue biology and pathology. Springer. Wien, New York.

Pearse A.G.E. (1985). Histochemistry. Theoretical and applied. 4th. ed. Vol. 2: Analytical technology. Churchill Livingstone. Edinburgh, London, Melbourne, New York.

Perrier C., Sprenger N. and Corthésy B. (2006). Glycans on secretory component participate in innate protection against mucosal pathogens. Biol. Chem. 281, 14280-14287.

Rajaram A. and Krishnamurthy V. (2003). Elephant temporal gland ultrastructure and androgen secretion during musth. Curr. Sci. 85, 1467-1471.

Rasmussen L.E. and Schulte B.A. (1998). Chemical signals in the reproduction of Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants. Anim. Reprod. Sci. 53, 19-34.

Rasmussen L.E. and Perrin T.E. (1999). Physiological correlates of musth: lipid metabolites and chemical composition of exudates. Physiol. Behav. 67, 539-549.

Rasmussen L.E., Hall-Martin A.J. and Hess D.L. (1996). Chemical profiles of male African elephants, *Loxodonta africana*: Physiological and ecological implications. J. Mammalogy 77, 422-439.

Rittman B.R. and Mackenzie I.C. (1983). Effects of histological processing on lectin binding patterns in oral mucosa and skin. Histochem J. 15, 467-474.

Schauer R. (2004). Sialic acids: fascinating sugars in higher animals and man. Zoology 107, 49-64.

Schneider R. (1956). Untersuchungen über den Feinbau der Schläfendrüse beim Afrikanischen und Indischen Elefanten, *Loxodonta africana* Cuvier und Elephas maximus Linnaeus. Acta Anat. 28, 303-312.

Spicer S.S., Horn R.G. and Leppi T.J. (1967). Histochemistry of connective tissue mucopolysaccharides. In: The connective tissue. Wagner B.M. and Smith D.E. (eds). Williams & Wilkins. Baltimore. pp 251–303.

Spicer S.S. and Schulte B.A. (1992). Diversity of cell glycoconjugates shown histochemically: a perspective. J. Histochem. Cytochem. 40, 1-38.

Stoeckelhuber M., Stoeckelhuber B.M. and Welsch U. (2004). Apocrine glands in the eyelid of primates contribute to the ocular host defense. Cells Tiss. Org. 176, 187-194.

Tunzi C.R., Harper P.A., Bar-Oz B., Valore E.V., Semple J.L., Watson-MacDonell J., Ganz T. and Ito S. (2000). ß-defensin expression in human mammary gland epithelia. Pediatr. Res. 48, 30-35.

Tsukise A. and Meyer W. (1982). Histochemistry of complex carbohydrates in the apocrine glands of the scrotal skin of the goat. Zbl. Vet. Med. A 29, 688-693.

Tsukise A. and Meyer W. (1983). Histochemistry of complex carbohydrates in the hairy skin of the domestic pig. Histochem. J. 15, 845-860.

Weissengruber G.E., Kübber-Heiss A., Forstenpointner G. and Riccaboni, P. (2000). Ein Beitrag zur Morphologie der Schläfendrüse (*Glandula temporalis*) des Afrikanischen Elefanten (*Loxodonta africana*). Wien. Tierärztl. Mschr. 87, 303-308.

Wu D., Fujio M. and Wong C-H. (2008). Glycolipids as immuno-stimulating agents. Bioorg. Med. Chem. 16, 1073-1083.

Yamada K. and Shimizu S. (1977). The histochemistry of galactose residues of complex carbohydrates as studied by peroxidase labelled *Ricinus communis* agglutinin. Histochemistry 53, 143-156.

Yasui T., Tsukise A., Fukui K., Kuwahara Y. and Meyer W. (2005). Aspects of glycoconjugate production and lysozyme- and defensins-expression of the ceruminous glands of the horse (*Equus przewalskii* f. dom.). Eur. J. Morphol. 42, 127-134. .

Yasui T., Tsukise A., Miura T., Fukui K. and Meyer W. (2006). Cytochemical characterization of glycoconjugates in the apocrine glands of the equine scrotal skin. Arch. Histol. Cytol. 69, 109-117.

Yon L., Chen J., Moran P. and Lasley B. (2008). An analysis of the androgens of musth in the Asian bull elephant (*Elephas maximus*). Gen. Comp. Endocrinol. 155, 109-115.

Yon L., Faulkner B., Kanchanapangka S., Chaiyabutr N., Meepan S. and Lasley B. (2010). A safer method for studying hormone metabolism in an Asian elephant (*Elephas maximus*): Accelerator mass spectrometry. Zoo Biol. 28, 1-7.

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