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Skin Microbiota in Atopic Dermatitis

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Abstract : In human body, the skin is the largest organ that has the function of mediating contact with the outside world and providing our body first line of defense against all kinds of pathogens, poisons and dangerous environments. The role of skin which are physical and immunological, supported by the microbial community that inhabits the skin. Skin microbiota contributes to barrier function by competing with pathogens and dealing with immune cells in the skin, to modulate local and systemic immune responses. Skin microbiota and immune mediators, for example complement system, have two-way interactions, and this shows that commensal microbes must be considered an important part of healthy skin. Many evidence shows that the composition of microbiota, especially in the intestines and also on the skin, can have a major influence on an individual's health. The influence of gut microbiota and its influence on the immune response has been widely studied, but the link of skin microbiota, immune response and certain skin diseases has not been widely discussed in the literature. Skin microbiota is expected to be affected in certain dermatological conditions, such as in psoriasis and in atopic dermatitis, which further shows the importance of the skin microbial community for human health. Understanding of skin microbiota role in pathogenesis of atopic dermatitis is still needed.
Key words : skin microbiota, atopic dermatitis.

Introduction

Skin microbiome is defined as all microorganisms, genomes, including environmental conditions on the skin. The definition of skin microbiota is all microorganisms that live in human skin.[5, 9, 10] Skin microbiome research refers to research on the global cutaneous microbial community, not just bacterial and fungal isolates. Skin microbiota consists of 2 groups, namely resident microorganisms, a group of microorganisms that are usually found on human skin and transient microorganisms, which are not permanent residents of the skin. Grice et al grouped skin microbiota into 4 phyla namely *Actinobacteria, Firmicutes, Proteobacteria*, and *Bacteriodes*[7].

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Microorganisms that live on the skin have been an interesting topic for dermatologists and microbiologists. Knowledge of microorganisms to date has been obtained through culture-based studies. Historically, *Staphylococcus epidermidis* and other *Staphylococcus* have been considered as primary bacterial colonies on the skin. Other microorganisms that are generally considered to be skin colonies include *Coryneform* from the phylum *Actinobacteria*(genera *Corynebacterium, Propionibacterium and Brevibacterium*) and the genus *Micrococcus*.[8]

Non-bacterial microorganisms have also been isolated from the skin. The most common fungal isolate species is *Malassezia spp*., Which is particularly prevalent in the sebaceous region. *Demodex* mites (such as *Demodexfolliculorum* and *Demodexbrevis*), which are microscopic arthropods, are also considered part of normal skin flora. *Demodex* mites eat sebum and are more common after puberty, and prefer to colonize the sebaceous regions of the face. *Demodex* mites can also eat epithelial cells lining the pilosebaceous units, or even other organisms (such as *Propionibacterium acnes*) that inhabit the same space. The role of commensal viruses has not been studied, and knowledge about viruses is still limited. The cross section of the skin and its microbiota is shown in figure 1.

Historically, culture-based approaches have become the standard for characterizing microbial diversity. It is now proven that only a small proportion of bacteria can develop in isolation. In addition, the environment of the hair follicles and sebaceous glands is anoxic, which is low in oxygen and can accommodate anaerobic microorganisms. Anaerobic isolation using a routine culture-based approach has limitations. These organisms often grow slowly and require special conditions for growth, transportation, and sample processing. The development of molecular techniques to identify and measure microbial organisms has revolutionized our view of the microbial world.[10]

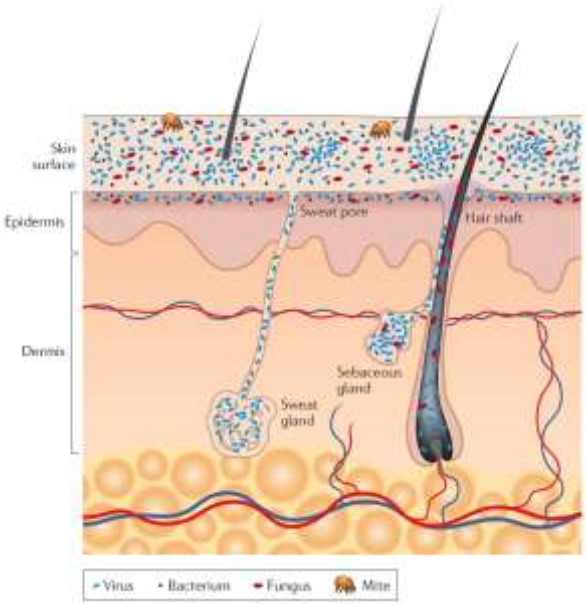

Figure 1. Histology cross section scheme of skin with microorganisms and adnexal tissue. (quoted from literature no. 10)

Characterization of bacterial genome diversity depends on analysis of the 16S ribosomal RNA gene sequence, which is present in all bacteria but not in eukaryotes. The 16S rRNA gene contains very specific species-specific regions, which allow taxonomic classification, and act as molecular markers for PCR. The advent of new technologies (such as pyrosequencing) has increased accessibility while reducing sequencing costs. So that an organism does not need to be bred to determine its type with 16S rRNA.[11]

Habitat

The physical and chemical features of the skin have their own unique set of microorganisms that are adapted to the location they inhabit. The microbiota distribution at its location is shown in Figure 2. In general,

skin is cold, acidic and dry, but different habitats are determined by skin thickness, folds and density of hair follicles and glands. Structurally, the epidermis is a formidable physical barrier, preventing penetration by microorganisms and toxins while maintaining moisture and nutrients in the body.[9-11] The upper layer of the epidermis, the stratum corneum consists of nucleated keratinocytes that have differentiated at the terminal stage, known as squama. Squama consists of keratin fibrils and cross-linking, layers of horn which are embedded in lipid bilayers, forming a 'brick and mortar' layer in the epidermis. [11] The skin is a self-renewing organ, where the squam continuously results from the surface of the skin at the final stage of terminal differentiation, after migration from the basal layer 4 weeks before[12].

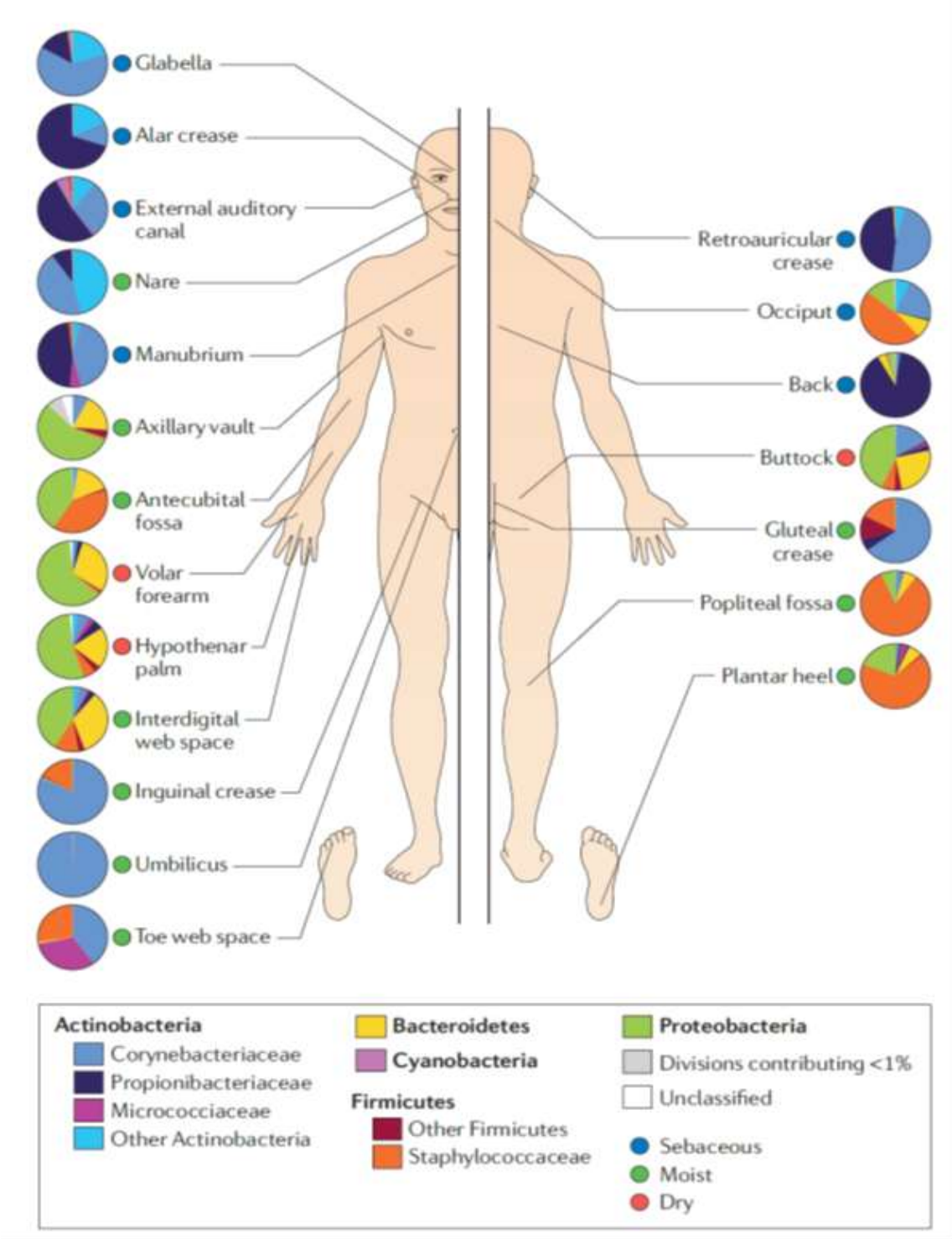

Figure 2.Distribution of skin microbiota to the body (quoted from literature no.11)

Skin surface varies topographically due to regional differences in skin anatomy. According to culture-based research, this region is known to have a different set of microorganisms. Some occluded skin areas such as the groin, armpits and toes, have high temperatures and humidity, which encourage the growth of microorganisms that thrive in humid conditions (for example, Gram-negative bacilli, *Coryneforms* and *S. aureus*). The density of the sebaceous glands is another factor that affects the skin's microbiota, depending on the region. Areas with high density of sebaceous glands, such as face, chest and back, encourage the growth of lipophilic microorganisms (eg Propionibacterium spp. And Malassezia spp.)[16]. The skin of the arms and legs is relatively dry and experiences fluctuations in surface temperature compared to other skin locations. These areas are found in fewer organisms quantitatively than moist areas on the surface of the skin using culture-based methods[17].

Invagination

Invagination of the skin and adnexa, including sweat glands (eccrine and apocrine), sebaceous glands and hair follicles, may be related to each other's unique set of microbiota[13]. The eccrine gland, which is more numerous than the apocrine glands, is found on almost all surfaces of the skin and continuously soaks the surface of the skin with its secretions, which are mostly composed of water and salt. The main role of eccrine sweat is thermoregulation through the release of latent heat from evaporation of water. Additional functions of the eccrine gland include water and electrolyte excretion, and acidification of the skin, which prevents the colonization and growth of microorganisms. The apocrine glands, which are located in the axilla (armpits), nipples and genitoanalarea will respond to adrenaline by producing thick secretions such as odorless milk. Apocrine secretions have long been thought to contain pheromones, which are molecules that trigger certain behaviors (for example, as sexual signals) in individual recipients[14]. The stereotypical odor associated with sweat originates from bacterial processing and the utilization of apocrine glandular secretions [15].

Sebaceous glands are anoxic and support the growth of anaerobic facultative bacteria, such as Propionibacterium Acne. This bacteria hydrolyzes triglycerides in the sebum, releasing free fatty acids in the skin, which will help the colonization of the sebaceous glands[16].

Skin Microbiota Molecular Analysis

Microbiome was analyzed by utilizing the universal presence of small subunit (16S) ribosomal RNA genes in all prokaryotes. The 16S rRNA gene contains regions that can facilitate PCR, whereas regions with high variability can be used for phylogenetic categorization. Users can search data in online databases, such as the ribosomal database project, which has hundreds of thousands of 16S rRNA gene sequence catalogs[18]. Thus, sequencing from 16SrRNA genes makes it easy to identify bacteria from the samples obtained. The technique for conducting this analysis is shown in the figure[3,19].

Metagenomic refers to genetic material in the microbial community and its aggregate function. If sequencing the 16s rRNA gene facilitates the phylogenetic categorization of the bacterial community, the metagenomic method performs sequencing of all genomic DNA randomly in a given sample. This sequence is then analyzed for phylogenetic (identification of microorganisms) and for functional activities so that the biological function of the whole community can be known. This approach has added human insight into health issues. However, metagenomic techniques require an adequate amount of DNA, so that low biomass (a small amount of DNA) obtained from skin samples currently limits the ability to do skin metagenomics[19].

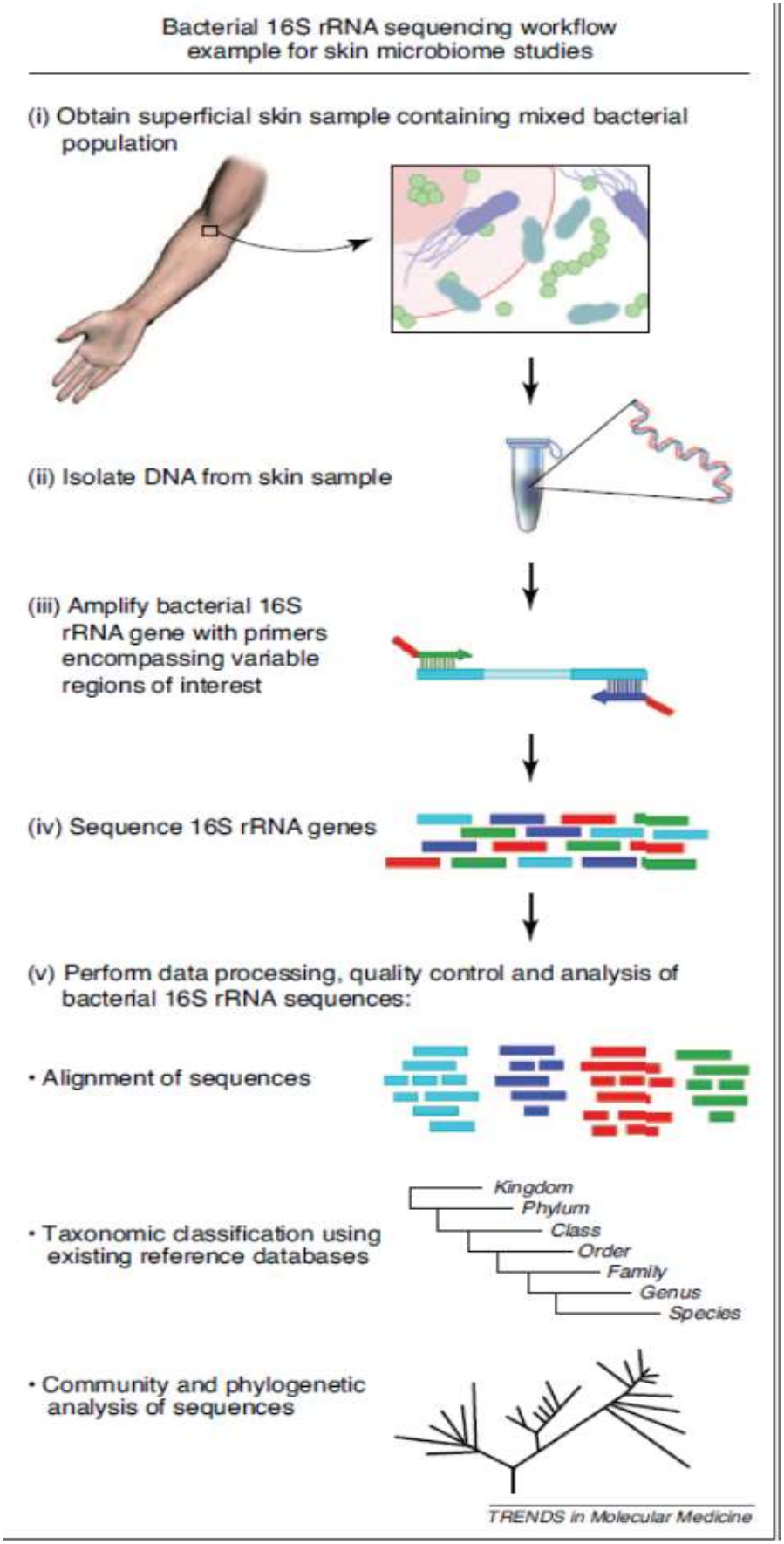

Image 3. 16RNA sequencing analysis technique (quoted from literature no 19)

The Role Of Skin Microbiota In Atopic Dermatitis

Atopic dermatitis (AD) is a chronic disease that affects 15% of children and 2% of adults, and is also associated with microbial colonization and infection. The prevalence of ADhas doubled or tripled in industrialized countries over the past three decades without a clear reason. This led to the hypothesis that fluctuating skin microbes modulate the interaction of genes and the environment on the surface of the skin, which results in an episodic exacerbation of AD[18].

The classic manifestation of AD, in the antecubital fossa and popliteal fossa, is a location that holds the same organism when compared to other body locations[20]. More than 90% of AD patients have more *S. aureus* in lesions and healthy skin, compared with <5% in healthy individuals. No specific relationship has been identified between virulence factors in S. aureus and flare-ups in patients with AD. However, in a mouse model with damaged skin barrier function (NC / Nga strains, which lack ceramid production), the application of *S. aureus* immunoglobulin G-binding protein A (also known as Staphylococcus protein A) along with agitating agents results in a similar lacquer to AD severe cases[21]. The relationship of skin microbiota and AD is shown in the figure 4.

Atopic Dermatitis is classically considered a childhood disease mediated by an imbalance of the T helper 2 (Th2) immune response, leading to an increase in IgE response to allergens. However, it is now recognized that the pathophysiology of AD is more complex than previously thought. AD is associated with defects in the epidermal barrier, by mutations inherited in keratinocyte proteins such as filaggrin that increase susceptibility to AD[22]. Another prominent feature of AD is the relationship between AD lesions and *S. aureus* colonization. Specifically, 90% of AD patients have excessive *S. aureus* in skin lesions while most healthy individuals do not store these bacteria on the skin[23]. Furthermore, an increase in *S. aureus* load in affected skin correlates with eruption in AD. Sequencing of 16S rRNA bacterial genes in skin samples of children with AD has revealed that the structure of the bacterial community, specifically the proportion of S. aureus, dramatically changes during DA eruption. The number of commensal bacteria of S. epidermidis skin also increased significantly during DA eruption. In addition, an increase in *Streptococcus, Propionibacterium, and Corynebacterium* species was also found after therapy. These findings indicate that increased colonization by *S. aureus* is a characteristic of skin lesions in AD and is associated with disease flares. However, whether the role of S. aureus is causative in AD is still unknown[24].

Early studies indicate that IgE is specific to *S. aureus* protein can be detected in the serum of ADpatients[25]. This study revealed that the presence of specific IgE antibodies against several *S. aureus* antigens was associated with severe skin manifestations in AD patients. Consistent with this observation, skin reactivity can be induced in sensitive AD patients with *S. aureus* extract. IgE Anti-S reactivity.aureus against various bacterial antigens is detected in patients with AD, but not in those who suffer from other atopic disorders including asthma, allergies and conjunctivitis. These studies show that AD patients show an immune response to S. aureus. However, the importance of the body's immune reactivity to bacteria remains unclear, and further studies are needed to understand why and how immune reactivity against S. aureus is selectively triggered in AD patients but not in other individuals whose skin also contains these bacteria[26].

Several AD mouse models have been developed to understand the pathogenesis of AD. Filaggrin-deficient mice (Flgft / ft), carrying mutations in the filaggrin gene, exhibit changes in skin barrier function and suffer from spontaneous dermatitis that resembles AD. Mutations in the Filaggrin gene in AD are strong predisposing factors for the development of AD in humans[27]. Bacterial entry into the epidermis is increased in the skin of ovalbumin-sensitive Flval mice and the amount of *S. aureus* that is highly correlated directly with increased expression of IL-4, IL -13, IL-22, TSLP and other cytokines associated with AD. This study explains the mechanism linking defects in the skin barrier and *S. aureus* colonization which causes anincrease in the production of inflammatory cytokines and AD exacerbations[28]. The Nc / Nga mouse is another mouse model in AD. Skin changes that are very similar to human skin that suffer from DA only develop when Nc / Nga mice are kept under conventional conditions, especially when the mouse skin is invaded by mites[29].

Several genetically engineered AD models of mice have been developed to study the pathogenesis of DA. These mutant mice include transgenic mice that overexpress IL-4, IL-31, TSLP and IL-18. IL-4 transgenic mouse skin specific keratinocytes are spontaneously colonized by *S. aureus*. Genetic deficiency ADAM17, a transmembrane metalloproteinase, is associated with AD in humans. Mice lacking ADAM17 exhibit skin

barrier dysfunction and show a dysbiosis characterized by decreased bacterial diversity and increased skin colonization by bacteria including *Corynebacteriummastitidis, C. bovis and S. aureus* during the onset of dermatitis. Treatment with antibiotics greatly reduces dysbiosis and skin inflammation. Collectively, these studies show that some AD model animals also have a characteristic skin colonization by *S. aureus*, which shows similarities to human AD. However, the origin and role of S. aureus, a human bacterium, in a mouse model with allergic skin disease needs further investigation[30].

Virulence of *S. aureus* is defined by many factors. One of them includes phenol-soluble modulins (PSM), which is a peptide that is regulated by the *S. aureus* accessory gene regulatory (Agr) virulence system. PSM peptides form amphipathic a-helical which are capable of forming pores on artificial membranes and are highly cytotoxic for various cells including PSM keratinocytes also contribute to the development of biofilms, an activity that may be important for staphylococcal colonization[31].

Quorum sensing (QS) in bacteria, including *S. aureus* regulates gene expression in response to variations in cell population density. Cell-to-cell communication through inducing molecules occurs within and between bacterial species as a strategy for bacteria to estimate the density of other cells and regulate gene expression, thereby maintaining the balance of the entire bacterial community. In *Staphylococci,* PSM is encoded at three different locations in the genome and is strictly regulated by QS through the Agr system. lociAgr produces RNAII andRNAIII transcripts. RNAII is produced through agrBDCA, which encodes the factors needed for the synthesis of automatic induction peptides (AIP) and the agr-regulation cascade. AIP, a peptide pheromone, is translated from agrD while AgrB transports AIP to the extracellular space allowing binding to the AgrC extracellular domain (Fig. 4*)*.

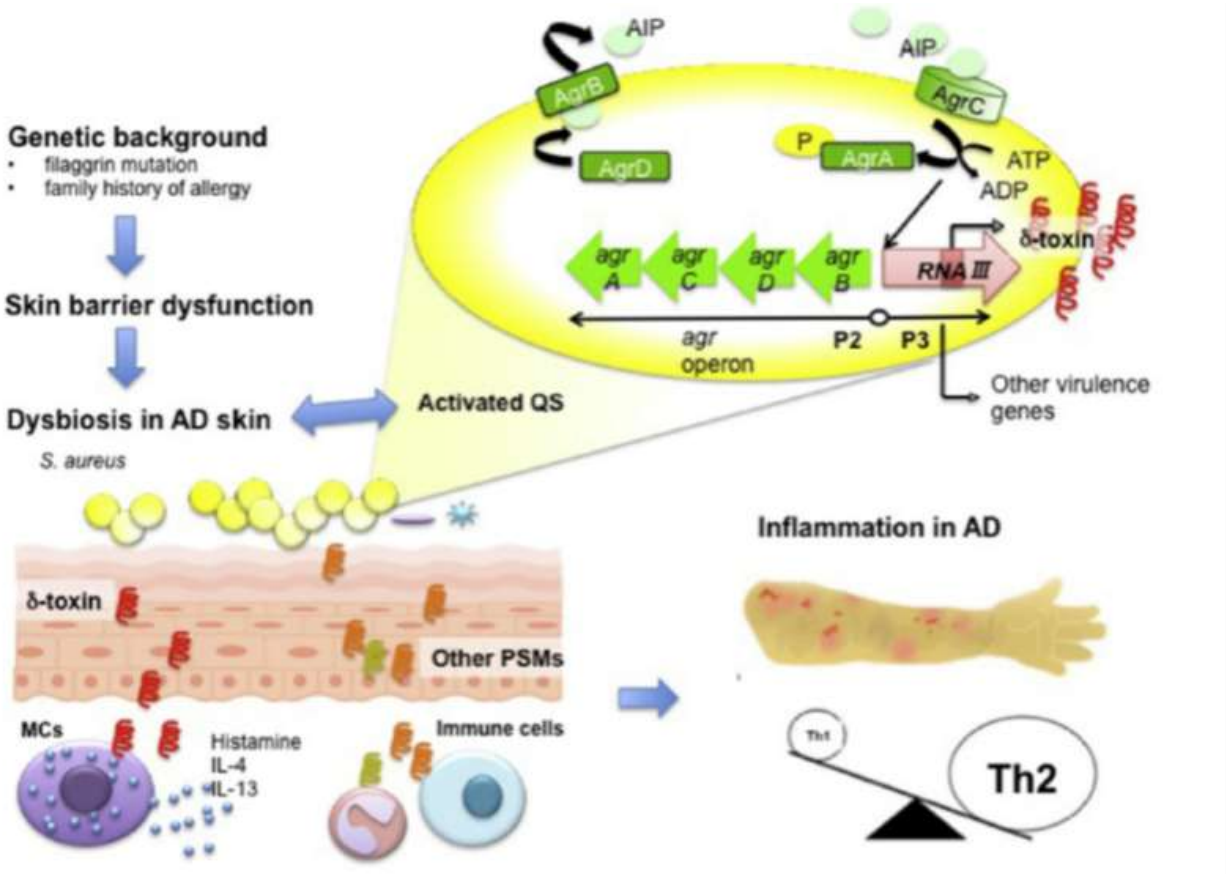

Figure 4. Pathogenesis of skin microbiota causes exacerbation of AD (quoted from literature no. 21)

AgrA and AgrC form a two-component signal transduction system that regulates signaling including RNAIII production, which regulates virulence factors and also contains the hld gene sequence that produces d-toxin. This arrangement is important for the time of expression of virulence factors during infection and the development of acute disease, while low Agr activity is associated with chronic staphylococcal infections such as those involving biofilm formation. Although additional clinical and basic studies are needed to determine the role of S. aureus in AD, strategies to inhibit the QS of *S. aureus* may be beneficial for the treatment of AD[29,31].

All mouse models with skin infections by *S. aureus* depend on subepidermal inoculation after physical disruption in the epidermis. However, recently, a follow-up model of *S. aureus* colonization without physical disruption in the epidermis triggers strong inflammation without epidermal disruption. Using this newly developed *S. aureus* model, in skin inflammation that causes the vir virulence, it was found that d-toxin, a PSM peptide also known as PSM g, induces mast cells (MC) degranulation of membrane-bound cytosol granules, including histamine, IL-4 and IL-13, which trigger the release of many molecules that are important in triggering Th2-type skin inflammation, including IgE production. Furthermore, S. aureus isolates obtained from skin lesions in AD patients produce high levels of d-toxin. This discovery not only provides an understanding of the relationship between S. aureus skin colonization and AD pathogenesis, but also identifies potential *S. aureus* colonies that trigger Th2 type skin inflammation, which is a hallmark of AD[28,31].

Conclusion

Under normal conditions, human skin can accommodate commensal microbes that have the potential to prevent dangerous external invasion. Disruption of this balance will result in a change in the composition of the skin microbiota, disrupt the immune response and can lead to an inflammatory disease as occurs in atopic dermatitis.

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