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Interactions between immune system and the microbiome of skin, blood and gut in pathogenesis of rosacea

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ABSTRACT

The increasingly wide use of next-generation sequencing technologies has revolutionised our knowledge of microbial environments associated with human skin, gastrointestinal tract and blood. The collective set of microorganisms influences metabolic processes, affects immune responses, and so directly or indirectly modulates disease. Rosacea is a skin condition of abnormal inflammation and vascular dysfunction, and its progression is affected by *Demodex* mites on the skin surface. When looking into the effects influencing development of rosacea, it is not only the skin microbiome change that needs to be considered. Changes in the intestinal microbiome and their circulating metabolites, as well as changes in the blood microbiome also affect the progression of rosacea. Recent research has confirmed the increased presence of bacterial genera like *Acidaminococcus* and *Megasphaera* in the intestinal microbiome and *Rheinheimera* and *Sphingobium* in the blood microbiome of rosacea patients. In this review we discuss our current knowledge of the interactions between the immune system and the skin, gut and blood microbiome, with particular attention to rosacea diagnostic opportunities.

KEYWORDS

blood microbiome, gut microbiome, immune system, rosacea, skin microbiome

INTRODUCTION

Rosacea is a chronic inflammatory skin disease that primarily affects women over 30 years of age [1]. The prevalence of rosacea is around 5% in the adult population worldwide [2]. The disease progresses in episodes. Diagnostic criteria of rosacea include primary features, such as transient or persistent facial erythema, telangiectasias, papules and pustules (usually in centrofacial distribution) and secondary features, such as a stinging or burning of the skin, oedema, tarsal conjunctivitis, ocular dryness and rhinophyma. Rosacea can be classified into four basic stages: erythemato-telangiectatic, papulopustular, phymatous, and ocular rosacea, with the less prevalent form of granulomatous rosacea [3]. Possible pathological mechanisms responsible for rosacea include dysregulation of the innate and possibly the acquired immune system [4], disrupted neurovascular signal transmission [5], degeneration of connective tissue elements, nutritional and chemical factors, UV and sun exposure, functional disorders of the pilosebaceous unit and infective microbes [6].

The highly sensitive and complex interaction between the immune conditions and the intestinal, blood or cutaneous microbes possibly influences the progression and therapeutic options of rosacea.

Dysregulation of innate and acquired immune system

The intact skin surface as a physical barrier is important for the integrity of the innate immune system. The sensitive skin of rosacea patients results in decreased tolerance to commonly used topical agents. The increase of trans-epidermal water loss, or the directly caused breaches by *Demodex* mites lead to disturbances in the epidermal barrier which represent the major contributing factor in the pathogenesis of rosacea [7].

The cells of the innate immune system in the skin express pattern recognition receptors (PRRs) that recognize damage-associated or pathogen-associated molecular patterns. Among the PRRs, toll-like receptor 2 (TLR-2) expression is highly increased in rosacea patients in contrast to healthy controls or psoriatic patients [8]. TLR-2 is activated by external stimuli such as the chitin of *Demodex* mites or bacterial products. Activation of TLR-2 triggers keratinocytes to produce proinflammatory cytokines and chemokines including IL-8, IL-1 β , and TNF- α . Elevated levels of cytokines and chemokines lead to chemotaxis of neutrophils into the skin and vascular hyper-reactivity [9]. Additionally, TLR-2 is known to increase the expression of serine protease KLK-5.

Cathelicidin is an antimicrobial protein (AMP) that can be found in neutrophils, mast cells, macrophages and monocytes and in lamellar bodies of keratinocytes [10]. KLK-5 serine protease together with proteinase-3 are responsible for cleaving inactive cathelicidin into the active

peptide form LL-37. In contrast to healthy people, rosacea patients have an increased baseline expression of cathelicidin and KLK-5 [11, 12]. LL-37 works against bacteria, fungi and parasites. In addition to its antimicrobial activity, LL-37 modulates neutrophil chemotaxis, and stimulates cytokine and chemokine release from mast cells [13]. The degranulation of mast cells leads to the exhibition of clinical features of rosacea, such as telangiectasia, erythema, and inflammation [14]. Moreover, LL-37 affects angiogenesis through enhancing the proliferation of endothelial cells and promotes expression of extracellular matrix components [15].

The adaptive immunity likewise exhibits dysregulated functions in rosacea patients. Inflammatory infiltration in rosacea leads to an increase in the amounts of Th1 and Th17 cells. The cytokine production of Th17 induces angiogenesis and also affects the elevated expression of LL-37 [4].

Approximately 10–20% of inflammatory cell infiltration in rosacea is composed of CD20+ B cells [16]. B cells through the production of fibrogenic cytokines such as IL-6 and TGF- β via TLR can stimulate fibrotic changes in the skin as it was shown in phymatous rosacea [17] (Fig. 1).

The role of microbes in the pathogenesis of rosacea

Novel microbiological diagnostic methods unearthed the association between certain microbes and rosacea disease. The simplest microscopic method, methylene blue stained adhesive tape preparation from rosacea skin surface clearly shows the accumulated amount of *Demodex* mites (Fig. 2.).

Fig. 1. Interaction between microorganisms and immunoreactions in rosacea affected skin

Fig. 2. Microscopical picture of *Demodex folliculorum*, methyleneblue staining

The correlation between the presence of mites and rosacea were described in 1932 [18].

Using traditional aerobic culture methods, *Staphylococcus epidermidis* was frequently isolated from folliculi of biopsy samples of rosacea patients. As *S. epidermidis* is one of the most common bacterial species of the normal skin flora, its pathogenic role in rosacea is questionable. Because the facial skin is warmer in rosacea patients than in healthy individuals and *S. epidermidis* strains cultured at higher temperature secrete different proteins in different quantities, the pathogenic role of these proteins can likely be confirmed [19].

A passive transfer of additional micro-organisms from *Demodex folliculorum* was speculated after *Bacillus oleronius*, a Gram-negative rod-shaped bacterium had been successfully isolated from the mite obtained from a papulopustular rosacea patient [20]. Patients with erythematoteleangiectatic rosacea show serum reactivity to the 62- and 83-kDa proteins of *B. oleronius*, suggesting that these highly immunogenic proteins may play a role in the induction of this condition [21]. Exposure of neutrophils to proteins from *B. oleronius* resulted in degranulation and the production of inflammatory cytokines [22, 23]. The increased temperature of the inflamed skin of rosacea patients alters the growth and increases the 62-kDa protein production of *B. oleronius* [24] and thus, continuously re-stimulates the inflammatory response.

Helicobacter pylori infection was suggested to play a role in the aetiopathogenesis of rosacea. However, the relationship is debated arguments ranging from coincidental, plausible or undecided, depending upon the examination method and the patient population in which the presence of the bacterium was tested. Using C [14]-urea-breath test as diagnostic tool the colonization rate of *H. pylori* was significantly higher in rosacea patients than in the control group [25, 26]. Several reports showed seropositivity to *H. pylori* in rosacea individuals [27–29], although this claim is not universally accepted [30–32]. *H. pylori* produces ROS

(reactive oxygen species) including NO (nitric oxide), and rosacea patients showed higher ROS in plasma than controls [33, 34]. NO is important in a number of different physiological processes in the skin, including vasodilatation, inflammation, and immune modulation. It was postulated that NO produced by *H. pylori* might cause the flushing and erythema associated with rosacea, or that it may have a pathogenic role in the inflammation seen in rosacea [35]. Furthermore, *H. pylori* can express *cagA* cytotoxin which induces express of TNF- α and IL-8, and subsequently a series of inflammatory reactions [36].

New perspectives with New Generation Sequencing

DNA sequencing studies have expanded our insights and understanding of the ecological and functional characteristics of the microbiome associated with different sites of the human body. It is estimated that humans harbour approximately 100 trillion microorganisms belonging to various domains, including viruses, bacteria, archaea, fungi, and protozoans [37]. Most of our knowledge of microbial communities in areas of the human body was obtained through non-culture-based testing of bacterial DNA. The bacterial 16S rRNA gene region consists of hypervariable and conserved loci allowing the construction of universal primers for bacterial detection and identification [38]. In addition to traditional detection methods, this new generation sequencing method has highlighted the importance of some bacterial genera from the skin, gut and blood microbes in the pathogenesis of rosacea.

Relationship between skin microbiome composition and rosacea

The three different skin microenvironments - dry, moist and sebaceous - and their associated microbiomes have a characteristic topography in each individual [39]. In these three skin microenvironments three bacterial phyla - *Firmicutes*, *Actinobacteria* and *Proteobacteria* - represent more than 90% of all the resident taxa [40]. The bacterial species alpha diversity is widest in dry and narrowest in sebaceous skin environments [41]. In healthy individuals *Staphylococcus* and *Corynebacterium* genera preferring high humidity occur more frequently in moist areas, whereas lipophilic *Propionibacterium* genus tends to colonize sebaceous areas [42].

Skin disorders may be influenced by microbiome associated with different microenvironments. Symptoms of acne and rosacea occur in sebaceous areas, body odour and atopic dermatitis in moist areas and psoriasis in dry microenvironments [39].

While acne and rosacea may display similar clinical characteristics in sebaceous areas, the clinical courses of each condition are quite distinct. Suspected by Thompson and co-workers, differences in the skin microbiota may be related to different pathophysiological courses of acne and rosacea. In the case of microbiome analysis the distribution of bacterial taxa showed that *Proteobacteria* was the most abundant phylum in acne patients, whereas *Actinobacteria* was the most abundant phylum in rosacea patients.

According to species-level comparison *Serratia marcescens* and *Cutibacterium acnes* (formerly *Propionibacterium acnes*) were each present in significantly higher mean relative abundance in rosacea patients compared to acne patients [43].

In erythematotelangiectatic rosacea and papulopustular rosacea increased density of *Demodex* mites on the skin has long been observed. This mite utilizes sebum as a food source and other nutritional sources may be cellular debris or bacteria such as *Cutibacterium acnes* that reside in the pilosebaceous unit [39]. *Demodex* mites and the associated *Bacillus oleronius* bacteria trigger inflammatory pathways in rosacea patients [23].

Zaidi and co-workers tested the correlation between bacterial species relative percentage abundance (RPA) and the severity of rosacea using the score established by the National Rosacea Society (NRS). A significant ($P < 0.05$) correlation was found between NRS score and the RPA of 9 genera: *Gordonia*, *Janibacter*, *Jeotgalicoccus*, *Tepidimonas*, *Dietzia*, *Wautersiella*, *Mycobacterium*, *Geobacillus* and *Weissella*. Multivariate random effect Poisson regression (REPR) analyses demonstrated that a 10% increase in RPA of *Gordonia* or a 10% decrease in RPA of *Geobacillus* significantly increase the NRS score [44].

Rainer and co-workers compared the skin microbiome of rosacea-affected skin and healthy skin in a study based on 19 rosacea patients. *Campylobacter ureolyticus*, *Corynebacterium kroppenstedtii* and *Prevotella intermedia* were found more common in patients with a mixed papulopustular-erythema-allergic form of rosacea [45].

Relationship between gut microbiome composition and rosacea

A link between rosacea and gastrointestinal diseases – such as gastroesophageal reflux, irritable bowel syndrome or small bowel bacterial overgrowth (SIBO) – have been demonstrated mainly by population based studies [46–49]. In a prospective study with a three-year follow-up of Agnoletti et al., SIBO significantly prevailed in papulopustular rosacea, while *H. pylori* infection in erythrosis [50]. The association is also confirmed by studies that show an improvement in rosacea in connection with SIBO antibacterial therapy [51, 52].

Using the NGS method, only a few studies have so far examined the relationship between intestinal microbiome composition and rosacea. Chen and co-workers have observed significant reduction of faecal microbial richness in rosacea patients, with increased abundance of *Rhodochlamydia*, *Bifidobacterium*, *Sarcina*, and *Ruminococcus* genera. In this study the discriminating reduced abundant genera were *Lactobacillus*, *Megasphaerae*, *Acidaminococcus*, *Haemophilus*, *Roseburia* and *Clostridium* [53]. In contrast the study of Nam et al. described an increased incidence of *Lactobacillus*, *Megasphaerae* and *Acidaminococcus* genera in rosacea patients [54]. The altered microbiome also influences the quantitative change of bacterial metabolic products. Chen and co-workers speculated that faecal microbial composition might be related to sulfur metabolism, cobalamin transport, and carbohydrate transport [53]. The

elevated hydrogen sulfide (H_2S) concentration in blood might shift the thiol/thiol disulfide balance toward thiol disulfide excess, resulting in a less effective protection against oxidative stress in rosacea patients. Sener and co-workers confirmed that the mean disulfide/native thiol ratio is significantly higher in rosacea patients than in controls [55]. The induction of rosacea symptoms is also known as an adverse side effect of B12 (cobalamin) therapy [56]. Not only overdosed therapy, but also altered intestinal microbiome can develop this detrimentally elevated cobalamin level.

Relationship between blood microbiome composition and rosacea

The circulation is a closed system and the blood in healthy organisms was first believed to be a sterile environment. Recent studies have detected microbial DNA in the blood of healthy humans, but it is still questioned whether they were derived from living or dead microbes or they simply contaminated the samples [57–59]. Additional studies have described significant differences in the blood microbiome of healthy individuals and individuals with various diseases including diabetes mellitus, schizophrenia, asthma or acute pancreatitis [60–63].

Only a limited number of studies of blood associated microbiome in dermatologic diseases like hidradenitis suppurativa [64], psoriasis [65] or rosacea [66] have been conducted. Faith's phylogenetic alpha diversity analysis showed a marginally significant difference between the blood microbiome of rosacea vs control patients. The relative abundance of *Chromatiaceae* and *Fusobacteriaceae* family was significantly elevated in the blood of rosacea patients. At the genus level 14 bacterial taxa had different abundance between the rosacea and healthy patients. The *Rheinheimera* genus differed increasingly across groups with significantly elevated abundance in rosacea patients [66].

Rheinheimera is a genus of Gram-negative aerobic bacteria. The occurrence of various species of the genus *Rheinheimera* has also been detected in marine and freshwater, industrial and urban wastewaters, plants and soil [67–71]. The exact pathophysiology of these bacteria in humans has not yet been fully clarified [72]. As Gram-negative bacteria, *Rheinheimera* contain lipopolysaccharides in their outer membrane, which may be involved in the pathogenesis of chronic inflammation in rosacea through the activation of TLR.

CONCLUSION

Rosacea is a chronic relapsing skin condition which can be controlled but does not have a traditional 'cure'. Among many other options (avoiding provocation factors, sun protection, vasoconstrictive topical care, azelaic acid, ivermectin, isotretinoin, laser or surgical measures), topical and oral antibiotic treatment are significant parts of anti-rosacea therapy. At this point, more information about the relationship between the triggering altered microbiome and the immune function involved in inflammation is needed.

Accurate assessment of the protective and harmful microbiome composition is essential for targeted antibiotic or probiotic use in rosacea therapy.

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