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The microbiome

THE ECOLOGICAL COMMUNITY OF COMMENSAL, SYMBIOTIC AND PATHOGENIC MICROORGANISMS THAT SHARE OUR BODY SPACE AND CONTRIBUTE TO OUR HEALTH AND DISEASE

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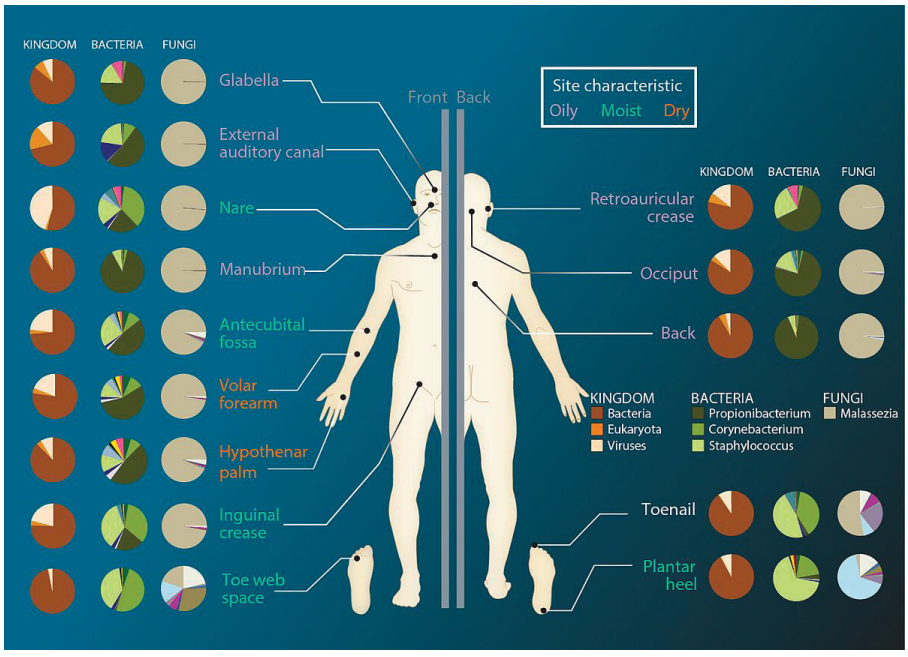
Introduction

In the last few decades, it became clear that the number and diversity of bacterial and fungal cells observed microscopically from medical samples (skin, faeces, etc) was much larger than that grown in culture, until it became clear that >90% of microorganisms do not grow in routine laboratory conditions, leading to a huge underestimations of the number and diversity of microbial communities. Today these communities can be investigated by means of a new method, called *next-generation sequencing* (Jo et al, 2016). Microorganisms can be identified to the species, classified for taxonomy, quantity, diversity, and multiple samples can be compared, e.g. from different areas on the same subject or from the same area of different subjects. From an initial group of 11 bacterial phyla in 1987, reference database called "Ribosomal Database Project" (accessed July 24 2019) has now reached the incredibly high number of 3,356,809 different bacterial sequences.

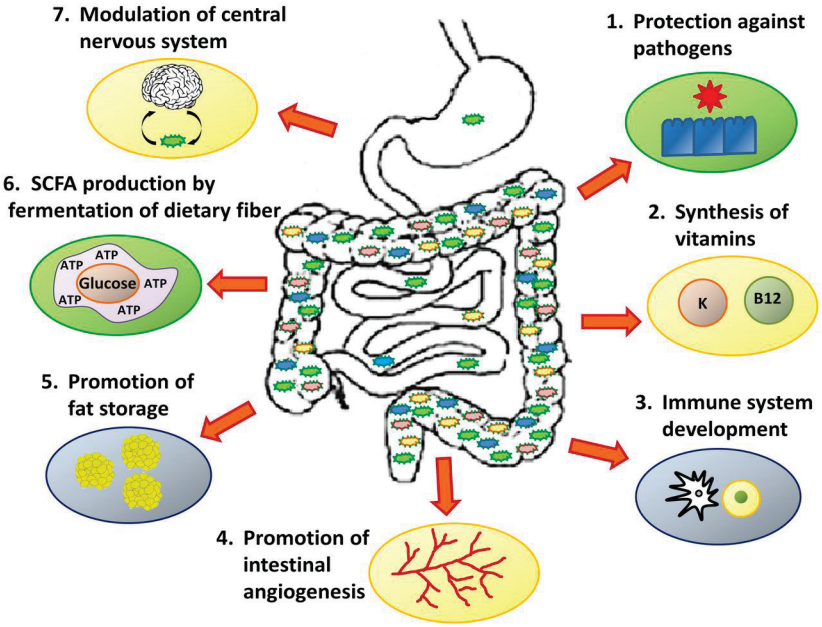
Numbers and importance of the microbiome

We know now that over >40[13] microorganisms live on and in our body, and that 90% of the cells in our body are not of human origin (Grice and Segre, 2012). It is estimated that 1 to 2 kilogram of our body weight is made by bacteria, and every square centimeter of our skin is covered by 1000 to 1,000,000 germs.

The Human Microbiome Project sampled the oral cavity, skin, GI and vagina in humans and found about 4000 bacterial species in the intestine with the genera *Bacteroides*, *Prevotella* and *Lactobacillus* predominant; 1200 bacterial species on the skin with the genera *Propionibacterium*, *Staphylococcus*, and *Corynebacterium* predominant; 800 bacterial species in the oral cavity with the genus *Streptococcus* predominant; and 300 bacterial species in the vagina with the genus *Lactobacillus* predominant. This variety of microorganisms is extremely important, as it provides an important genetic variation. The human genome has only 23,000 genes and is 99,99% identical among individuals, while in microorganisms of the



microbiome there are over 1,000,000 genes which provide diversity and functions that human cells do not have. Some examples are vitamin or digestive enzyme production, so that microbes and hosts have a symbiotic relationship: the host supports the microbes, and the microbes contribute to the function of the organs and systems. In particular microorganisms play an important role in interacting with the immune system, so that dysbiosis of the microbial flora is often associated with disease. One example is that perturbances of the cutaneous microbiome are associated with atopic dermatitis (AD) (Kong and Segre, 2012).



multiple functions of intestinal microbiome

The skin microbiome, its interaction with the immune system, dysbiosis and disease
(Belkaid and Segre, 2014; Grice et al, 2011)
Keratinocytes constantly sample skin surface for microorganisms, by means of receptors that recognize the microbes. The activation of these receptors stimulates the innate immune response, causing the production of antimicrobial peptides (AMPs) and pro-inflammatory cytokines. AMPs directly kill bacteria, fungi and enveloped viruses, but are also able to activate the adaptive immune response. Our skin is covered by millions of germs, but only rarely we develop inflammation, because keratinocytes can discriminate between harmless commensal microbes and harmful pathogenic ones. It is known that commensal species, like *Staphylococcus epidermidis* and Propionibacterium, are able to stimulate the production of cytokines and AMP against pathogenic microorganisms (Lai et al, 2010; Nagy et al., 2005). In AD there is a lower AMP production and in more than 90% of patients (compared with <5% of healthy individuals) the skin becomes colonized by the pathogenic bacterium *S. aureus*, of which 50% are toxin producing (Salava and Lauerma, 2014). These toxins can cause inflammation and skin barrier dysfunction, leading to disease flares and skin lesions. Since staphylococci produce themselves antibacterial compounds, there is a decrease in the protecting commensal flora, a dysbiosis which leads to a vicious circle (Wollina 2017).

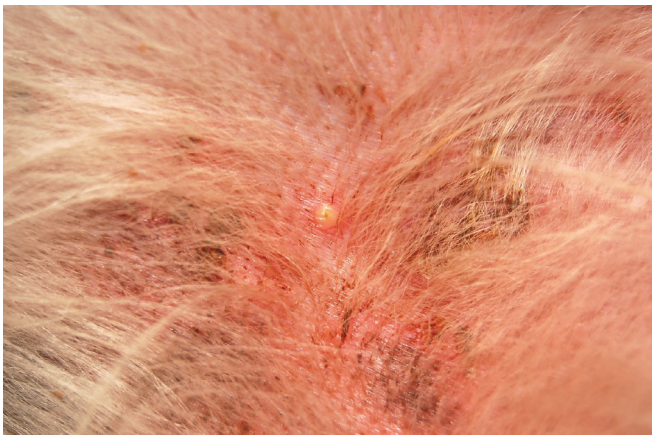
The Skin-Gut axis and the influence of the fecal microbiome on atopic dermatitis
There is a close relationship between the gut and skin, as most probably the intestinal microorganisms and their metabolites are able to influence skin physiology, and some dermatological disorders could be linked to gut dysbiosis (Vaughn et al, 2017). Studies have even postulated that gut microbiota produce neurotransmitters in response to stress that can influence skin function.
These neurotransmitters cross the intestinal epithelium, enter the bloodstream and induce systemic effects. In humans it is known that IBD is linked to pyoderma gangrenosum, celiac disease to vitiligo and Chron's disease with psoriasis. The same applies to atopic dermatitis: low diversity in the faecal microbiota has been demonstrated in patients with AD compared to healthy controls (Watanabe et al, 2003, Melli et al 2016), and the administration of systemic antibiotics increases the risk of AD, possibly due to changes in intestinal microbiota.

Modifying the gut microbiota has been considered a possible therapeutical option for treating AD, as the restoration of the microbial diversity leads to reduction of cutaneous flares. To this end probiotics have been extensively used, as they help modulate the immune system to shift away from pro-inflammatory immune reactivity and synthesize metabolites with anti-inflammatory effects (Butel 2014). Several studies determined that probiotics such as *Lactobacillus casei var. rhamnosus*, *Bifidobacterium animalis subsp lactis*, *Lactobacillus salivarius* and *Bifidobacterium breve* are able to alleviate pruritus and quality of life in adult AD patients and normalize the immune reactions towards allergens (Drago et al, 2015; Iemoli et al, 2012).

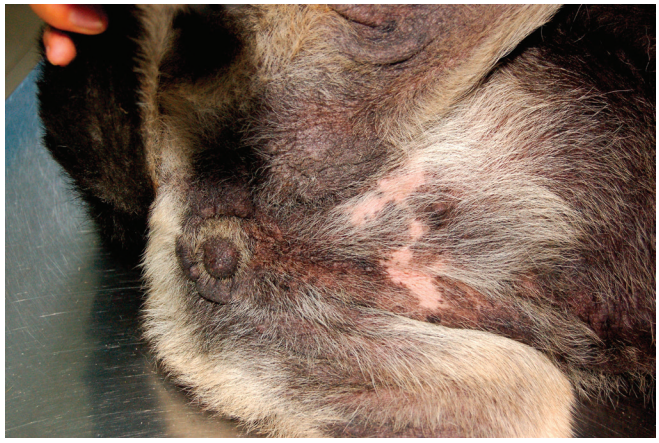
The canine skin microbiome in health and disease

The great importance of the microbiome in health and disease has been quickly recognized also in veterinary medicine, and several studies have been conducted to investigate it. Bacteria and fungi in canine and feline skin were found to be different than those of human skin. Canine and feline skin is dominated by *Proteobacteria, Firmicutes, Fusobacteria, Bacteroides* and *Actinobacteria*, and by environmental fungi such as Alternaria and *Cladosporium*, whereas human skin is colonized more abundantly by *Actinobacteria*, *Firmicutes* and *Malassezia* (Rodrigues-Hoffmann et al, 2014).

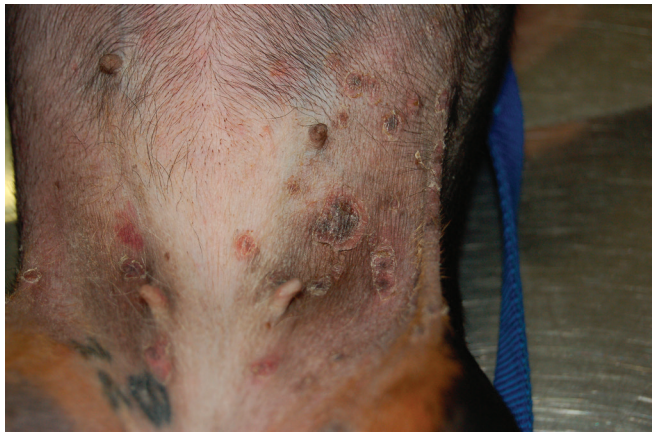
As in humans cutaneous dysbiosis was found to be associated with skin abnormalities. For example, clinically healthy dogs with strong odour were found to have reduced diversity of their skin microbiota with increased abundances of *Malassezia* and staphylococci (Jeffreys et al, 2017), similar to allergic dogs. As in humans, allergic dogs have increased proportions of *Staphylococcus* (*S. pseudintermedius* in particular) and *Corynebacterium* compared with a healthy control, and these proportions correlate with disease severity.



Pustule: typical lesion of pyoderma, a frequent consequence of allergic dermatitis in dogs

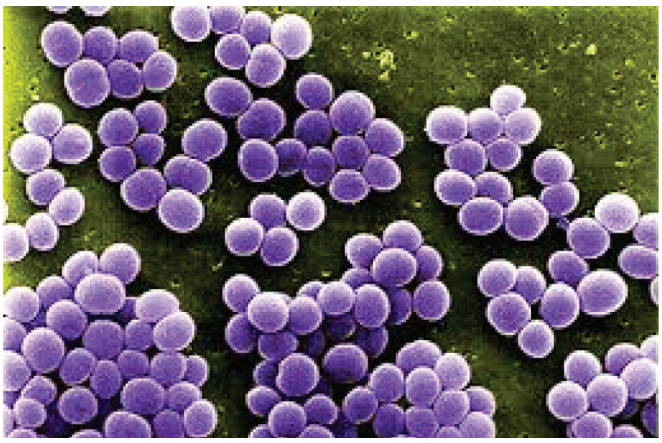


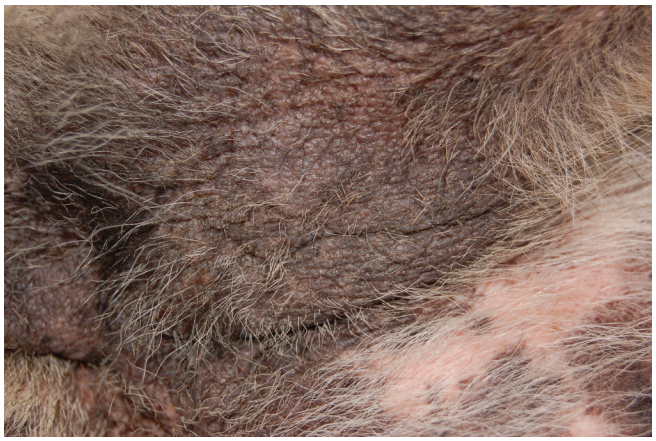
Ventral and perineal erythema in an allergic dog



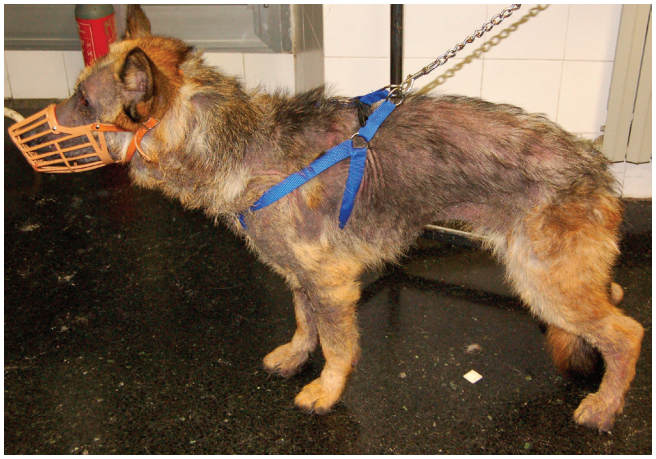
Collarette on abdomen in an allergic dog

Treatment is able to restore bacterial diversity in parallel with improvement of lesion severity and pruritus (Bradley et al, 2015). Recently proactive protocols were suggested for the treatment of canine AD, based on maintenance therapy between flares, rather than reactive treatment after each relapse episode (Olivry et al. 2015, Olivry and Banovich 2019). Controlling pruritus is very important, as scratching and rubbing leads to disruption of the skin barrier and favours colonisation of pathogenic microorganisms and cutaneous dysbiosis, leading to the need of antibiotic therapy, which further damages the normal microbioma and favours development of baterial resistance. Anti-inflammatory and antipruritic molecules, such as oclacitinib and lokivetmab, when given on long term basis are able to prevent pruritus and lesions due to allergy and help restore the necessary cutaneous bacterial diversity necessary for a healthy skin (Bradley et al, 2015). In parallel, as previously described in humans, the administration of oral probiota can also prevent and improve the clinical manifestations of canine AD, as recently determined in a model of allergic disease using *Lactobacillus rhamnosus* (Marsella 2009, Marsella et al, 2012). Similarly, the oral administration of tyndallised *Lactobacillus rhamnosus* was found to be associated with decreased clinical signs, cutaneous mast cell numbers and IgE production, compared to placebo, in a mouse model of atopic dermatitis (Lee et al. 2016). Probiota can thus represent a safe and useful integration of the classic pharmacological therapy.

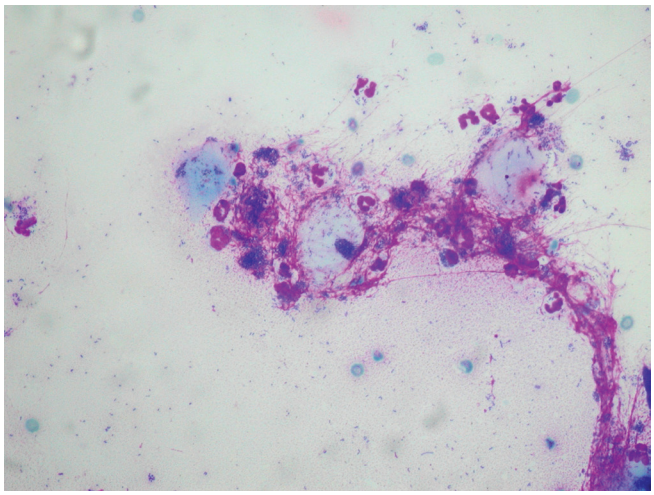




Advanced lichenification in an allergic dog



Allergic dog with widespread erythema, alopecia and lichenification



Cytological finding obtained from skin apposition with multiple coccoid and rod-shaped Bacteria

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