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Factors shaping the composition of the cutaneous microbiota

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What's already known about this topic:

-Microbes are integral components of the human ecosystem.

-The cutaneous microbiota plays an important role in the regulation of skin homeostasis.

-The composition of skin microbiota is influenced by many factors.

What does this study add?

-The dominance of *P. acnes* in the postadolescent sebum-rich skin regions and its role in acne pathogenesis may be explained by the disappearing microbiota hypothesis.

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Abstract

From our birth, we are constantly exposed to bacteria, fungi and viruses, some of which are capable of transiently or permanently inhabiting our different body parts as our microbiota. The majority of our microbial interactions occur during and after birth, and several different factors, including age, sex, genetic constitution, environmental conditions and life style, have been suggested to shape the composition of this microbial community. *Propionibacterium acnes* (*P. acnes*) is one of the most dominant lipophilic microbes of the postadolescent, sebum-rich human skin regions. Currently, the role of this bacterium in the pathogenesis of the most common inflammatory skin disease acne vulgaris is a topic of intense scientific debate. Recent results suggest that Westernization strongly increases the dominance of the *Propionibacterium* genus in human skin compared to natural populations living more traditional lifestyles. According to the disappearing microbiota hypothesis proposed by Martin Blaser a few years ago, such alterations in the composition of our microbiota are the possible consequences of socioeconomic and lifestyle changes occurring after the industrial revolution. Evanescence of species that were important elements of the human ecosystem might

lead to the overgrowth and subsequent dominance of others because of the lack of ecological competition. Such changes can disturb the fine-tuned balance of the human body and, accordingly, our microbes developed through a long co-evolutionary process. These processes might lead to the transformation of a seemingly harmless species into an opportunistic pathogen through bacterial dysbiosis. This might have happen in the case of *P. acnes* in acne pathogenesis.

Introduction

Our microbiota is the result of constant exposure to bacteria, fungi and viruses, which transiently or permanently inhabit our body parts. Many of these microbial species are not simply passive bystanders, but, together with various human cells, form a complex ecosystem [1-3].

The first identified human-associated bacterium, *Escherichia coli*, was isolated from stool samples of healthy and diseased children by pediatrician Theodor Escherich in the 1880's [4]. His contemporary Louis Pasteur had already hypothesized that normal human flora were essential for life (reviewed by Mackowiak, et al.) [5]. The idea that microbes can act as important integral components of the human body received particular attention a decade ago, and subsequently, studies on microbial communities inhabiting various organs have become increasingly popular. The Human Microbiome Project (HMP) was launched in 2007 to identify and characterize these microorganisms [6]. Much interesting data has been gathered by internationally coordinated research efforts in the last few years; however, we are still far from completely understanding the exact role of these microbes.

The microbiota

Various parts of the human body provide appropriate environments for colonization by several microbial species even in healthy individuals. Surfaces that come into direct contact with the external environment provide constant temperature, moisture and nutrient availability, allowing bacterial and fungal species to be selected through a long co-evolutionary process [7;8]. Colonization of our skin, regions of the alimentary canal and parts of our urogenital tract has been long known. However, organs previously considered sterile, such as lung and placenta, may also have their own resident community [9-11].

The relationship between resident microbial communities and human cells is very complex. Previously it was thought that resident microbes inhabit the available niches and use the nutrients that are present without pathogenesis. Today it is clear that these species and their metabolic products also play important roles in a wide range of biological functions. In fact, they may regulate the development of cellular and histological features of colonized human organs and help to maintain their proper functions [12-14].

Factors shaping the composition of the skin microbiota

The cutaneous microbiota populates the epidermis and the pilosebaceous unit of human skin [15;16]. To date, approximately 1000 bacterial species belonging to 19 phyla, as well as fungal (dermatophytes) and viral species have been identified as members of this community [15]. *Actinobacteria (Propionibacterium* and *Corynebacterium species)*, *Proteobacteria*, *Firmicutes (Staphylococcus species)* and *Bacteroidetes* are the most common representatives of the four dominant bacterium phyla. Many factors (individual, lifestyle, environmental) influence the microbial diversity of our skin, and changes in any of these conditions can result in rapid alterations of the species composition within the community [15;17;18].

Early colonization

Cutaneous colonization generally starts at birth. Individual differences in the composition of the microbiota of the gut and possibly of the skin may be caused by the mode and manner of birth (vaginal or cesarean delivery, hospital or home setting, use of antibiotics, etc.) [19-22]. During vaginal delivery, babies come into contact with their mothers' vaginal microbes, and this encounter will determine the composition of the pioneer colonizers, including *Lactobacillus*, *Prevotella*, *Atopobium* and *Snethia* spp. Children delivered by C-section acquire their first inhabitants from their environment (mainly *Staphylococcus* spp. and other skin bacteria), which do not necessarily originate from their mothers. Initially the flora of a newborn is completely identical and undifferentiated at various anatomical sites (e.g., gut, mouth, skin), regardless of the mode of delivery [20].

Early colonization is a critical event and may have long-term consequences, as microbes that most efficiently adapt to an environment will subsequently become dominant. When two species cannot coexist and compete for the same resources, even a slight advantage (e.g., faster growth, more effective use of the available nutrients, or more efficient binding to the available attachment sites of the surrounding tissues) may allow one species to out-compete the other. In ecology, this phenomena is referred to as competitive exclusion [23]. In cases of less severe competition, marked delays in the colonization of beneficial species may occur: babies delivered through C-section exhibit delayed colonization of *Lactobacillus*, *Bifidobacterium* and *Bacterioidetes* spp. in the gut [24;25]. Dominant microbes of a community actively modify the properties of their environment and, as a result, the microbial ecology. Many commensals secrete factors, such as phenol-soluble modulins and bacteriocins from *Staphylococcus epidermidis* and acnecin from *Propionibacterium acnes* (*P. acnes*), that are bacteriostatic or antibacterial for other species [26-28]. Early colonizers may also change their microenvironment to enhance their own growth and inhibit the growth of other microbes.

Another factor that may also influence early colonization is the presence of vernix caseosa (VC) on the newborn skin. This white, creamy substance is synthesized during the third trimester of neonates [29;30]. It is highly cellular: polygonal, water-filled corneocytes are embedded in an amorphous, lipid-rich material. The structure is somewhat similar to the cornified envelope layer called stratum corneum (SC) of postnatal skin, although corneocytes are not interconnected by desmosomal cellular contacts, and the lipid matrix does not possess a lamellar architecture. As a result, VC is considered a mobile, fluidic SC [31,32].

VC is mainly composed of water (81%), lipids (9%) and proteins (10%) [33]. Its lipid content is mostly of sebaceous origin, synthesized from the third trimester onward, marking an important step of neonatal epidermal barrier maturation. Other important components are proteins, many of which exhibit antimicrobial properties. As a result of its complex composition and structure, VC exhibits multifaceted biological functions. It acts as a mechanical barrier, offers lubrication during birth, has important waterproofing properties, and may aid thermoregulation after delivery. Because of its viscous and hydrophobic nature and its protein constituents, VC also has important antimicrobial functions, protecting the baby from the colonization and growth of pathogenic microbes [34;35]. The pH of the skin surface is 6.0 at birth and becomes slightly more acidic (pH=5.1) during the first 6 weeks of life. VC appears to facilitate these events, further favoring the early colonization of skin commensal microbes as opposed to pathogens [36;37]. It also provides an anti-oxidant shield and aids wound healing of newborn skin. Overall, because of its complex functions, VC is an important

substance providing a smooth transition between intra– and extrauterine life [31] and aiding the formation of a balanced, human–microbial ecosystem.

Changes in the pattern of early colonization may lead to unfavorable consequences. Early stimuli can critically affect the developing immune system of the baby, and might lead to the development of atopic, chronic inflammatory and allergic diseases later. These effects are well studied in the case of the gut microbiota, but little is known about the exact nature and effect of the cutaneous microbiota on the pathogenesis of such diseases [38].

Host factors

After early colonization and stabilization on the skin, *Streptococcaceae* and other *Firmicutes*, *Bacteroidetes*, *β* and *γ-Proteobacteria* dominate the microbiome of children. The composition of this community changes during puberty, when endocrine-induced events result in hyperplasia of sebaceous glands and subsequently enhance sebum excretion [30;39]. The most pronounced alterations affect areas where the density of sebaceous glands is the highest (face, shoulders, chest and back). These locations likely experience the largest shift in composition of the resident microbes. By analyzing the composition of microbial communities, investigators have noted that the species diversity clearly decreases with sexual maturation and that lipophilic microbes, including members of the *Corynebacteriaceae* and *Propionibacteriaceae* families, gain dominance on the face [39;40].

P. acnes, one of the most dominant lipophilic microbes of human postadolescent skin, is a Gram-positive, anaerobic fermenting, rod-shaped bacterium. *P. acnes* has been shown to secrete various enzymes, including lipases, that generate fatty acids from sebum lipids, and might compromise the growth of other microbes [28;34]. *P. acnes* also secretes short-chain fatty acids (SCFA) during anaerobic fermentation, one of which, propionic acid, clearly exhibits antibacterial effects [41-43]. The generated free fatty acids together with the secreted SCFAs may contribute to the maintenance of a skin pH that is acidic enough to restrict many microbes [44]. These data together explain why this bacterium is dominant in sebum-rich skin. The generation of an environment that is hostile to other microbes suggests that the observed decrease of microbiota diversity during puberty is a direct consequence of *P. acnes* expansion.

After this transitory period, the core composition of the cutaneous microflora stabilizes by early adulthood. The type and number of bacterial groups that become accustomed to our body is intriguingly limited, suggesting the presence of strong selective forces and co-evolution [45]. Marked differences in microbiome composition can, however, be detected in samples originating from

different anatomical locations of the same individual, suggesting that physiological properties of a given niche lead to site-specific differences in the local composition [15;39;46]. According to Grice *et al.*, the dominant phyla in sebum-rich regions are the *Actinobacteria* (*Propionibacteria ssp.*) and *Firmicutes* (*Staphylococci ssp*). Moist areas (e.g., armpit, interdigital areas, inguinal crease) are mostly populated by *Corynebacteria* and Staphylococcus. Dry regions (forearm, buttock) host the most diverse, mixed population of *Actinobacteria*, *Proteobacteria*, *Firmicutes* and *Bacteriodetes* [15, 17].

Gender also has a noteworthy impact on the microflora. Sex-specific differences likely manifest directly after birth, as recent data suggest that the lipid composition of VC in newborn boys and girls differs: the VC of girls seems to contain higher proportion of wax esters and triacylglycerols with longer hydrocarbon chains than those found in the VC of boys [47]. Fine anatomical and physiological properties of the skin (thickness, pH, composition and rate of sebum secretion, cosmetic use) might also contribute to gender-specific differences even when comparing the same regions [18;46;48]. Skin surface pH is generally lower and sebum secretion higher in males compared females in age-matched cohorts [49].

The reproductive organs also host a specialized microbiome due to their large differences in anatomical and physiological properties (e.g., chemical composition, pH). Specific species that normally populate the genitals have been shown to spread to other anatomical regions: thus, bacteria previously characterized as genital (*Lactobacillus* and *Gardnerella* in females, *Corynebacterium* in males) have also been detected in samples originating from other areas, such as the upper buttock [50].

Geographic, environmental, socio-economic and lifestyle factors

Individual habits together with the properties of the surrounding environment are important determinants for the composition of human skin microbiota. Westernized lifestyles have clearly reduced the microbial load and diversity in our environment. Epidemiological studies at the end of the twentieth century revealed that changes occurring after the industrial revolution, including personal and household hygiene as well as declining family size, led to enormous increases in the prevalence of atopic and other diseases. In 1989, David P. Strachan proposed the hygiene hypothesis [51], which suggests that the quantity and diversity of the environmental microbes with which we come into contact are crucial for the development of our immune systems. In addition to the effects of contact with environmental microbes, microbial components of the human ecosystem have also

been suggested to play important roles in the maintenance of our healthy and balanced states [52;53]. Important questions remain as to whether and how all these changes can affect our body and homeostasis.

Agricultural development, urbanization, the industrial revolution and Westernization represent prominent shifts in human cultural development that have resulted in changes in individual lifestyles, and most probably, in our microbiota. Analysis of these events is rather difficult, as archive or archeological materials preserving ancient microbiomes are not readily available. A recent study of historic samples excavated from a monastery in Germany examined dental tissues of human skeletons (ca. 950-1200 CE) exhibiting signs of periodontal disease. Results suggests that currently known oral pathogens (e.g., *Tannerella forsythia*, *Porphyromonas gingivalis*, *Treponema denticola*) have long been associated with the development of periodontal disease, regardless of changes in diet and personal oral hygiene [54], and might have become part of the human oral flora in parallel with the introduction of farming in the early Neolithic period [55]. It is also intriguing that these bacteria included sequences similar to antibiotic-resistance (AR) genes, long before antibiotics were available. The presence of such sequences were also identified in another study conducted on members of a contemporary population (Yanomami) in the Amazonian jungle, in Venezuela, who lives a seminomadic, hunter-gatherer lifestyle that is presumably very similar to the lifestyle of our ancestors. These individuals have been secluded from Westernized lifestyle and, as reported, have not been exposed to medical doses of antibiotics throughout their history [56]. Still, AR gene-like sequences are present in their microbial genomes, suggesting that our microbiome may have been serving as a reservoir and source of antibiotic resistance [54,56].

To model changes in the cutaneous microflora throughout human history, several groups now focus on the analysis of contemporary populations with traditional, less industrialized lifestyles. Comparing these groups with Westernized populations might elucidate conditions that can be associated with historical lifestyles [56;57]. Strikingly, the results from these studies also indicate that more traditional living conditions mostly correlate to higher microbiome diversity [54;56;58]. The most complex composition reported to date was discovered on Yamomami individuals, except for the oral samples, where species diversity was comparable to US individuals living a Westernized lifestyle [56;57].

The cutaneous microbiota of these individuals appears to be highly complex, but *Staphylococcus*, *Propionibacterium*, *Corinebacterium* and *Neisseria* species are much less dominant for Yamomami and South American Amerindian individuals than for Westernized populations [56;58]. Generally, Westernization seems to be associated with an increasing dominance of the *Actinobacteria* phylum and, particularly, the *Propionibacterium* genus on the skin [58].

These data corroborate nicely with the disappearing microbiota hypothesis proposed by Martin Blaser. According to this proposition, the two major routes to acquire resident microflora are vertical acquisition (by maternal transmission to the offspring) and horizontal transfer (from the surroundings through contaminated environment, food, drinking water and physical contact). Because of changes in hygiene, housing and family models, the latter route has gradually become less prominent in the human population. As a consequence, loss of particular microbes in the maternal generation could be inherited by subsequent generations, and, thus, the loss could become permanent. The net effect would be gradually decreasing variability in our resident microbes, or, in other words, disappearing microbiota [45;59]. The combined effects of decreasing diversity for environmental microbes (hygiene hypothesis) and drastic changes in human ecology might also lead to declining diversity in our resident flora (disappearing microbiota hypothesis). These events might be linked to the gradual increase in the prevalence of Westernization diseases, including atopic diseases, such as asthma, as well as obesity and metabolic syndrome [45;59;60].

Clearly, socioeconomic changes and the resulting lifestyle differences have a great impact on our microbiota; however, it is less clear how seasonal changes, climate and ethnicity affect our cutaneous microbial community. Most of the currently available studies are associated with the HMP [61] and have been conducted in Western countries. Only a handful of reports have investigated populations living under different climatic zones in different geographical areas [6;57;58;62]. According to the available data, while the core composition of the microbiome is similar for different populations (*Proteobacteria, Firmicutes, Actinobacteria* phyla), clear differences have been detected. Relative abundances of various genera can be diverse — perhaps even unique — and population-specific microbes have been identified, such as the *Enhydrobacter* genus in the cutaneous samples of Chinese individuals [62]. It should be noted that dissimilarities among populations may not be entirely caused by geographic differences. The lifestyle and socioeconomic differences described above should also be taken into consideration when comparing geographically distinct populations. It is difficult to assess how substantial the impact of environmental differences on the skin microbiome composition is, as such a comparison should include, for example, equally modernized populations exhibiting a very similar lifestyle. One investigation compared groups living in two states within the United States, Colorado and New York. Even though socioeconomic differences were likely to be small, subtle alterations in the microbiome composition were apparent, suggesting that geographic and climatic factors may also have some effect [58].

Nonetheless, these studies clearly indicate the importance of well designed and large-scale investigation of different populations to further increase our understanding of pan-microbiome composition.

What can we learn about the role of the cutaneous microbiota in acne pathogenesis?

A balanced interaction between microbial and human cells is important for the maintenance and promotion of healthy functions [12;13;50]. Microbes can synthesize and release nutrients from our food for use by human cells, protect us from the colonization of pathogenic or harmful invaders, beneficially modulate our immune system and even facilitate differentiation and renewal of certain tissues (e.g., gut mucosa) [12;63]. When this delicate and intricate equilibrium is disturbed in our ecosystem, dysbiosis develops, leaving us vulnerable to microbial diseases. Changes in the microenvironment and colonization by an extraneous microbe can contribute to dysbiosis and, together with other pathogenic factors, might lead to diseases such as seborrheic dermatitis (*Malassezia spp*), atopic dermatitis (*Staphylococcus aureus*), post-operative infections (*Staphylococcus epidermidis*) or acne vulgaris (*P. acnes*) [12;17;64].

In many cases it is difficult to clearly distinguish between commensal, symbiotic and pathogenic microbes, as the behavior and impact of a microbe can be strongly context dependent [45]. For these reasons, the pathogenic roles of several species, including *P. acnes* and its involvement in acne vulgaris, are a matter of intense scientific debate [28;65-67].

Several changes occurring in puberty, such as hormonal changes, androgen excess, sebaceous gland hyperplasia and subsequently enhanced sebum secretion, create a permissive environment for lipophilic bacteria. At this time, a shift from the "childhood" microbiome, in which *Streptococcaceae*, *Firmicutes* β- and γ *Proteobacteria* predominate, to a more "mature" composition dominated by *Corynebacteriaceae* and *Propionibacteriaceae* occurs [39]. Changes in the skin microenvironment drive these events as well as the possible strong competitive exclusion generated by the "newcomers." During this transitory period, dysbiosis can occur before the stabilization of the adult ecosystem.

Keeping in mind these natural, developmentally driven changes in microbiome composition, it is interesting to consider the microbial consequences of Westernization. In natural populations, the composition of the cutaneous microflora is more complex and balanced compared to Westernized groups, where *P. acnes* clearly dominate the postpubertal microbiota [56;56]. What causes these

differences is currently not known. During puberty, enhanced sebum secretion may provide a growth advantage for the lipophilic *P. acnes*, and the bacterium can subsequently modify its environment by lowering the pH as a result of SCFA secretion. In contrast, the disappearing microbiota hypothesis may also provide some explanation. Competing microbes controlling *P. acnes* growth might have been gradually lost as a result of Westernization. The consequence of such a loss might be the dominance of *P. acnes*, its enhanced growth and the resulting dysbiosis leading to acne pathogenesis during puberty.

This could also imply that acne is a disease of Westernized populations. Although no reports are available on the incidence of acne in natural populations, earlier reports suggest that acne does not equally affect all populations: acne vulgaris is present in 80 to 90% of the adolescents living in developed countries [68;69], whereas, in isolated communities, this ratio can be much lower. Some reports suggest that acne is or was nonexistent in the inhabitants of the island of Okinawa before World War II [70], the Bantus in South Africa [71;72], isolated South American Indians [73], and Pacific Islanders [74], the lifestyles of all of these populations are considerably less Westernized than in developed countries [75].

Whether *P. acnes* load is higher in the skin of acne patients than unaffected individuals is currently not clear. Earlier reports provided conflicting results [76-78]; the reason for this disagreement is likely that the bacterium is located deep in the pilosebaceous units, that it exhibits different culturing properties and that it often presents in a biofilm form. It seems, however, that increased incidence of *P. acnes* biofilms is detectable in the lesional skin samples of patients [79]. How and why exactly this happens is currently not known. Bacterial quorum sensing could possibly explain this discrepancy: by reaching a threshold density, the bacterium may start to form a biofilm in the pilosebaceous unit and express molecules contributing to bacterial pathogenicity [80]. Such transformations might lead to dysbiosis and, subsequently, also to acne pathogenesis.

Even if the relative abundance of the bacterium is similar, there are indications that the *P. acnes* population structure of controls is different for acne patients. Strains that preferentially present in lesional skin samples might have altered genetic and microbiologic properties, as well as pathogenicity [81].

Although it has not been explicitly proven, we believe that all these data strongly suggests that *P. acnes* has a role in acne pathogenesis.

How can this knowledge be put into practice?

Can we somehow overcome the potentially deleterious effects of the disappearance of various species from our microbial ecosystems? Is it possible to artificially modify the pattern of microbial transfer or aid the transfer of a complex, balanced microbiota to prevent various diseases? It may be possible. There are already exploratory clinical studies that are implementing this idea. Alteration of the gut flora by fecal microbiota transplantation (FMT) has already shown efficacy in severe *Clostridium difficile* infections and has been proposed for the treatment of other conditions (e.g., inflammatory bowel disease, irritable bowel syndrome, metabolic syndrome), in which the composition of the gut microbiome differs from the healthy state [82]. Another, widely used method to restore a balanced intestinal microflora is to use probiotics, which are clearly beneficial after antibiotic use. Probiotics might also have beneficial effects for diseases such as obesity, insulin resistance syndrome, type 2 diabetes and non-alcoholic fatty liver disease, although this conclusion requires further, well designed, rigorous clinical investigation [83].

Attempts are also being made to provide an appropriate pioneer flora to babies who are not delivered vaginally. In a recent article, Dominguez-Bello and colleagues reported a clinical trial to establish a healthy microbiota in C-section-delivered babies by wiping them with a gauze previously exposed to the vaginal fluids of their mothers. Although the results are preliminary, the analyzed sample size is relatively small and only partial microbiome reconstitution was achieved, the results clearly suggests that during the analyzed time period the microbiota of wiped, C-section delivered babies are more similar to vaginally delivered children compared to untreated, C-section delivered neonates [84]. The consequences of such procedures on the overall health and the prevalence of, for example, atopic and chronic inflammatory diseases is of interest for following in long-term studies.

If the above proposed model of acne pathogenesis and the role of *P. acnes* is confirmed, would it be possible to treat this condition by artificially modifying the composition of cutaneous microbiota of the teenager population? Currently it is difficult to answer this question. Further studies of natural populations living more traditional lifestyles would be very useful to define a core "ancient" cutaneous microbiota composition. From such knowledge, we could select microbes that might provide an appropriate control over *P. acnes* dominance but were most likely lost during our socioeconomical development. Topical formulations including these selected species are envisioned for application in a way analogous to probiotic use. Naturally, detailed and rigorous *in vitro* and *in vivo* experiments would be needed to test the interaction of the different microbes with one

another, their effects on skin cells and, finally on the whole organism. To support methods for replacing microflora lost by Westernized lifestyles, it will also be necessary to examine lifestyle and other changes that maintain healthy microflora complexity and balance as, presumably, the forces in our modern environments that reduce complexity are still in effect.

Conclusion

A complex interplay between a host and its microbiota is important for the maintenance of healthy skin function. Because of the polygenic and multifactorial nature of the disease, there are many possible alternative paths leading to the pathogenesis of acne vulgaris. One possible mechanism might be a change in the interaction between the skin cells and the cutaneous microflora leading to an imbalanced state and subsequently causing a “harmless” commensal, such as *P. acnes,* to become pathogenic.

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