Microbial Ecology of Human Skin in Health and Disease

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Cultivation of human skin reveals numerous bacteria and at least one fungus to be normal inhabitants of this ecosystem; however, most of our knowledge about the microbiology of human skin was acquired decades ago. Modern techniques employing nucleic acid-based microbial identification methods demonstrate the limitations of cultivation for appreciating microbial diversity in many ecosystems. The application of modern molecular methods to the study of skin may offer new perspectives on the resident microfora, and new insights into the causes of antibiotic responsive dermatologic conditions, such as acne and rosacea. ***Key words: acne/microbiology/microflora/rRNA. Journal of Investigative Dermatology Symposium Proceedings 6:167–169, 2001***

In the medical sciences, we tend to view the skin as an organ, and focus on the host cellular responses to disease. Although this view is accurate, it is incomplete. The skin is also a complex microbial ecosystem, with interactions between microbial constituents, and between microbes and the host. In this broader view, disease may result from ecologic shifts in microbial inhabitants or community structure.

SKIN AS ECOSYSTEM

There are multiple niches within the ecosystem of the skin. The axilla may be as different from the trunk as a tropical rain forest is from a desert (Marples, 1969). The various regions of the skin are noted to have different populations of microbial inhabitants, reflecting their different niches. Colony counts of aerobic bacteria from moist areas such as the axilla or toe web spaces can reach 10^7 bacteria per cm^2, whereas dry areas such as the forearm or trunk may harbor 10^2 or fewer bacteria per cm^2 (Leyden *et al*, 1987). Anaerobic bacteria are also present on human skin, with colony counts up to 10^6 bacteria per cm^2. In addition, skin structures within a specific skin zone may harbor unique microbes. The stratum corneum, cellular layer, hair shaft and follicle, eccrine, apocrine, and sebaceous glands may each have associated microflora.

The ecosystem of the soil is a good analogy for the ecosystem of the skin. In her *Scientific American* article ''Life on the human skin'', Mary Marples (1969) noted: ''Both the soil and the skin lack producer organisms and obtain their organic material from without: the soil from above (in the form of dead plant material) and the skin from below. In both soil and skin there is an extensive nonliving matrix that is permeated by solutions, and the living organisms in both are grouped around structures that penetrate the surface to deeper layers. In the soil the densest populations of microorganisms are in the rhizosphere, the region that surrounds plant roots. The comparable region in the skin is the hair follicle.''

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The skin provides nutrients for selected colonizing microbes in the form of lipids and protein (keratin). This dry and slightly acidic environment may limit the types of microbes that can survive on normal skin. To colonize, a microbe must compete with other microbes of the normal flora for organic molecules and space. In stable ecosystems, microbes tend to maintain a state of equilibrium, resisting abrupt changes in community structure. This process may protect the host from microbial pathogens by excluding them from the cutaneous ecosystem.

Many external factors can alter the ecosystem of the skin, with resulting changes in microbial populations (Roth and James, 1988). Environmental factors include temperature, humidity, salinity, and light exposure. Host factors include age, sex, immune status, hospitalization status, hygiene, use of medications (antibiotics, steroids), use of soaps and cosmetics, and presence of trauma. Activities that seem trivial to us, such as taking a shower, may be the equivalent of a hurricane to the microbes inhabiting the skin, with changes in landscape and population structure.

A few studies have described the interactions between microbes on the skin. Microbial lipases generate free fatty acids from secreted triglycerides, which may have antimicrobial properties, limiting the types of microorganisms that can exist on the skin (Hentges, 1993). Some resident skin bacteria also secrete bacteriocins that have antimicrobial properties (Roth and James, 1988).

MICROFLORA OF SKIN

Our knowledge of the microflora of the human skin is based mostly on cultivation studies. Predictably, the results of cultivation studies depend very much on how the studies are performed. Are microbes removed from the skin by swabbing, scraping, washing into a detergent solution, or plating directly on agar with a touch method? Is the resulting sample processed by incubating aerobically or anaerobically, in liquid media or on agar plates? What are the cultivation conditions, including pH, osmolarity, temperature, carbon dioxide concentration, and types of organic substrates. The conditions selected will determine the number and types of microbes found on cultivation. No *in vitro* cultivation system can duplicate the exact environment found on the skin.

Numerous bacteria have been cultivated from normal skin (Leyden *et al*, 1987; Roth and James, 1988). These include staphylococci, micrococci, corynebacteria, brevibacteria, propionibacteria, and acinetobacter species. The yeast *Pityrosporum* (or

Malassezia furfur in hyphal form) is a fungus that is normal flora. One microscopic animal, the follicle mite Demodex folliculorum, is also considered part of the normal flora. Skin flora maps such as those done by Bibil and Lovell demonstrate anywhere from 0 to more than 100 000 colony forming units of aerobic bacteria can be isolated from each square centimeter of skin from different regions of the body (Bibel and Lovell, 1976). Other microbes, such as *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Psuedomonas aeruginosa*, may be transient colonizers in abnormal conditions.

DOES CULTIVATION REFLECT BACTERIAL REPRESENTATION?

There is evidence to suggest that cultivation may provide an inaccurate assessment of microbial diversity in various niches. When natural ecosystems are surveyed, the percentage of bacteria that can be cultivated tends to be quite low. Usually less than 1% of bacteria can be cultivated from such environments as seawater, lakes, sediments, and soil (Amann *et al*, 1995). These figures are calculated by comparing the number and types of bacteria directly visible by microscopy to the number and types of bacteria that are cultivated from the same sample. The discrepancy between the directly observed and the cultivated microbes has been named the ''great plate count anomaly''.

Another approach to assessing microbial diversity relies on the detection of microbial DNA. The most widely used gene for identifying microbes is the small subunit ribosomal RNA gene, such as the 16S rRNA gene in bacteria. This gene is present in all known bacteria, has conserved regions that make good priming sites for polymerase chain reaction, and has variable regions that allow one to identify bacteria or infer phylogenetic relationships to known bacteria. By knowing the distribution of 16S rRNA gene types in a sample, one can create a census of microbes without cultivation.

When the 16S rRNA profiles of environmental microbial communities are compared with the cultivation-based profiles, a significant discrepancy is revealed again. Numerous 16S rRNA sequence types are usually discovered that do not match cultivated members of the community. These results have been reproduced in seawater, hot springs, and soil (Giovannoni *et al*, 1990; Ward *et al*, 1990; Stackebrandt *et al*, 1993).

One might ask if the same discrepancies occur in human microbiology. At least two studies have addressed this question. In a study of normal human feces, investigators found that 76% of the 16S rRNA sequences generated from a fecal sample did not match any known bacterial sequences present in public databases (Suau *et al*, 1999). In another study of bacterial diversity in the human mouth, 52% of polymerase chain reaction amplified 16S rRNA sequences from a subgingival scraping did not match sequences present in public databases (Kroes *et al*, 1999). More than 13% of these sequences were sufficiently different from known sequences to suggest the presence of unique genera. The same methods have not been applied to the human skin, so our understanding of this ecosystem is incomplete.

DO MICROBES PLAY A ROLE IN IDIOPATHIC DERMATOLOGIC DISEASES?

The pathogenesis of numerous dermatologic conditions remain unexplained. The hypothesis of infection has been proposed for diseases such as scleroderma, psoriasis, eczema, seborrheic dermatitis, dyshidrosis, acne, and rosacea. Is it possible that specific microbes, or changes in the microbial community on the skin, initiate or sustain some of these diseases? If so, microbes might exert their pathogenic effect by release of toxins, invasion of cells, alteration in host cell regulation, induction of allergic or inflammatory responses, or alteration in the microbial community.

If microbes play a role, the failure to link microbes to these diseases may be explained by several factors. First, one must advance the hypothesis of an infectious cause if one hopes to find a causative microbe. Second, previously used microbial detection technologies are not adequate to detect all microbes, as detailed above. Third, microbes may initiate a pathologic process, yet be absent at the peak of disease when they are most likely sought (hit and run). Fourth, it is difficult to link ubiquitous microbes to a disease when only a subset of susceptible patients may develop disease manifestations (e.g., *H. pylori* and peptic ulcer disease). Fifth, multiple microbes forming a microbial community may be necessary to produce disease. In this setting, trying to link a single microbe to the disease may prove futile.

ACNE VULGARIS AND ROSACEA: THE ANTIBIOTIC EFFECT

Acne vulgaris is the most common skin condition, and is marked by open and closed comedones, papules, pustules, and cysts. The condition is associated with androgen exposure and puberty. Numerous studies have confirmed that patients treated with antibiotics tend to experience acne remission; however, this response does not consistently correlate with changes in the microflora of the skin as determined by cultivation (e.g., *P. acnes* counts) (Holland *et al*, 1977; Thomsen *et al*, 1980; Al-Mishari, 1987; Till *et al*, 2000). Weeks to months of antibiotic therapy are required to induce a response. Many antibiotics from different classes have been successfully used in acne treatment, including tetracyclines, clindamycin, macrolides, and trimethoprim/sulfamethoxizole. Several mechanisms have been proposed to explain the salutary effects of these antibiotics, including antianabloic effects on sebaceous glands, antilipolytic effects on bacterial lipase, anti-inflammatory effects on host cells, and lastly antibiotic effects on bacteria.

Rosacea is another common dermatologic disorder. Rosacea tends to affect the face of middle aged and older people, and is marked by erythema, telangiectases, inflammation, and hyperplasia, particularly of the nose. This condition also responds to antibiotic treatment with many different antibiotics, including metronidazole, macrolides, clindamycin, and tetracycline.

The reason that antibiotics are effective in these skin diseases is not clear. Without convincing evidence of a specific microbe to blame, emphasis has been placed on host cell effects; however, the fact that many different classes of antibiotics are active in these diseases argues for an antibacterial mechanism. Molecular methods may provide a clearer picture of the bacteria present in these conditions, and how changes in bacterial representation correlate with antibiotic use.

CONCLUSIONS

We currently know more about the molecular microbiology of activated sludge from sewage treatment plants than we know about the molecular microbiology of the skin. Our knowledge of skin microflora in health and disease is likely biased by our reliance on cultivation. Molecular methods may provide new opportunities for understanding the microbial ecology of the skin, and for studying the role of novel microbes or microbial communities in disease pathogenesis. Dermatologists know that antibiotics are effective in many dermatologic conditions. It is time for microbiologists to explain why.

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