

## REVIEW ARTICLE

# The microbiome and atopic eczema: More than skin deep

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### ABSTRACT

Discoveries in the defective molecular composition of the epidermal barrier, such as the epidermal protein filaggrin, in those with atopic eczema (or atopic dermatitis [AD]) have proved crucial in understanding this disease, but its aetiology remains to be fully elucidated. The epidermal barrier is just one interface between the microbial world and our immune system. Recent advances in molecular technology have demonstrated for the first time the true scale of the normal human microbiome and changes seen in disease states. In this review article we discuss the role of the human microbiome in the aetiology and maintenance of AD. The role of *Staphylococcus aureus* within the skin microbiome is examined, in addition to the role of other bacteria and fungi, identified using novel culture-independent methods. The significant contribution of the gut microbiome and its manipulation via probiotic use is also reviewed. We emphasise that the microbiome of separate systems, including the gut, has a significant role to play in the manifestation of this cutaneous disorder. To date, there has been a lack of studies investigating whether changes to the lung microbiome may play a role in AD. An early interaction between the microbiome and immune system via multiple routes (skin–gut–lung) could feasibly affect the risk of a subsequent development of atopic diseases. When making management decisions for AD patients, clinicians must be mindful of the role of the microbiome.

**Key words:** 16s rRNA, atopic dermatitis, atopic eczema, atopy, gut, hygiene hypothesis, lung, microbiome, probiotic, *Staphylococcus aureus*.

### INTRODUCTION

Atopic eczema (or atopic dermatitis; AD) is often the first manifestation of the ‘atopic march’, which refers to the typical natural history of the atopic disorder. AD is often followed by allergic rhinitis and subsequently asthma, though conditions may overlap.<sup>1</sup> The prevalence of AD is estimated at 20% in developed countries.<sup>2</sup> As intriguing as this high overall figure is the increase in its prevalence over recent years, particularly in developing nations,<sup>2</sup> together with the many mechanisms purported to underlie this explosion of atopic disease. While genetic factors are clearly implicated; for example, monozygotic twins have a higher concordance for atopic disorders than dizygotic twins,<sup>3</sup> the changing environment (including changes in diet and subsequent obesity, urbanisation, exposures to chemical agents, the use of antibiotics, improved sanitary conditions and the absence of helminth parasites) in westernised nations is considered contributory.<sup>2</sup>

Initial hypotheses described AD as an inside-outside disease, with the clinical picture stemming from an intrinsic immune system abnormality.<sup>4</sup> An exaggerated pro-inflammatory type 2 helper T-cell pathway (Th-2) response to external antigens, characterised by the production of interleukins (IL) IL-4, IL-5 and IL-13, has frequently been found in AD. Dupilumab, a human monoclonal antibody that blocks an IL-4 receptor subunit and inhibits IL-4 and IL-13, has demonstrated efficacy in patients with moderate to severe AD.<sup>5</sup> The discovery of a genetic basis for epithelial barrier dysfunction was a significant breakthrough. Loss of function variant mutations encoding the epidermal barrier protein filaggrin (*FLG* R501X and 2282del4) have been seen in individuals with AD.<sup>6</sup> Persistent antigen entry through an impaired skin barrier could prime the Th-2 response classically seen in AD. A defective skin and antimicrobial barrier provides a feasible route for pathogenic and non-pathogenic organisms to contact and influence

### Abbreviations:

|      |                          |
|------|--------------------------|
| AD   | atopic dermatitis        |
| Ig   | immunoglobulin           |
| IL   | interleukin              |
| PID  | primary immunodeficiency |
| Th-2 | type 2 helper T-cell     |
| Treg | regulatory T-cell        |

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the immune system. In a mouse model, epicutaneous exposure to a protein antigen led to a Th-2 like response and sustained raised specific immunoglobulin (Ig)E levels.<sup>7</sup> Moreover, IL-4 and IL-13 have been shown to reduce filaggrin expression during keratinocyte differentiation,<sup>8</sup> demonstrating a possible route of acquired filaggrin disruption secondary to local inflammation.

The hygiene hypothesis suggested that early childhood infection might prevent the development of atopic disease, stemming from the finding that allergic rhinitis was less common in children with several older siblings.<sup>9</sup> The notion that exposure to microbes during a critical period in the development of the immune system affects future immune system functioning has since been distilled. Rather than particular viral or bacterial infections being protective against atopy, it is now thought that overall exposure to the microbial community may shape the development and regulation of the immune system in health.<sup>10</sup> Not only could a defective skin barrier in AD give rise to susceptibility to infections, but it could facilitate microbial contact with the immune system. Much recent interest has focused upon the role of the skin microbiome in health and disease, giving rise to numerous insightful reviews.<sup>11–15</sup> Here we present the current evidence on the role of the human microbiome in the aetiology and maintenance of AD.

## THE HUMAN MICROBIOME

The human microbiome consists of the global array of microorganisms and their genetic material living on and in an individual. The full human microbiome has become accessible only relatively recently, through the use of novel molecular techniques for taxonomic classification. 16S rRNA gene-based analysis exploits the universal presence of the 16S ribosomal RNA gene in bacteria. The conserved region of the 16S rRNA gene allows amplification by polymerase chain reaction primer binding and the variable regions can then be analysed to identify organisms by comparison with reference databases. The Human Microbiome Project has exploited such methods to identify and characterise microorganisms living on and in the healthy human body though the detection of genetic material across five major body areas, including the skin and the gut.<sup>16</sup>

### The normal skin microbiome

The skin microbiome in health needs to be understood to appreciate changes in disease. Early attempts to identify organisms employing culture-based methods gave rise to a selection bias, identifying only those organisms able to thrive in standard lab conditions,<sup>16</sup> and vastly underestimated the true scale and diversity of the microbiome.

The density of microorganisms in the skin has been found to be up to  $10^7$  per  $\text{cm}^2$ .<sup>18</sup> 16S rRNA gene-based analysis has noted that the skin is a significantly diverse microbial habitat. Grice and colleagues<sup>19</sup> detected 19 different bacterial phyla on normal human skin, most of which fall into the following phyla: Actinobacteria, Firmicutes, Proteobacteria and Bacteroidetes. Fierer and

colleagues largely corroborated this work, detecting on the palms of individuals over 150 operational taxonomic units that were assigned taxonomy to the species level. Of these organisms, 94% were from the phyla Actinobacteria, Firmicutes and Proteobacteria.<sup>20</sup> At a genus level the most abundant organisms were *Propionibacteria* (32%), *Streptococcus* (17%), *Staphylococcus* (8%), *Corynebacterium* (4%) and *Lactobacillus* (3%). This method also detected a number of novel species inhabiting the skin microbiome, such as *Dietzia maris*<sup>21</sup> (Fig. 1).

Skin fungal diversity is also high. Using molecular methods analogous to those used to identify bacteria (using different conserved regions: 18S rRNA gene and internal transcribed spacer region), Findley and colleagues<sup>22</sup> identified over 150 fungal operational taxonomic units from healthy adult skin. This included over 60 from the genus *Malassezia*, which were the dominant organism at varied body sites (including species *M. globosa*, *M. restricta* and *M. sympodialis*). In addition to *Penicillium* and *Aspergillus* species *Candida* was present but at considerably lower levels.<sup>22</sup> 16S rRNA gene-based analysis was performed alongside this method to assess the interplay between fungal and bacterial commensals. Patterns varied by site, arms showed higher bacterial diversity and lower fungal diversity while the feet showed the opposite pattern.<sup>22</sup>

Skin microorganisms are seen to vary between and within individuals over space and time. The skin microbiome is established soon after birth, with the birth method strongly affecting composition – those born by vaginal delivery are colonised by organisms from their mother's vagina (such as *Lactobacilli*), but those delivered by Caesarean section are colonised by those from their mother's skin, such as *Staphylococci*.<sup>23</sup> Sebaceous sites are dominated by *Staphylococci* and *Propionibacteria* while *Corynebacteria* are more prevalent in moist sites such as the antecubital fossa and interdigital spaces.<sup>19</sup> Moreover, longitudinal skin sampling suggests variability to be site dependent – with the more stable colonisers being found in sebaceous sites.<sup>19</sup>

## THE SKIN MICROBIOME IN AD

### *S. aureus*

Early culture studies linked the presence of a single organism, *S. aureus* to AD.<sup>24</sup> The abundance of *S. aureus* is greater in lesional than in non-lesional skin in AD,<sup>25</sup> in a dose-dependent manner – with higher colonisation rates in severe eczema.<sup>25</sup> However, observational studies detecting *S. aureus* on the skin of individuals with AD cannot distinguish cause and effect.

Treatment with clinical improvement is associated with a reduction in the abundance of *S. aureus*. Twice-weekly dilute sodium hypochlorite solution (bleach baths) combined with nasal mupirocin in children with AD led to marked improvement in eczema area and severity index scores 3 months later.<sup>26</sup> Moreover, improvements were localised to areas that were in contact with the bleach bath – head and neck scores did not improve.<sup>26</sup> Topical steroids alone produce a therapeutic effect equivalent to the



**Figure 1** Classification of organisms of interest from the skin microbiome in health and atopic eczema.

combination of topical mupirocin and topical steroids,<sup>25</sup> even if the microbiome is central to the pathophysiology of AD, and antibiotics may not be the most appropriate treatment.

Strain-specific diversity is seen. However, many studies have not characterised organisms to this level. In AD compared with controls, *S. aureus* strains are more likely to produce toxins. In one study 62% of lesional skin samples produced at least one identifiable exotoxin.<sup>27</sup> Exotoxins from *S. aureus* strains isolated from AD patients often have superantigenic properties and stimulate T-lymphocytes directly. Superantigens (*S. enterotoxin B*) have been shown to trigger the proliferation of CD4+ and CD8+ T-cells and the subsequent production of IL-5 and IL-13.<sup>28</sup>

### Other organisms

*S. aureus* is by no means the only importantly player in AD. In Japan, *M. restricta* and *M. globosa* were detected in more than 90% of patients with AD and a new species – *M. dermatis* was also isolated in lesser quantities<sup>29</sup>; the contribution of fungal organisms must be considered.

Using molecular technology, *Stenotrophomonas maltophilia* was more commonly detected on the facial skin of individuals with AD than on that of controls.<sup>21</sup> In contrast, common commensals *P. acnes* and coagulase negative staphylococci were not isolated from a number of individu-

als with AD. This suggests that the absence of specific microorganisms may be just as important as the presence of others.

The skin microbiome of patients with primary immunodeficiencies (PID) characterised by AD-like skin (hyper-IgE syndrome and Wiskott–Aldrich syndrome) were compared with that of true AD and normal controls. A high frequency of *S. aureus* was found in the antecubital fossae of the AD group, while in patients in the PID group, *S. epidermidis* and *S. haemolyticus* were more abundant.<sup>50</sup> Among immunodeficient patients, *P. acnes* abundance was negatively correlated with disease severity, while the opposite was true for *S. aureus*.<sup>50</sup>

*S. epidermidis* is abundant on the skin and the nature of its interaction with *S. aureus* has been debated. Particular strains of *S. epidermidis* appear to inhibit the formation of *S. aureus* biofilm.<sup>51</sup> The mechanism of inhibition of *S. aureus* is unclear. Some suggest inhibition occurs via the upregulation of antimicrobial peptides produced by keratinocytes,<sup>52</sup> while others suggest that the secretion of the serum protease Esp is critical in the inhibition of *S. aureus*.<sup>51</sup>

### Changes in the skin microbiome during flares

Shannon diversity, a measure that considers both the total number of organisms of a particular species and their

relative abundance, is reduced in the antecubital fossae of AD patients with an untreated flare.<sup>55</sup> However, there was no difference between Shannon diversity in the antecubital fossae of patients with treated flares, controls and non-flare AD patients. Moreover, *Staphylococcal* spp. dominated areas of high disease predilection in AD patients with flares, compared with controls and AD patients without flares. Reduced Shannon diversity correlated with the abundance of *S. aureus*, highlighting the organism's importance in this cohort<sup>55</sup> and its interplay with other microorganisms. To date, no published study has assessed changes in fungal diversity in flares.

### MORE THAN SKIN DEEP: THE GUT MICROBIOME IN AD

When considering AD it is tempting to focus on the skin as the site of primary pathology but immunological changes are more than skin deep. Various geographically separate surfaces (such as the skin, the gut and the lungs) could act as a shared immunological interface. Stimulatory antigens crossing any barrier could give rise to the same end-point – learned immune tolerance or inappropriate immune stimulation.

#### Normal gut microbiome

The gut microbiome harbours the largest pool of foreign antigens, with 100 trillion resident bacteria<sup>54</sup> Development of tolerance to external antigens mainly occurs via the gut. Gut colonisation by beneficial organisms could confer protection against atopy, with repeated early exposure to particular groups of bacterial antigens enhancing immune regulation and development.

The gut microbiome in individuals from the developed and developing world show marked differences. Using 16S rRNA gene-based analysis technology, Firmicutes and Proteobacteria were found to be more abundant in the stool of African children from Burkina Faso, whereas a higher abundance of organisms from the phyla Actinobacteria and Bacteroidetes was seen in European children.<sup>55</sup> Numerous specific organisms were found exclusively in the African children and Shannon diversity was higher in that population.<sup>55</sup> The authors suggest the differences in diet in the two populations shape these differences, but other variables between the populations, such as sanitation and hygiene, may play a role. Though this study cannot provide a causal link, some have suggested that such fundamental differences in the microbiome composition can explain the differential incidence of atopic disease in developed and developing countries.

#### The gut microbiome in atopic disease

Numerous studies have confirmed the differences in gut microbiota between those with atopic diseases and controls (Fig. 2). In a case-control study, children and young adults with AD had significantly lower gut concentrations of *Bifidobacteria* than controls (measured by stool sample

analysis), and the concentration was inversely correlated with AD disease severity.<sup>56</sup>

Further work suggests that differences in gut microflora precede the development of atopic diseases (though a causal relationship cannot be directly inferred from this). Atopic infants (based on positive skin prick testing at 12 months) have higher numbers of faecal *Clostridium* and lower numbers of *Bifidobacteria* in their stools at the age of 3 weeks.<sup>57</sup> Early gut colonisation with *Clostridium difficile* was also associated with AD development in other studies.<sup>58</sup> Ismail and colleagues<sup>59</sup> demonstrated that diverse gut microbiota in infants 1 week old was associated with a decreased risk of AD development (but not with other atopic diseases) in those at high risk of atopic disease. Low total gut and Bacteroidetes diversity at 1 month has been associated with the development of AD at 2 years of age.<sup>40</sup> However, some studies have failed to replicate the link between low *Bifidobacterium* and consequent atopic disease development,<sup>41</sup> or have failed to demonstrate a broader association between a dysregulated early gut microbiome and AD development.<sup>10</sup>

As with the skin commensals, differences at species level are thought to be relevant. Allergic infants are more frequently colonised than those who are not allergic by 'adult-like' *Bifidobacterium* (such as *B. adolescentis*), while in healthy infants the *Bifidobacterium* population was dominated by *B. bifidum*.<sup>42</sup> Indeed, various *Bifidobacteria* species can differentially affect cytokine induction by immune system cells *in vitro*,<sup>43</sup> providing a physiologically feasible link between alternations at the species level and a predisposition towards atopy.

#### Treating the aberrant gut microbiome with probiotics

As low total *Lactobacillus*, *Bifidobacteria* counts or the lack of species-specific *Bifidobacteria* are thought to be strongly associated with AD, there is good reason for attempting to modify gut bacterial composition and subsequent atopic risk by increasing the abundance of such bacteria.

Probiotics are compounds containing live microorganisms that, when ingested, confer a health benefit upon the host.<sup>44</sup> The role of probiotics in the prevention and treatment of atopic diseases has been widely studied but has been limited by methodological restrictions such as small sample sizes. Pretreatment of women during pregnancy in a double-blind placebo controlled trial with *L. rhamnosus* GG reduced the risk of development of AD in high-risk infants by half at 2 years,<sup>45</sup> and this effect was maintained at follow up with children aged 4 years.<sup>46</sup> In a modest sized sample, one group demonstrated that weaning infants onto formula supplemented with either the probiotic strains *B. lactis* Bb-12 or *L. strain* GG reduced the severity of AD as measured by scoring atopic dermatitis scores at 2 months.<sup>47</sup> However, overall, probiotics have been shown not to have a significantly beneficial effect in the treatment of AD, as highlighted in a Cochrane Review.<sup>48</sup> Together, these data suggest that modulating gut bacteria may protect against the development of AD,

perhaps during a critical period when the gut environment can tip the immunological balance away from an aberrant allergic pathway, but not treat established AD.

What are the plausible mechanisms of gut microbial manipulation and reduction in AD risk? The presence of appropriate commensal organisms have been purported to help establish the Th-1/Th-2 equilibrium of the developing immune system,<sup>49</sup> which at birth is skewed towards the Th-2 response.<sup>50</sup> The presence of important commensals in infants may result in regulatory T-cell (Treg) induction and immune tolerance development. Gut colonisation of germ-free mice by *Bacterioides fragilis* has been shown to normalise this imbalance through the promotion of Th-1 cytokines.<sup>51</sup> Moreover, the absence of important commensals may have a direct effect by the lack of Treg induction but also an indirect effect by allowing opportunistic pathogens to thrive.

### THE LEAST RESEARCHED FRONTIER: THE RESPIRATORY TRACT

Historically, the lungs were thought to be a sterile environment. Next-generation techniques have confirmed that the lungs of healthy individuals harbour a diverse microbiome, similar in nature, but lower in density to the upper

respiratory tract.<sup>52</sup> As it was not one of the chosen sites for the Human Microbiome Project, studies of the lungs in this respect is relatively scant. Appropriate sampling of the lung microbiome is also technically difficult to achieve. However, there is some evidence that organisms from the phylum Bacteroidetes are more common amongst controls than those with respiratory diseases such as asthma, while Proteobacteria were high in asthmatics.<sup>53</sup> While it is feasible that the early interaction of the microbiome and immune system via various routes (skin–gut–lung) could affect the risk of the subsequent development of atopic diseases, no study has investigated the link between the lung microbiome and AD development. The respiratory tract could represent another important portal for exposing the developing immune system to microbes during a critical period.

### LIMITATIONS IN CURRENT KNOWLEDGE

The use of novel, culture-independent methods has led to an explosion in published studies on the microbiome. However, caution needs to be employed in reaching conclusions. For example, when examining the gut microbiome, study outcomes are biased by access to important gut microbes. The stool is the most commonly

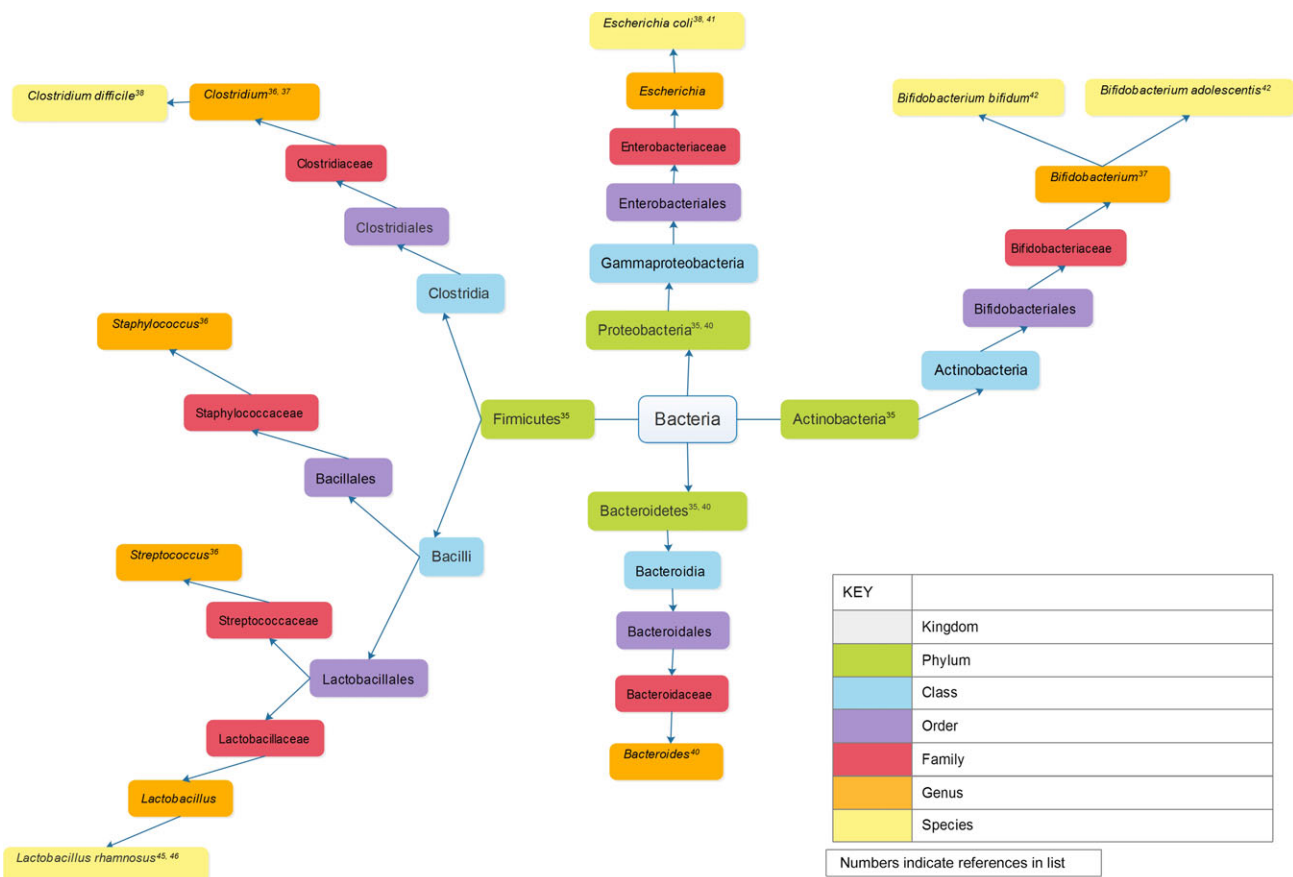


**Figure 2** Classification of organisms of interest from the gut microbiome in health and atopic eczema.

used sample for gut evaluation, however, this sample best reflects luminal organisms of the large intestine<sup>58,40</sup> rather than mucosal organisms with a greater ability to influence the immune system. Moreover, stool samples do not reflect the organisms of the small intestine, which significantly outnumber those in the large intestine.

Studies employing different methodologies cannot be directly compared and subsequently, there has been a lack in the replication of results. It can be confusing when studies identify microorganisms at different classification levels; reporting findings at the phylum, order, genus or species level. Species or even strain-level classification may be needed to understand important interactions. There are also limitations in the markers used. 16S rRNA gene-based analysis may arrive at ambiguous results for identifying organisms at genus or species level. For fungi, 18S rRNA gene-based methods are unable to classify beyond phylum or class level. Given these limitations, methods should be selected carefully based on the aims of the study. 16S rRNA gene-based methods may need to be combined with metagenomics to gain further functional information. The use of various diagnostic criteria for AD also lead to further variability. Some studies require only parent-reported outcomes of the presence of an itchy rash in their infant, while others use stringent clinical criteria. Using standard diagnostic criteria in future studies should strengthen outcomes.

## CONCLUSIONS

The pattern of microbial exposure in early life appears to be critical for the development of normal immune regulation and may play a central role in the development of atopic disease. While individual bacterial (such as *S. aureus*) and fungal species may be crucial to the microbiome and immune development, the balance of the overall microbial community is likely to be key. While both low diversity and the presence or absence of particular microorganisms appear to be important, we still do not know which variable is critical.

We see that the microbiome changes dynamically during AD flares and that current treatments already modulate the microbiome. However, current antiseptics and antibiotics are non-selective. When we make management decisions for AD patients we must be mindful of how we are influencing the microbiome to avoid the indiscriminate selection of microorganisms. We understand that the composition of the fungal community may be important in AD, yet we do not know how this pattern changes in flares. Understanding the complex interactions of the human microbiome will aid the development of novel and more targeted treatments to modulate the microbiome and influence AD outcomes. Principles gleaned from the success of faecal transplantation in the treatment of *C. difficile* infections<sup>54</sup> may be translated to AD with organism transplantation used to normalise the skin or gut of those with, or at high risk of developing AD.

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