The skin microbiome and canine atopic dermatitis

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1. Introduction

As the interface with the external environment, the stratum corneum (SC) is constantly exposed to commensal and environmental microorganisms [1]. Recent advances in bacterial 16S ribosomal RNA (rRNA) gene sequencing have revealed that the cutaneous microbiota is more diverse than previously observed in bacterial cultures [2]. It is also shown that resident microbials play an integral part in the function of the skin, by interacting with innate immunity and the cutaneous barrier function. Accumulating evidence suggests that cutaneous dysbiosis may drive or exacerbate the inflammatory reaction in certain skin diseases, such as atopic dermatitis (AD). This lecture aims to explain the current knowledge of the cutaneous ecosystem and its relationship to innate immunity and atopic skin diseases in humans and companion animals.

2. Cutaneous bacterial microbiome in normal skin

Bacterial microbiota in mammalian skin primarily consists of four phyla: Firmicutes, Actinobacteria, Proteobacteria and Bacteroidetes [3]. Most bacteria belonging to the Firmicutes phylum are gram-positive strains. The *Staphylococcaceae* family, which includes *Staphylococcus*, is a well-known bacterial genus that inhabits the skin [4]. *Actinobacteria* are also a phylum of Gram-positive bacteria [4]. The families *Corynebacteriaceae* and *Propionibacteriaceae* belong to this phylum and are also known as commensal bacteria in the skin. *Proteobacteria* is a major phylum of Gram-negative bacteria; the family *Pasteurellaceae*, which includes *Haemophilus* spp., belongs to this phylum [5]. *Bacteroidetes* are also known to be frequently isolated in the feline oral cavity [2].

Topographical areas, environment and disease status influence the diversity of bacterial microbiota in the skin. In humans, topographical areas can be classified into three areas based on the cutaneous environment and bacterial microbiota: (i) sebaceous areas wherein *Propionibacteriaceae* and *Staphylococcaceae* predominate, (ii) moist areas wherein *Corynebacteriaceae* predominates and (iii) dry areas that have higher bacterial diversity than seen in other areas [6]. In contrast, the bacterial microbiota in canine and feline skin is more diverse than that seen in human skin and is highly valuable across individuals and body sites [7, 8]. Another study demonstrated that the structure of the bacterial community varied among seasons, and *Propionibacterium acnes*, *Haemophilus* sp., *Corynebacterium* sp. and *S. epidermidis* were key members of the bacterial community in dog skin [9]. Feline skin appeared to have a more abundant *Bacteroidetes* population, suggesting that the grooming behaviour of cats affects the cutaneous flora [8].

Among the commensal bacteria from mammalian skin, possible roles of *Staphylococcaceae*, *Corynebacteriaceae* and *Propionibacteriaceae* in the cutaneous immunity have been reported:

2.1 *Staphylococcus epidermidis*

Accumulating evidence suggests that *S. epidermidis* has a negative impact on the proliferation of *Staphylococcus aureus* in human atopic dermatitis (AD). Serine proteases produced by *S. epidermidis* have the potential to inhibit and destroy *S. aureus* biofilm [10, 11]. There is *in vitro* evidence showing that small molecules produced by *S. epidermidis* activate Toll-like receptor (TLR) 2 signalling and enhance human β-defensin (hBD) 2 production in cultured human keratinocytes [12]. In addition, a study using experimental mice showed that *S. epidermidis* enhanced the production of IL-17A and IFN-γ by skin γδT cells [13], which promotes protective immunity not only against *S. aureus* but also against other pathogens, such as *Leishmania* sp [14]. IL-17A is also known to encourage the recruitment of neutrophils to the skin and to enhance the production of antimicrobial peptides at local sites [15]. Furthermore, *S. epidermidis* promotes the activity of regulatory T cells and suppresses the proliferation of CD4+ lymphocytes [16]. Hence, *S. epidermidis*, a major commensal organism of humans and canines, plays a protective role in the skin by directly inhibiting pathogens or by activating innate and adaptive immune functions.

2.2 *S. aureus*

Besides the beneficial effects of *S. epidermidis* on cutaneous immunity, *S. aureus* is considered to enhance inflammatory reactions. Previous studies have demonstrated that *S. aureus* colonisation in the skin of mice with aberrant cutaneous barrier function, including filaggrin (FLG)-deficient flaky tail mice, is recognised [17]. Percutaneous entry of *S. aureus* in flaky tail mice directly correlated with increased expression of IL-4, IL-13, IL-22, thymic stromal lymphopoietin (TSLP) and other cytokines associated with AD, and with decreased expression of an antimicrobial peptide cathelicidin. In addition, *S. aureus* colonisation was also recognised in mice lacking a disintegrin and metalloproteinase 17 (ADAM17), which regulates epidermal growth factor receptor ligand-dependent terminal keratinocyte differentiation [18]. The ADAM17-deficient mice showed cutaneous bacterial dysbiosis and spontaneous dermatitis associated with *S. aureus* colonisation. It has been reported that mice lacking ADAM17 in keratinocytes exhibit skin barrier defects, characterised by the disintegration of cornified envelopes [19]. Additionally, δ-toxin produced by *S. aureus* induces mast cell degranulation, leading to the release of histamine and the Th2 cytokines IL-4 and IL-13 [20]. Conversely, production of IL-17A, but not that of IL-4, in $CD4^+$ cells from lymph nodes increased in ADAM17-deficient mice inoculated with *S. aureus* [18]. Additional information revealed that Langerhans cells (LCs) play a crucial role in Th17 and γδTh17 responses induced by the percutaneous inoculation of *S. aureus* [18].

2.3 *Staphylococcus pseudintermedius*

Despite the frequent isolation of *S. pseudintermedius* from the skin of dogs with AD and secondary superficial pyoderma, the role of this strain in innate immunity has been insufficiently investigated. However, increased serum IgE against *S. pseudintermedius* in

dogs with spontaneous AD suggests the role of this strain in the adaptive immune reaction of canine AD [21].

2.4 *Corynebacterium* sp.

It has been reported that *C. bovis* enhances the production of IFN-γ, IL-4, and IL-17A in $CD4^+$ cells from the lymph nodes of ADAM17-deficient mice [18], suggesting a combinational effect of this bacterial strain with S. aureus in the development of AD.

2.5 *P. acnes*

The bacterial strains are well known as the causative microorganism of acne vulgaris in humans. Previous studies have indicated that *P. acnes* inhibits the growth of *S. aureus* by fermenting glycerol into a variety of fatty acids, which decrease the pH [22]. Additionally, *P. acnes* induces the production of hBD2 and proinflammatory cytokines and chemokines in cultured human sebocytes [23].

3 Cutaneous bacterial dysbiosis and AD

3.1 AD in humans

Skin colonisation by *Staphylococcaceae* has been reported in human, canine and feline allergic skin diseases. In 2012, Kong et al. first reported that increased *S. aureus* in skin lesions was associated with disease flares in human AD [24]. The same group also reported that commensal *S. epidermidis* increased during disease flares, suggesting that this strain possesses antagonistic mechanisms against pathogenic *S. aureus* [24]. Of particular interest is that skin colonisation by *S. aureus* was recognised in the vast majority of human patients with AD. In contrast, the colonisation was identified in only a minority of healthy people [24, 25]. Moreover, increased proportions of *Streptococcus*, *Propionibacterium*, and *Corynebacterium* species were observed following therapy for AD in humans [24].

3.2 AD in animals

It has been reported that bacterial diversity in the axillary and pinna skin decreased, while the proportion of *Staphylococcaceae*, particularly *S. pseudintermedius*, and that of *Corynebacteriaceae* increased in dogs with spontaneous atopic dermatitis (AD) compared to healthy dogs [26]. It was also reported that dysbiosis and the proportions of *Staphylococcaceae* were restored following therapy [26]. Moreover, bacterial diversity was negatively correlated with transepidermal water loss, a quantitative measure of the skin's inside-to-outside barrier function [26]. Skin colonisation by *Staphylococcaceae* and *Corynebacteriaceae* was also observed in experimental atopic dogs subjected to percutaneous sensitisation by house dust mites. In the experimental dogs, an increased proportion of *Corynebacteriaceae* was recognised shortly after the development of skin lesions. In contrast, an increased proportion of *Staphylococcaceae* continued after the skin lesions had remitted [27].

Increased proportion of *Staphylococcaceae* in the skin was also recognised in cats with allergic skin diseases, although bacterial diversities did not differ among healthy and allergic cats [8].

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