RHEUMATOLOGY

Review

The bacterial skin microbiome in psoriatic arthritis, an unexplored link in pathogenesis: challenges and opportunities offered by recent technological advances

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Abstract

The resident microbial community, harboured by humans in sites such as the skin and gastrointestinal tract, is enormous, representing a candidate environmental factor affecting susceptibility to complex diseases, where both genetic and environmental risk factors are important. The potential of microorganisms to influence the human immune system is considerable, given their ubiquity. The impact of the host−gene−microbe interaction on the maintenance of health and the development of disease has not yet been assessed robustly in chronic inflammatory conditions. PsA represents a model inflammatory disease to explore the role of the microbiome because skin involvement and overlap with IBD implicates both the skin and gastrointestinal tract as sources of microbial triggers for PsA. In parallel with genetic studies, characterization of the host microbiota may benefit our understanding of the microbial contribution to disease pathogenesis—knowledge that may eventually inform the development of novel therapeutics.

Key words: skin microbiome, psoriatic arthritis, next-generation sequencing, metagenomics.

Introduction

The term microbiome has been used to describe the microorganisms that are present on and in the human body along with their genetic information and their ecological milieu [1]. The human body harbours 10 times more microbial cells than human cells and there is increasing acknowledgement that this represents a second genome, which contributes to tissue homeostasis [2]. The term metagenome takes into account the contribution of the genetic material from the host and all the genes and genetic elements of the microorganisms in and on the host.

Metagenomics refers to the study of the structure of the complex communities of the microbial flora inhabiting the human host, the functional information about these communities and the interactions with the human host [3]. The National Institutes of Health (NIH) Human Microbiome Project has endeavoured to characterize and establish a standardized reference database of the human microbiome in healthy individuals [4−8] in an effort to understand the interactions of microorganisms with the human host in health. The goals of all the studies characterizing the human microbiome are to define the complex microbial community and explore their functional consequences in human health and, ultimately, to translate this to understanding disease.

The emerging impact of the microbiome in inflammatory disorders

Indeed, host−microbe−gene interactions have been implicated in the pathogenesis of inflammatory disorders [3]. The hypothesis is that the normal immune system develops through harmonious relationships between the host and the normal microbes. An imbalance in the

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immune system due to genetic variation thus leads to inappropriate levels of inflammation in response to microbial products either as a failure to establish the normal microbial flora or because of the presence of a key organism that triggers pathology [9]. The mutual effect of the human immune system on the composition of the microbiota and the effect of the microbiota on the immune system is well described [10].

In terms of inflammatory arthritic diseases, Costello *et al.* [11] have discussed the potential role of the gut as a physical as well as an immune barrier in AS and the impact of the gut microbiota on disease. A recent study [12] comparing the faecal microbiota in early RA with those seen in FM reported a difference in the faecal bacterial composition but was limited by the number of bacterial species that could be investigated. The studies, to date, have been cross-sectional in nature and have not been able to determine whether changes in the microbiome are cause or effect. To determine the causality of an observational analysis of the human microbiome requires evidence of the consistency, specificity, temporality and biological plausibility of a relationship. The exemplar is the discovery and eradication of *Helicobacter pylori*, which changed the management of peptic ulcer disease and demonstrated the pathogenic role of this bacterium. Nonetheless, even cross-sectional metagenomic studies will inform hypotheses about causality. Advances in technology mean it is now possible to efficiently study the community structure and characterize the functional metagenomics of this important, potentially modifiable, environmental factor.

Recent technological advances in the investigation of the microbiome

Studying the microbiome was previously limited to culture-dependent methods that resulted in difficulties in isolating and identifying the fastidious (difficult to culture) microbes. Culture-independent methods of microbial identification are usually based on characterizing the bacterial flora by sequencing of the 16S rRNA gene. The 16S rRNA gene is found in all bacteria, but not in human cells; it is highly conserved with nine hypervariable regions (V1–V9) that can be used to determine the species of the bacteria. It is large enough (1500 bp) for informatics purposes, and the function of the gene has not changed substantially over time, which means that any changes within the gene reflect new mutations or evolution. Culture-independent methods have facilitated the identification of even the most fastidious bacteria and, consequently, have significantly expanded the numbers of bacteria identified in a wide range of environmental and clinical niches.

All sequencing-based methods use primers that bind to the unique DNA sequence within or around the 16S rRNA gene. Thus all amplification products should be of bacterial, not human, origin. The length of the amplified sequence depends on the site of primer binding, and this is determined by the primers used. The bacterial DNA

sequence can then be determined using a choice of platforms. Metzker [13] has elegantly described the sequencing technologies that are currently available. The first-generation technology, employing automated Sanger sequencing, is slow and relatively expensive, but produces long reads (segments of DNA that are being analysed). The advantage of sequencing the entire 16S rRNA gene using the Sanger sequencing technology is that it reduces the possibility of primer bias (selective loss of bacterial groups due to the inability to accurately sequence certain bacteria because of the region that the selected primer pair binds), which may be introduced in the newer next-generation sequencing (NGS) platforms, such as the Roche/454 GS Junior, Illumina/MiSeq and Life Technologies/Ion Torrent PGM. The newer NGS platforms are faster and more cost effective, but they have shorter mean read lengths of DNA (between 150 and 550 bases).

However, for taxonomic assignment of bacteria using the 16S rRNA gene, read lengths as small as 250 bp have been shown to be sufficient, with relatively little benefit in increasing the read length [14]. The shorter read lengths that are the norm of the NGS platforms have the disadvantage that, depending on the hypervariable region selected for amplification, not all groups of bacteria can be classified. Conversely, NGS technologies have a greater depth of sequencing (with larger number of reads) and in general have lower sequencing errors. Hence the choice of platform is moving towards NGS, especially with the increasing read lengths that all providers are striving to achieve and approaches that combine data generated using a collection of primers targeting several variable regions. The use of barcodes in NGS allows pooling of samples, facilitating analysis of larger numbers of samples simultaneously. NGS technologies are being extensively used in the investigation of the microbiome [6, 7, 14, 15], including the NIH Human Microbiome Project [4].

Aetiological hypothesis: PsA and the microbiome

Psoriasis is a chronic inflammatory skin condition that is prevalent worldwide, with varying incidence and prevalence rates depending on the geographic region and age group studied [16]. Nearly one-third of individuals with psoriasis develop PsA, with an average of 8–10 years from the onset of skin disease [17–23]. Studies have shown that patients with PsA have greater cardiovascular morbidity than the general population [24] and poor quality of life [25]. Given these co-morbidities, it is imperative that early effective intervention is instituted in patients with PsA. However, the treatment of PsA patients is often based on knowledge and evidence from studies investigating patients with RA. A recent study [26] exploring the synovial histopathology of PsA compared with undifferentiated spondyloarthropathy (USpA), AS and RA showed that PsA is more similar to USpA and AS than RA. These discrepancies highlight the need to understand

the pathogenesis of PsA for development of targeted therapies.

The 40-fold increased recurrence risk ratio observed by Karason *et al.* [27] in first degree relatives of patients with PsA suggests a strong and complex genetic susceptibility component along with an important environmental contribution. Among the environmental triggers, infections have been investigated as infectious triggers of PsA in the past. For example, Vasey *et al.* [28] found elevated levels of antibody to streptococcal exotoxin anti-deoxyribonuclease-B in PsA compared with patients with psoriasis without arthritis, RA or other forms of dermatitis and normal controls. In a study that investigated surrogate markers for viral infection, an increased incidence of serological markers for HCV in PsA cases compared with patients with RA have been reported [29], although the finding was not replicated in a later study [30].

More recently, Eder *et al.* [31] reported the association of infection requiring antibiotic treatment with the onset of PsA in a case–control study. With the advent of the HIV epidemic in sub-Saharan Africa and the *de novo* appearance of psoriasis and PsA in a population with previously low incidence and prevalence rates [32, 33], there is renewed interest in the dysregulation of innate immunity due to infection or an alteration in the microbial community structure as an aetiological pathway.

However, the identification of a clear-cut contribution from a pathogen to PsA susceptibility has been elusive. Histologically there is evidence of inflammation in the gut [34, 35] and psoriatic skin lesions [36], therefore the possibility of variations in the microbiological milieu at these sites of immune regulation has been considered. Support for involvement of the skin microbiome comes from reports of overexpression of endogenous antimicrobial peptides such as cathelicidins [36] and human beta-defensin 2 (hBD-2) [37] in psoriatic skin lesions, and simultaneously increased serum HBD-2 levels have been found in active psoriatic skin disease [37]. High hBD-2 levels have an immune-modulatory effect as well as chemotactic properties [37].

Furthermore, a recent review [38] of the functional and genetic studies in psoriasis and PsA have implicated genes affecting the skin barrier function, as well as the innate and adaptive immune systems in the pathogenesis of these diseases. Such studies add to the increasing evidence of the influence of the IL-23/IL-17 axis in psoriasis [39] and PsA [40], and the advent of effective treatments targeting the downstream IL-17 cytokine implicates the central role of the Th17 subset of T cells.

Interestingly, Ivanov *et al.* [41] demonstrated the importance of local microbiota in driving the Th17 cell repertoire and maintaining the balance between regulatory T cells (Treg) and Th17 cells in the gut, while Belkaid *et al.* [42] demonstrated the importance of local cutaneous microbiota in the modulation of T cell effector function in germ-free and specific-pathogen-free mice: administration of an antibiotic cocktail to the mice did not affect the skin microbiota but did affect the gut. In germ-free mice the introduction of a skin commensal rescued the IL-17A production in the skin but not in the gut. Restoration of the immune response in germ-free mice by introduction of a skin commensal underlines the importance of the host–skin–commensal cross talk.

The skin microbiome is therefore being investigated to assess the host–microbe symbiotic relationship and to gain an understanding of its contribution to the pathology of skin diseases [43]. As PsA develops in individuals with psoriasis, examining the difference in skin microbe composition in those who develop PsA and those who do not may offer unique insights into its pathogenesis. This information may also help in stratifying individuals who may be predisposed to developing arthritis from among the psoriatic group. The potential for the discovery of variations in the pathogenesis depending on the combined host–microbiome characteristics may contribute to improving our understanding of the underlying mechanism of the disease in different subgroups and could ultimately lead to potential therapeutic targets for prevention and control of PsA.

Investigation of the skin microbiome: progress so far

Skin, the largest organ of the human body, is a complex and dynamic ecosystem hosting a multitude of microorganisms and is one of the main physical barriers of defence in humans. The interactions occurring in the skin, as an organ, include those between the human host cells, external microbes, other environmental factors and the existing resident microbial components. Cogen *et al.* [44] describe the various positive (where both species benefit) and negative (where one species benefits at the cost of the other) potential relationships between the skin flora and the human host. Technically the skin microbiome is the most challenging microbial milieu to study due to the difficulty of DNA extraction from a low bacterial biomass. Table 1 describes the main features of previous studies of the skin microbiome using culture-independent methods. These studies compared the skin microbiome between populations of different ethnicities and regions, between healthy and diseased skin, have explored inter- and intra-personal variation of the skin microbiome, have assessed differences between various sampling and DNA extraction techniques and consequently have established a database of the healthy core skin microbiome.

Different techniques of sampling of the skin microbiome, namely skin swabs, scrapes and punch biopsies, have been assessed [45]. All three methods were able to capture a representative profile of the microbial community. Robust statistical analysis supported the existence of a core skin microbiome among healthy human subjects. In a follow-up study, the microbiome from 20 distinct skin sites in healthy individuals was analysed and showed that physiologically comparable sites harbour similar bacterial communities, but sebaceous skin sites are less diverse, less even and less rich than moist and dry sites [46].

The temporal variation of the skin microbiome has been explored in several studies [46–50]. Although the time

TABLE 1 Studies on the bacterial microbiota of the skin using culture-independent methods

Author/year	Aims/objectives of the study	Number of subjects	Site of sampling	Specimen collection	Platform used
Dekio *et al.* (2005) [56]	Analysis of skin microbiota	*n* = 5 healthy individuals	Forehead	Swab scrub	Sanger sequencing
Dekio *et al.* (2007) [50]	Comparison of skin microbiota between atopic dermatitis patient and healthy controls	*n* = 1 atopic dermatitis patient and 10 healthy controls	Facial skin	Swab scrub	Terminal restriction fragment length polymorphism
Gao *et al.* (2007) [49]	Skin microbiota on the volar forearm to determine bilateral conservation	*n* = 6 healthy volunteers	Volar forearm bilaterally midway between the wrist and elbow	Skin swab	Sanger sequencing
Gao *et al.* (2008) [52]	Compare bacterial populations in psoriatic lesions, from unaffected skin in subjects with psoriasis and from skin from healthy persons	*n* = 12; 6 healthy subjects and 6 subjects with psoriasis	Psoriatic plaques and normal skin from forearm	Skin swab	Sanger sequencing
Grice *et al.* (2008) [45]	To determine resident skin microbiota of the antecubital fossae in healthy humans	*n* = 5 healthy volunteers	Bilateral non-overlapping regions of the antecubital fossa	Skin swab, scrape and punch biopsy	Sanger sequencing
Fierer *et al.* (2008) [48]	Analysis of the diversity of hand surface bacteria and factors such as sex, handedness and time since last hand-washing	*n* = 59 healthy volunteers	Palmar surface of the hand	Skin swab	454
Costello *et al.* (2009) [47]	Obtain an integrated view of the spatial and temporal distribution of the human microbiota	*n* = 9 healthy volunteers	18 skin sites	Skin swab	454
Grice *et al.* (2009) [46]	Analysis of intrapersonal variation between microbiota at different skin sites	*n* = 10 healthy volunteers	20 skin sites	Skin swab	Sanger sequencing
Gao *et al.* (2010) [57]	Develop qPCRs to enumerate total bacterial and fungal populations and determine the most common bacterial and fungal genera in different locations on human skin	*n* = 8 healthy adults	11 skin sites	Skin swab	Sanger sequencing
Fierer *et al.* (2010) [58]	Use skin-associated bacteria as fingerprints for forensic identification	*n* = 14 healthy individuals	Ventral surface of the distal joint of each fingertip, axilla or entire palmar surface	Skin swab	454
Egert *et al.* (2011) [59]	Identify active members of the human axillary microbiota	*n* = 10 healthy males	Bilateral axilla	Skin scrub specimen	Sanger sequencing
Staudinger *et al.* (2011) [60]	Assess the microbiota on the forearm and forehead and the influence of make-up	*n* = 11 healthy individuals	Forehead and forearm	Skin scrape (cotton swab for 1 min)	Sanger sequencing

(continued)

TABLE 1 Continued

Author/year	Aims/objectives of the study	Number of subjects	Site of sampling	Specimen collection	Platform used
Blaser *et al.* (2013) [61]	Describe and compare the composition of the cutaneous microbiome of Amerindians in Venezuela and subjects in the USA	*n* = 72 healthy individuals	Forearm skin specimen	Skin swab	454
Fahlen *et al.* (2012) [53]	Comparison of microbiota between psoriatic and normal skin	*n* = 10 psoriatic patients and 12 control subjects	Unmatched skin sites (trunk and limbs)	Skin biopsy	454
Flores *et al.* (2012) [54]	Assessment of different DNA extraction approaches prior to PCR	*n* = 4 healthy individuals	Forehead, forearm and underarm skin	Skin swabs	454
Huse *et al.* (2012) [51]	Describe the core microbiome—NIH Human Microbiome Project	*n* = 242 healthy volunteers	Skin sites: bilateral antecubital fossae and retroauricular creases	Skin swab	454

qPCR: quantitative PCR; 454: next-generation high-throughput DNA sequencing platform.

intervals varied from a few hours to several months, different methodologies were employed and the sites investigated varied in these studies, the skin microbiome was found to be stable in an individual over time. Furthermore, analysis of two analogous sites (left and right side of the body) [49] showed a high level of conservation of the bacterial community at any moment in time. The more extensive sample size from the Human Microbiome Project [51] is now providing the database for the core microbiome of healthy humans. In that study, the core operational taxonomic units (OTUs; defined as the number of distinct 16S rRNA sequences at a certain cut-off level of sequence diversity) were defined as those that were present in at least 95% of all the samples for a given body site. The relative abundance (84–0.021%) of the core OTUs varied between subjects, but the notable feature was that the most abundant OTUs tend to be present in more subjects than the less abundant OTUs overall.

Preliminary assessment of psoriatic plaques has shown that there is a difference in the skin microbiome between the plaques and the normal skin in individuals with psoriasis [52]. The Blaser lab [52] compared the microbiota in psoriatic plaques with that of normal skin in six individuals with psoriasis and also the normal skin in six healthy controls. Fahlen *et al.* [53] compared the skin biopsies from psoriasis plaques from 10 individuals with psoriasis with skin biopsies from control individuals undergoing wide excision of skin lesions without any past or family history of psoriasis. Both studies found similar results, with increased Firmicutes (one of the major bacterial phyla including most of the Gram-positive genera) in the psoriatic plaques and a higher ratio of *Streptococcus/Propionibacterium* in the psoriatic plaques compared with controls. Some differences between the studies were noted, but were thought to arise because of variations in the sampling methods and the unmatched nature of the control skin obtained for analysis in the study conducted by Fahlen *et al.* [53]. In both studies, psoriasis patients had stopped topical treatment for at least 2 weeks prior to the sampling and were not on any systemic treatment, thereby reducing the possibility of treatment confounding the results.

Non-invasive skin swabs were the sampling method of choice in most studies. However, the yield of bacterial DNA from swabs can be low, reflecting the low biomass of the bacteria resident on the skin. Several DNA extraction techniques have been used and Flores *et al.* [54] compared the direct PCR approach against the DNA extraction/purification approach to determine if yields were similar. The consensus was that the direct approach was faster but resulted in lower bacterial yields, so it may not be appropriate if multiple PCR-based assays are required. However, the newer, commercially available DNA extraction kits have improved the yield of bacterial DNA from skin swabs.

The PCR primers used in the different studies varied with respect to the regions selected for amplification in the 16S rRNA gene. The majority of the published studies have used primers that cover nearly all the hypervariable

regions in the 16S rRNA gene. The latest studies [51, 53, 54] have restricted the choice of region to V3–V5. This is because sequences from the V3–V5 primers were significantly more accurate relative to data generated from V1–V3 or V6–V9 primers when tested using the Roche 454 platform [55]. The primary NGS platform to be utilized for microbial work was the Roche 454, due to its ability to produce long maximum (1000 bp) read lengths; when this platform was used, the sample size studied was greatly increased (a few hundred individuals) compared with the use of Sanger sequencing technologies (tens of subjects). The cost, throughput, speed, flexibility and high volume of data output of the newer benchtop sequencers (MiSeq, Ion Torrent), although generating shorter read lengths, are promising and warrant their use in larger-scale studies to provide greater power.

Discussion

The studies that are currently available in the literature with respect to the skin microbiome are limited in number. The majority have attempted to assess the normal skin microbiome using culture-based methods and to answer basic questions regarding the spatial and temporal association and stability of the microbiological milieu. The Human Microbiome Project is now developing a database of the core microbiome of different ecological niches of the human body. The temporal stability of the microbiome has been demonstrated and studies have ascertained the existence of differing microbial communities in the different ecological niches of the skin. This information is important to enable characterization of the normal composition of the microbiome. The studies that have looked at disease conditions have shown variations from the normal in a number of conditions. Further work needs to be done to build upon these important results. The establishment of causality of a microorganism would require replication of previous work and further detailed functional characterization of the microbiome.

The explosion in NGSs will certainly be helpful for undertaking high-throughput parallel sequencing at a reduced cost and should pave the way for genotypic and functional characterization of the complex microbial communities in a relatively short time. However, being aware of the shortcomings of these methods, namely short read length, primer bias, sequencing errors and depth of sequencing in the different methods employed, is essential to develop robust databases. The Human Microbiome Project has attempted to standardize procedures to enable researchers worldwide to replicate and continue to expand this work. Computational capacity and constant improvements in the pipelines available for the analysis of the immense datasets generated by high-throughput sequencers is an essential requirement for obtaining valid data. This latter aspect is currently the bottleneck in many studies and is a problem that requires concerted effort in terms of development of user-friendly tools for data analysis and wider training of suitably qualified personnel.

Just as knowledge of the inflammatory pathways in RA paved the way for the use of the biologic anti-TNF treatments, knowledge of the skin microbiome in PsA may be the first step towards future progress in therapeutics and early identification. Our current knowledge of the skin microbiome in psoriasis suggests an alteration in the microbiota of psoriatic plaques. Investigation of the psoriatic plaques in PsA will determine if there is further compromise in the skin barrier that may explain the drivers of inflammatory arthritis. This may also help in developing strategies for PsA prevention in patients with psoriasis or delaying the onset of joint disease. Extending the application to characterization of the microbiome in the gut and determination of the microbial variation between inflammatory bowel disease with or without PsA may also be enlightening.

In comparing psoriasis and PsA, however, potential confounders such as therapy, ultraviolet light exposure and skin type pose a challenge to the interpretation of these findings. Functional characterization work through metatranscriptomic studies, such as those currently being undertaken in the NIH Human Microbiome Project, will help to clarify the significance of microbial flora. As described by Grice and Segre [2], characterizing and analysing the human microbiome is vital for an understanding of this essential yet overlooked component of human immune regulation and important contributor to tissue metabolism and homeostasis. Defining the role of the microbiome in complex diseases like PsA, which has a very strong genetic contribution, but where the causality cannot be explained solely by host genetics, has the potential to impact our understanding of the disease pathogenesis.

Rheumatology key messages

- The skin microbiome is an intriguing candidate environmental factor in PsA.
- Different approaches have been used to study the skin microbiome, and each has its strengths and weaknesses.
- 16S rRNA gene sequencing is ideal for bacterial microbiome hypothesis generation in PsA.

Acknowledgements

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