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NOTE

# Further aspects of the general antimicrobial properties of pinniped skin secretions

**W. Meyer[1,*], U. Seegers[2], J. Herrmann[1] , A. Schnapper[1]**

[1]Anatomical Institute, School of Veterinary Medicine, Bischofsholer Damm 15, 30173 Hannover, Germany
[2]Institute of Pharmacology, Toxicology and Pharmacy, School of Veterinary Medicine, 30559 Hannover, Germany

ABSTRACT: In 2 pinniped species with varying hair densities (the northern fur seal *Callorhinus ursinus*, and the common seal *Phoca vitulina*), the enzyme lysozyme and the peptide group β-defensins are demonstrated for the first time as products of the apocrine glands and the sebaceous glands in the common integument of mammals. These antimicrobial substances are also found in the epithelial lining of the hair follicles, between the corneal lamellae of the epidermis, and in dermal histiocytes. Thus, it becomes obvious that another general defense mechanism against microbes, in addition to free sugars (Meyer et al. [2000] Aspects of general properties of skin secretions in the common seal *Phoca vitulina*. Dis Aquat Org 41:77–79), is active on the skin surface of haired marine mammals to which glandular lysozyme and defensins are regularly transported, so that this biotope is protected against the proliferation of potential pathogens, e.g. bacteria and fungi.

KEY WORDS: Antimicrobial substances · Seals · Skin surface · Skin defense



Fishes use numerous substances in their skin mucus to reduce hydrodynamic drag as well as for protection against different microbial or parasitic invaders of the epidermis (Wirth 1999, Ellis 2001). Non-cetacean marine mammals lack such multifunctional proteins and glycoconjugate mixtures, although their skin surface is the biotope for micro-organisms, such as, for example, bacteria and fungi (e.g. Rand 1979, Wells et al. 1990, Guillot et al. 1998). For this reason, an effective defense strategy against the microbial challenge, and/or a proliferation control of the microflora present seems to be a basic need for mammalian skin biology not only under terrestrial conditions, but also in the aquatic medium. Recent studies of pinniped skin have indicated that mucus analogous antimicrobial functions can be exerted on the skin surface, at least in part, by free sugars as derivatives of glycoconjugates containing secretions of the apocrine tubular skin glands (Meyer et al. 2000). However, recent research has also revealed that additional antimicrobial defense strategies, particularly regarding the use of antibiotic peptides and proteins, are generally detectable within the skin of aquatic and terrestrial vertebrates, including various mammalian groups (Bos et al. 2001, Yang et al. 2001). Based on these findings, the present immunohistochemical study was designed to provide preliminary information on the production and, particularly, the extra-corporal use of such substances in the integument of haired marine mammals.

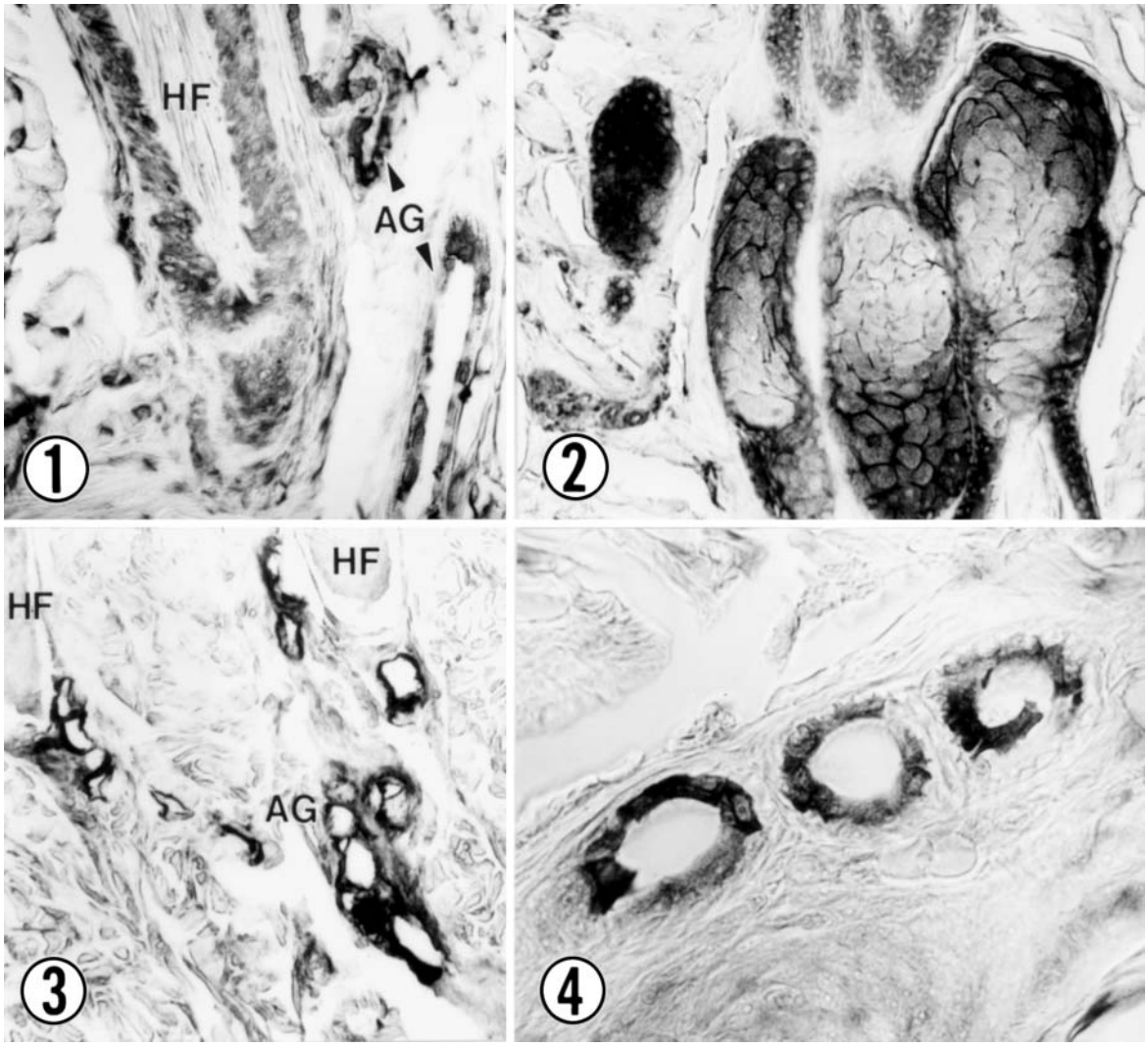
Skin specimens were excised from the dorsum, the flanks, and the abdomen of 4 adult common seals *Phoca vitulina* and 2 adult northern fur seals *Callorhinus ursinus*. The material was fixed in Bouin's fluid, embedded in paraffin wax, and hydrated 8 µm sections were stained immunohistochemically for the determination of lysozyme (dil. 1:100; anti-human, from rabbit; Dako), α-defensin-1-3 (dil. 1:25, 1:50; anti-human, from mouse, Quartett), and β-defensin-3 (dil. 1:50, 1:100; anti-human/anti-mouse, from rabbit; Biologo). Following incubation for 60 min at room temperature, the reaction was detected by an ABC system (Vector Elite Kit) using biotinylated second antibodies and peroxidase-conjugated streptavidin; before the reaction, all sections were digested for 30 to 60 min with 0.1% trypsin (for a detailed protocol including controls see Meyer 1997, 2001).

In the integument of the northern fur seal *Callorhinus ursinus* and the common seal *Phoca vitulina*, positive medium to strong reactions were visualized immunohistochemically for lysozyme and β-defensin-3, especially in the apical portion of many secretory cells of the endpiece of the apocrine glands, but not in the excretory duct of these glands (Figs. 1, 3 & 4). In the sebaceous glands, strong reaction staining for

*Email: wilfried.meyer@tiho-hannover.de

lysozyme was detectable in intercellular substances and/or the cell membrane of immature as well as disintegrating sebocytes (Fig. 2). Lysozyme and β-defensin were demonstrated in the epithelial lining of the hair follicles (Fig. 1), more weakly in the cells of the vital epidermis and between the lamellae of the stratum corneum, and distinctly in numerous free cells (histiocytes) of the dermis. Positive staining for lysozyme was also visible in the endothelia of smaller integumental blood vessels, mainly arterioles. All immunohistochemical staining intensities were especially strong in the apocrine tubular glands and dermal free cells of the abdominal body region of the common seal, whereas in the northern fur seal the staining generally appeared somewhat weaker with no differences among the body regions studied. The reaction for α-defensin was weakly to moderately positive only in single free cells of the dermis of both species studied.

Thus, our results clearly demonstrate that the antibiotic cationic enzyme lysozyme as well as the multifunctional and likewise antibiotic peptide group of defensins are present as glandular secretions on the skin surface of pinnipeds. It is likely that these compounds serve as a non-specific defense of the common integument of haired marine mammals against bacteria, fungi and, perhaps, algae. The occurrence of such substances in this biotope was unknown until now for the hairy skin of aquatic as well as terrestrial mam-




Figs. 1 to 4. Immunohistochemical staining. Fig. 1. *Phoca vitulina*. Lysozyme, dorsum, distinct reactions in the hair follicle (HF) and apocrine glands (AG), ×235. Fig. 2. *Callorhinus ursinus*. Lysozyme, dorsum, distinct reaction in the sebaceous glands, ×260. Fig. 3. *C. ursinus*. Lysozyme, abdomen, strong reactions in strongly coiled secretory portions of the apocrine glands (AG) below the hair follicles (HF), ×250. Fig. 4. *P. vitulina*. β-defensin, abdomen, distinct reactions in several secretory cells of the apocrine gland, ×240

mals. Previously, a positive demonstration of lysozyme had only been obtained for the apocrine tubular glands of specific body regions of humans like the axilla, but the eccrine tubular glands of the human or hominoid hairy skin reacted negatively (Ezoe & Katsumata 1990). The use of lysozyme as a non-specific antimicrobial immune response has been shown, however, to exist in fishes as a normal feature of the mucus elaborated by the epidermal cells (Smith et al. 2000, Ellis 2001, Sarder et al. 2001).

Furthermore, β-defensin was demonstrated in this study for the first time to be an ingredient of the apocrine tubular gland secretion of the mammalian hairy skin. However, previous publications noted that this peptide group is expressed in the epidermis and the pilosebaceous units (without apocrine glands) of humans (Harder et al. 1997, 2001, Ali et al. 2001, Chronnell et al. 2001, Seo et al. 2001). Whether defensin (β-defensins-2 and -3) production in apocrine tubular glands of the common integument of medium-sized and large non-hominoid mammals (the hairy skin of small mammals lacks apocrine glands!) is a continuous process, particularly in rather sparsely haired groups, or is stimulated, for example, by invading microbes as shown for human skin (Harder et al. 2001, Yang et al. 2001, Liu et al. 2002), has still to be clarifed. Distinct reactions for α-defensin in free cells (obviously only in the macrophage group of histiocytes) of the abdominal skin of both pinniped species examined here may be related to the fact that this defensin group also increases its ability to resist local infection by enhancing neutrophil recruitment to infected tissues (Yang et al. 2001). In pinnipeds without a dense hair coat, in particular, the ventral body region is normally stressed by severe chemical and mechanical hazards caused by sliding and creeping movements on different types of grounds and, therefore, shows numerous macrophages with high cell metabolism (Meyer 1997, 2001).

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