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THE EPIDERMIS OF MAN: CO-EXISTING WITH COMMENSALS (SUMMARY OF THE 28TH OLD HERBORN UNIVERSITY SEMINAR)

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"A BODY WITHOUT SKIN PROVES THAT WE ARE ALL THE SAME, BUT WITHOUT THE SKIN THERE, THERE CAN BE NO US"
(*Jablonski*, 2006).

The statement reproduced above may be a colloquial way to express the importance of skin. From a biologic perspective, the story of human skin has also been considered to represent a multitude of critical functions integral to the survival of many species. Skin has been instrumental in the long history of human racial and cultural evolution and conflicts to date. The human skin has represented the beauty and the beast in its colours, shine, wrinkles, pimples, sweat and odour, and a reflection of emotions in its sensitivity to touch. The skin has also been viewed as an assemblage of the "wounds of knowledge, the scars of truth and the limits of power" (*Emberley*, 2008). Human skin has been an object of art from times immemorial with use of cosmetics, tattoos, insertion of jewellery and other pierced decorations (*Mayell*, 2002).

The skin constitutes the biggest and the single most visible organ of human body, estimated to weigh about 40 pounds and measuring about 21 square feet in a fully-grown man. The outer layer is sloughed off and regenerated continuously. Normal human skin is an organ with multiple folds, invaginations and creases, hair follicles, sebaceous and sweat glands and characterized by varying degrees of melanin pigmentation. Although infections of the skin, associated with neurologic and or immunologic imbalance with or without manifestations of systemic disease have been well known for thousands of years, it is only recently that careful investigations have been undertaken to explore in detail the interaction between the skin, and the diverse spectrum of environmental microorganisms including bacteria, viruses, fungi, mites and other parasitic organisms which colonize the skin under different environmental and cultural settings (*Sanford* and *Gallo*, 2013). The information available about the skin, at the time this seminar was being planned, is briefly summarized.

SKIN STRUCTURE

The epidermal surface of the skin is formed by a network of cross-linked cornified cell envelopes (CCE) embedded in a matrix of water repellent lipid containing extracellular material rich in ceramide, cholesterol and free fatty

acids. The skin is richly endowed with eccrine and apocrine sweat glands, hair follicles, sebaceous glands (often as pilo-sebaceous units) and secretes sebum, which lubricate the hair and the skin surface. The breakdown-products of sebum include free fatty acids, cathelicidin, β-defensins, and other antimicrobial histones (*Gallo* and *Hooper*, 2012*)*. The anatomic differences of texture between different parts of the skin strongly impact on the qualitative and quantitative nature of microbial colonization in different sites. Although the outmost layers of the skin are critical components of the barrier function, several layers of skin below the epidermis as well profoundly impact on the barrier function. An aqueous layer above the epidermis also contributes significantly to the ecologic balance of epidermis (*Segre*, 2006). The intercellular tight junctions play an important role in the barrier function of the skin, although its role in antimicrobial barrier has not been fully explored. The epidermis represents a robust physical barrier and its cool, acidic nature is generally not a welcome environment for microbial growth.

SKIN IMMUNE SYSTEM AND MICROBIOME

It is estimated that over one billion bacteria reside in a square centimetre of skin surface and its associated appendages and glandular tissues (*Hentges*, 1993; *Sanford* and *Gallo*, 2013). At the same time, the functional components of both innate and adaptive mechanisms of immunity exist within the skin (*Kupper* and *Fuhlbrigge*, 2004). The keratinocytes as epithelial cells express several Pattern Recognition Receptors (PRR) designed for interaction with specific conserved Microbial associated Molecular Patterns (MAMP) such as lipoproteins, nucleic acids, microbial cell wall components and other microbial determinants. Keratinocytes express several antimicrobial peptides, chemokines and cytokines. Langerhans cells function as a subset of dendritic cells as antigen presenting cells and participate in regulation of immune response, including induction of tolerance via activation of regulatory T (Treg) cells. In addition, macrophages, mast cells, NK cells, CD8+, CD4+ Th1, Th2, or Th17, γδ-T cells and Treg are present within and below the epidermis. This information has been reviewed extensively in several recent publications (*Nestle* et al., 2009; *Afshar* and *Gallo*, 2013). It has been suggested that skin has the greatest diversity of variables that influence its surface characteristics, and may be better viewed as a "melting pot" of different microenvironments shifting constantly in response to the external environment and host's internal milieu (*Sanford* and *Gallo*, 2013). Thus, the microbiome in different sites of the skin must be considered as a dynamic entity, involving constantly changing quality and the quantity of its content.

The skin microbiome is in general considered either as resident or transient. The majority of normal resident inhabitants belong to the following phyla, based on 16S ribosomal RNA gene sequencing: Actinobacteria, Bacteroidetes, Firmicutes, or Proteobacteria. However, their distribution is site-dependent and exhibit significant differences between moist, sebaceous or dry areas. Microbiome in moist sites is dominated by *Staphylococcus* and *Corynebacterium* species, in sebaceous sites by *Propionibacterium* species of Actinobacteria phyla (the least diverse population of microbes), and in dry

areas by the most diverse of the microbes with varying representation from all four phyla identified above (*Eckburg* et al., 2005; *Dewhirst* et al., 2010). The resident microbes are a relatively fixed group of organisms found routinely in normal skin and they re-establish themselves after being dislodged by a variety of environmental insults. The transient microbes do not generally reside constantly in a defined skin site. However under several pathologic conditions, many organisms exhibit abnormal colonization patterns, proliferate locally, and may result in clinical disease (*Bik* et al., 2006; *Turnbaugh* et al., 2006). Differences in skin microbiome have also been observed as a function of temporal patterns of testing and interpersonal variations secondary to culture and geographical differences (*Grice* and *Segre*, 2011). The skin is initially colonized at the time of birth with a microbiome of very low diversity and is largely shaped by the method of delivery. Subsequently, by 2-3 years of age the microbiota at various body sites acquire more diversity and specificity harbouring over 150 species of microbial phylotypes (*Grice* et al., 2009).

During the past few years, a number of investigators have begun to explore the functional association of resident commensal skin microbiota with skin-derived immunologic responses, the impact of competition between commensals and pathogens via induction of innate immunity and specific priming for adaptive immune response, and the clinical consequences of altered microbiome in atopic dermatitis, psoriasis, opportunistic infections, and other disease states of host-microbial interactions (*Fukao* and *Koyasu*, 2003; *Strober*, 2004; *Sanford* and *Gallo*, 2013; *Oh* et al., 2013).

SKIN NERVOUS SYSTEM AND MICROBIOME

Contact with the skin and the sense of touch have been crucial elements in the evolution of emotional bonding between the mother and her new-born baby, and between other individuals in the social group in virtually all mammalian species. A high degree of correlation exists between lack of physical contact in childhood and higher rates of aggression later in life. Additionally hospitalized premature infants exposed to frequent physical contact, appear to have better weight gain, earlier hospital discharges and reduced rates of depression (*Feldman* et al, 2014). There is now increasing evidence to suggest that the skin establishes contact with external environment via sensory neuron end-organs. A complex network of sensory nerve fibres has been identified in the skin, which terminate as free nerve endings or specialized end-organs such as Meissner corpuscles or Touch domes. These nerve fibres are responsible for the perception of touch, temperature and local pain. In addition, the Schwann cells, and the nerve bundles that they ensheath as Remak Bundles, have been shown to function in a manner similar to the Glial cells of the central nervous system. Recent immunohistologic studies have demonstrated the presence of other sub-epidermal nerve plexus *in situ*, and the existence of other markers of neurologic function on the epidermal and dermal sheets (*Tschacheler* et al., 2004). These include, cytokeratin-20, protein gene product 9S, and neurofilament nerve growth factor (*Johansson* et al., 1990; *Griffin* et al., 2001). Furthermore, cutaneous nerves have been shown to release neuropeptides which can activate target cells such as keratinocytes,

Langerhans cells, mast cells, and endothelial cells in the skin. Finally, peptides such as substance P, IL-1, calcitonin gene-related peptide (CGRP), and vascular cell adhesion molecule VCAM-1 have also been identified in the skin after appropriate stimuli. These observations clearly support the role of nervous system in the skin in mediating biologic functions in health and disease (*Ansel* et al., 1997).

The information summarized above provides a bird's eye view of the skin surface, its acquired microbiome, and their possible interactions with the immune system and the nervous system of the skin related sites. More detailed information is available in several recent publications (*Baker*, 2006; *Grice* et al., 2009).

Several dominant organisms, especially *Staphylococcus* and *Propionibacterium* constitute the major portion of resident microbial flora. However, the mechanisms underlying the development of transient and pathogenic flora and their association with disease remain to be defined. Furthermore, factors that affect the balance between different bacterial species in different skin sites are poorly understood. The extent and the nature of potential benefits offered by the commensal and resident organisms to the host remain to be fully explored. Finally, the precise nature of cellular interactions between the skin microbiome, skin immune system, and their impact on the host's health or outcome of disease remains to be elucidated (*Kong* and *Segre*, 2011).

28TH OLD HERBORN UNIVERSITY SEMINAR

This seminar was planned to explore some of the questions raised above and other, still to be resolved, issues. It was also hoped to update the current state of knowledge about the epidermis and its microbial endowments, especially as they relate to the development of several disorders of the skin.

The seminar began with an introductory overview of the skin and its appendages as a dynamic barrier by Prof. Peter Elias. He provided a holistic view of epidermal defences based on the structural aspects of the epidermis including events associated with intra-epidermal metabolism regulated by barrier requirements and the homeostatic signalling mechanisms responsible for such metabolic events (*Elias* et al., 2008, 2014). He reviewed in some detail the structure of stratum corneum, and stratum granulosum in the inner epidermal surface, and the functions of the defensive gradient in the outer epidermis relative to permeability barrier and antimicrobial barrier, antioxidant activity, cellular cohesion, cytokine activation, neurosensory mechanisms and their possible functions, and the impact of hydration on epidermal homeostasis. The principal message conveyed by Prof. Elias in his overview was that stratum corneum is metabolically highly active and the epidermal defence functions are inter-related, co-regulated and highly interdependent. Although antimicrobial and permeability barriers function independently, they share many common features and functions. Prof. Elias also discussed the role of hydration of the skin and the biochemical and structural basis for stratum corneum hydrolysis, and the possible role of glycerol, NMF-Fil-aggrin derived products, lactic acid, glucose, salts, and urea. An interesting observation highlighted by Prof. Elias was the development of mast cell hypertrophy and degranulation after prolonged exposure of the skin to dry environment.

Prof. Paul Forsythe provided a systematic review of the role of mast cells in linking microbiome to the development of allergy. He pointed out that the induction phase of most allergic disorders are characterized by events associated with alterations in the phenotype and function of APC, induction of Treg and shift in Th1/Th2 balance. Paul reviewed recent information which suggests that microbiota significantly determines the outcome of the effector phase of the disease. He discussed the role of mast cells in microbiota-allergy axis, in the context of regulation of mast cell function by commensal bacteria employing *Lactobacillus rhamnosus* strain JB-1 as an experimental model (*Forsythe* et al., 2012). He also discussed potential mechanisms of mast cell stabilization by bacteria, employing quorum sensing molecules, and evaluation of mast cell immuno-regulation and microbial interaction in other areas such as neural, immune and endocrine systems (*Kendall* and *Sperandio*, 2006; *deKivit* et al., 2012; *Forsythe* and *Bienenstock*, 2012).

These studies have demonstrated that mast cells play an important role in host-microbiome communications and influence the development, outcome and severity of allergic manifestation at a clinical level. Cutaneous microbiome appears to have a major role in modulating mast cell function, based on the observations that non-pathogenic *E. coli* and other commensals function via induction of mast cell degranulation. However, other microorganisms utilize other distinct and very different functional mechanisms to stabilize mast cell function (*Forsythe* et al., 2012).

Prof. Richard Gallo examined in his comprehensive review the essential immune functions for commensal bacteria of the skin. He discussed the role of resident commensals in maintaining skin homeostasis, specifically via the generation of many antimicrobial peptides (AMP) (*Lai* et al., 2009, 2010). He suggested that normal resident skin microbiome plays a critical role in the suppression of any overwhelming inflammatory tissue response following mucosal injury (*Naik* et al., 2012). Based on information generated in an experimental mouse model developed in his laboratory with *Staphylococcus epidermidis*, some AMPs such as Firmocidin, appear to exhibit antibacterial as well as anti-neoplastic functions. In addition, such commensals also induce defensins and other antimicrobials to provide protection against pathogenic organisms. In particular AMP from *Staphylococcus epidermidis* have been shown to activate TLR2/CD36-P38, strengthen the cellular tight junctions in keratinocyte culture system, and enhance antiviral activity in murine skin by enhancing mast cell derived AMP, especially cathelicidin

Prof. Gallo also suggested that epidermis might be more appropriately considered a modest antimicrobial filter rather than an absolute antimicrobial barrier, based on several recent studies (*Amann* et al., 1990; *Horz* et al., 2005). He also discussed a fascinating pilot study on atopic dermatitis (AD), using lesional and non-lesional skin from the fore-arm from patients with AD. His group has demonstrated that the microflora in non-lesional skin of patients with AD consisted mainly of Proteobacteria and/or Actinobacteria. On the other hand, lesional skin contained higher proportion of Firmicutes and increased *Staphylococcus aureus* (*Sanford* and *Gallo*, 2013). He concluded that human body is truly a collection of non-human and human cellular structures, hoping to work together towards a common good. As part of this balance, non-human structures such as bacteria and other microbial antigenic

determinants may enter deep into dermis in order to maintain homeostasis via the induction of specific immune responses.

Following the discussion of the basic cellular structures and the characteristics of skin microbiome and immune system, subsequent presentations during the seminar focused on major clinical disease states, namely Staphylococcal colonization of the skin and nose, atopic dermatitis and, psoriasis. These presentations were followed by a comprehensive review on vitamin D and sunlight, participation of cutaneous nervous system in immunologic functions in the skin, and a comprehensive review of available antimicrobial peptides (AMP).

Prof. Andres Peschel reviewed the colonization with *Staphylococcus aureus* as a paradigm for the ecology of endogenous pathogens. Epidemiologic studies have shown that 80% of all severe bacterial infections are caused by only few endogenous pathogens. These include *Enterococcus faecalis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter spp.*, *Pseudomonas spp.*, *E. coli* and *Enterobacter spp*. An individual is at a significantly higher risk of invasive infection after nasal colonization by *Staphylococcus aureus*, and for increased eczema relapses after skin colonization in patients with atopic dermatitis. Colonizing pathogenic organisms appear to exhibit a complex interplay with the resident microbiome and host defence mechanisms. These include alterations in the content of nutrients, overgrowth of the endogenous pathogens and factors involving the qualitative and quantitative nature of the resident commensal microbiome. Other studies have demonstrated the impact of microbiome generated Bacteriocins and host defence immune mechanism on the endogenous pathogens and the impact of microbiome induced immune conditioning on pathogen-induced inflammation in the host. Prof. Peschel reviewed studies on nasal colonization with *Staphylococcus aureus* which indicate that interactions between Wall Teichoic acid (WTA) and scavenger receptors (SREC-1) are essential for colonization. It appears that WTA structures also govern horizontal gene transfer among major bacterial antigens. Understanding the ecology of endogenous pathogens will be the key to the effective control and prevention of diseases with such organisms.

Prof. Adrian Gombart provided an extensive review of the vitamin D and its diverse spectrum of biologic functions in man. He began his presentation with a discussion of the cleavage of B-ring of 7-dehydrocholesterol in the skin to its conversion to calcitriol, the bioactive form of vitamin D which binds to vitamin D receptor (VDR). VDR is a ubiquitous receptor expressed on a variety of cells, including T and B cells, monocytes-macrophages, dendritic cells, and neutrophils. Prof. Gombart reviewed available information about the role of vitamin D in modulating immune response via its effects on TLR, inhibition of Th17, and Th1 T cells function, promotion of tolerance in DC, and expansion of Th2 helper and regulatory T cells. He also discussed the epidermal barrier function and the role of vitamin D, antimicrobial peptides, and the skin microbiota. Vitamin D has been shown to increase expression of cathelicidin gene via activation of TLR 2/1 signalling. Considerable information is now available regarding the impact of microbiota, AMP and epithelial homeostasis in the gut (*Hooper* and *Macpherson*, 2010; *Kellermayer* et al., 2011). It appears that loss of

cathelicidin may affect the composition of gut microbiota and thus alter gut metabolism (*Gombart*, 2009). Similar to the gut, the skin has dynamic microbial ecosystems in different parts. Changes in microbial composition are associated with many skin disorders, and in certain situations the decreased bacterial diversity correlates with disease severity (*Kong* et al., 2012). However, the precise relationship between abnormal AMP expression and possible shift in microbial composition and pathology of the skin disease remains to be determined. Of particular interest was the observation that the vitamin D-CAMP pathway is primarily human and primate specific (*Gombart* et al., 2009). Based on the observation reported here, Prof. Gombart concluded that cutaneous synthesis of vitamin D and induction of cathelicidin and other antimicrobial peptides offers a unique mechanism for modulation of skin microbiota by natural sunlight.

Prof. Thomas Bieber reviewed the current state of knowledge about atopic dermatitis (AD) and the associated skin microbiome. AD is a disease of complex phenotypes and severity. He discussed the natural history of the disease in the man and in the dogs, which exhibit similar phenotypes and precipitating factors for the expression of the disease. Available information suggests that the clinical expression of AD is characterized by loss of diversity of microbiome during flare ups, stray colonization with *Staphylococcus aureus*, lack of tolerance to allergens, altered DC phenotype (FcεR++), and abnormal expression of TLR in skin cells. It has been suggested that the nature of interaction between TLR2 and FcεR1 in the dendritic cells (DC), modulate their phenotypes and function, associated with induction of Th17 and reduced expression of TLR2 in the keratinocytes and Langerhans cells in the skin. However, it remains to be shown if the microbial microenvironment is solely responsible for the modification of LC phenotypes and function. It is also not known why TLR2 expression is reduced in LC and if the altered microbiome has any lasting epigenetic impact.

Prof. Michael Gilliet discussed the immunologic and microbiologic aspects of psoriasis, a chronic relapsing, inflammatory skin disorder, affecting 2-3% of the human population worldwide. Psoriasis is an autoimmune disorder characterized by increased epidermal proliferation, significant immune activation of auto reactive T cells, and increased production of IL-22, IL-17, and IL-23 cytokines. Studies carried out in his laboratory have shown that Plasmacytoid (pDC) dendritic cells play a critical role in the development and pathogenesis of psoriasis. The pDC bearing a unique phenotype (BOCA2+, CD123+, CD4+, HCADR+) represent about 0.2% of peripheral blood mononuclear cells. It has been proposed that skin pDC are activated following injury or damage to the skin as an initial insult, which results in expression of IFN-α by the pDC. Expression of IFN-α is followed by expression of TNF-α and subsequent activation of IL-22, IL-17A/F and IL-23 cytokine and associated genetic phenotypes. These events are responsible for the maintenance phase of the clinical disease. The pDC are activated by specific antimicrobial peptides, especially the highly cationic amphipathic human β-defensins (hBD2, hBD2), Iyz, LL-37. These peptides are produced by the keratinocytes and induce the breakdown of tolerance to self-DNA. These peptides also disrupt the bacterial membranes, form complexes with native DNA and

thereby promote their immunogenicity. Autoimmunity against native DNA also appears to be promoted by IL-26 with direct antimicrobial activity, and by IL-17 that appears to be bactericidal via the induction of IL-26. Based on these studies, Prof. Gilliet proposed that the pathogenesis of psoriasis is mediated in part by abnormally increased production of some AMPs, followed by increased pDC and IFN-α production resulting in persistent chronic inflammation and auto-reactive immune responses and specific increase in epidermal proliferation (*Reizis* et al., 2011).

Prof. Michael Zasloff provided an integrated perspective of the basic biology of the microbiome, nervous system and immunity in the skin as discussed by other speakers as it relates to infection-induced tissue damage in clinical situations: tissue injury in diabetes mellitus and anti-tumour effects of bacterial infections of skin. He reviewed the historical aspects of severe experimentally-induced erysipelas on the regression of sarcoma lesions in man (*Linder* et al., 2010). Based on available limited evidence, Prof. Zasloff proposed that the process of tissue damage in the ulcer in a diabetic foot begins with loss of sensory nerve fibres which results in:

a) impaired homeostasis of AMP expression and significant breakdown of antimicrobial barrier,
b) impaired release of neuropeptides such as substance P (SP) and CGRP, and loss of neuronal mediated pro-inflammatory responses, and
c) failure to communicate effectively with other functional elements of central nervous system.

The loss of sensory nerve fibre function is related to impairment of innate immune responses in the epidermis in such patients.

In an effort to explain the historical observation on the regression of sarcoma tissue following experimentally induced erysipelas, Prof. Zasloff proposed that invading microorganisms or their toxins stimulate central autonomic vascular responses, associated with vasoconstriction of pre-capillary arterioles surrounding the sarcoma mass, resulting in anoxia, and breakdown and liquefaction of the tumour mass (*Chiu* et al., 2013).

One of the principle reasons for organizing this seminar was the recent surge of academic interest in mucosal microbiome and in the identification of many antimicrobial peptides, In particular, the Magainin antimicrobial peptides in the Xenopus skin (*Zasloff*, 1987).

This seminar concluded with a special lecture delivered by Prof. Jens Schröder on the discovery of human epithelial antimicrobial peptides. He began with lessons learned about the AMPs identified in patients with psoriasis. As pointed out earlier, it is a disease with widespread and extensive breakdown in epithelial barrier, and yet, overt microbial infections are surprisingly rare in psoriasis. It is now evident that uppermost layers of epidermis generate neutrophil attracting chemotactic factors and a large number of other antimicrobial peptides. These include many defensins, psoriasins, calprotectin and others peptides. Human β-defensin-2 (hBD-2) has been shown to link innate and adaptive immunity through dendritic cells and T cell receptor CCR6 and specially target Gram-negative bacteria and Candida. Human β-defensin-3 (hBD-3) is a broad spectrum AMP directed against many Gram-positive organisms. Resistance to skin specific infections by *E. coli* is largely mediated via Psoriasin (s100A7) and calprotectin (s100A8/9),

which are expressed in site-specific patterns in different skin sites. RNase7 another important AMP specifically targets enterococcus for its antimicrobial effects (*Schröder* et al., 1998; *Schröder* and Harder, 2006; *Simanski* et al., 2010).

CONCLUDING REMARKS

Human skin has often served as a mirror of many systemic infections and autoimmune disease processes. These include, systemic infections associated with cutaneous exanthema, enteric diseases characterized by cutaneous manifestations, and allergic or autoimmune disorders in which different parts of the skin may serve as the as primary target of disease manifestations. It is also of interest to note that intra-cutaneous and sub-cutaneous sites have been employed with varying degrees of success for delivery of vaccines, induction of allergic desensitization and delivery of pharmaceuticals for prophylactic or therapeutic intervention against many systemic disorders. Recent evidence has suggested that immune system of skin is well integrated into the common mucosal immune system with significant circulation of APC, antigen sensitized T and B cells and other mediators between the mesenteric lymphoid tissue, gut mucosa and the skin (*Glenn* et al., 2007; *Lawson* et al., 2010).

Because of limited availability of time, it was not possible to discuss these areas in much detail during this seminar. However, during the open discussion next day, Prof. Richard Walker introduced recent studies supported by the program for appropriate technology in health (PATH), involving successful intradermal immunization with enterotoxigenic *E. coli* (ETEC) against an oral challenge with virulent coliform organisms. Other phase I trials with transcutaneous patches impregnated with vaccine antigens have also demonstrated effective development of mucosal immune responses in the gut. These observations provide further support for a common immune system involving the skin and the mucosal sites.

Several other important aspects of the skin microbiome and its interaction with its neural and immunoreactive cellular components were highlighted during the discussion. The spectrum of such interactions range on one hand, from the development of clinical disease with *Staphylococcus aureus* in atopic dermatitis and the diabetic foot, and on the other, to relative protection against bacterial infections in psoriasis. The role of the interactive skin associated innate and adaptive immune responses, cutaneous nervous system, and of antimicrobial peptides in the regulation of homeostasis of the skin under normal or pathological studies remains to be precisely defined. There is no clear explanation for the mechanisms underlying the existence and qualitative nature of human skin microbiome as it has been identified to date. It is not known if the skin microbiome has undergone significant alteration during cultural evolution of man to date. The extent to which antimicrobial peptides observed in the skin are host or microbiome derived is also not known. Hopefully, this seminar will stimulate sufficient interest to pursue these and many other important questions regarding the skin and its microbiome. Nevertheless, it is now abundantly clear that skin is a remarkable organ and its biologic complexity far exceeds its cultural and cosmetic perceptions. It represents a major habitat

of a multitude of organisms in a site-dependent colonization pattern at the surface, deeper stratum corneum and its associated appendages.

It was in the context of this information that Prof. Thomas Bosch provided a final thought provoking evolutionary perspective of the early life forms and their interaction with the native microbiome in the marine ecosystem, He elegantly explained the complex communications linking the rudimentary nervous system in *Hydra* as a "holobiont" to its marine microbial composition and the bidirectional communication between the microbiota and the components of host epithelium. Holobiont is a host organism (plant or animal), which is in constant interaction with all its associated microorganisms, as an entity for selection of evolution. Based on the lessons learnt from *Hydra* and the observations in human and other mammalian species, it is clear that humans represent the best example of a holobiont to date (*Matyssek* and *Luttge*, 2013). However, the course of their future evolutionary outcome will be ultimately determined by the nature and the critical balance, between its human elements and all associated non-human component microorganisms.

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