Archaea associated with human surfaces: not to be underestimated

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One sentence summary: This review highlights the recent knowledge on archaea's abundance in humans, their interactions with human immune cells and their potential impact on health and disease.

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ABSTRACT

Over 40 years ago, Carl Woese and his colleagues discovered the existence of two distinctly different groups of prokaryotes—Bacteria and Archaea. In the meantime, extensive research revealed that several hundred of bacterial species are intensely associated with humans' health and disease. Archaea, originally identified and described to occur mainly in extreme environments, have been shown to be ubiquitous and to appear frequently and in high numbers as part of human microbiota in recent years. Despite the improvement in methodologies leading to increased detection, archaea are often still not considered in many studies focusing on the interdependency between members of the microbiota and components of the human immune system. As a consequence, the knowledge on functional role(s) of archaeal species within the human body is mainly limited to their contribution to nutrient degradation in the intestine, and evidence for immunogenic properties of archaea as part of the human microbiota is generally rare. In this review, the current knowledge of human mucosa-associated archaeal species, their interaction with the human immune system and their potential contribution to humans' health and disease will be discussed.

Keywords: archaea; methanoarchaea; detection; human microbiome; immune homeostasis

INTRODUCTION

The human body forms one of the most complex ecosystems being host to taxa across the entire tree of life: Eukarya, bacteria, archaea and viruses, and is thus a prime example for a ‘metaorganism’ (Whitman, Coleman and Wiebe 1998; Turnbaugh et al. 2007; Haynes and Rohwer 2011; McFall-Ngai et al. 2013). However, currently only a minority of these microbiome members can be cultivated and studied in pure cultures. Hence, the development of high-throughput sequencing techniques during the last decade has greatly improved the knowledge on microorganisms present in the human ecosystem (reviewed in Achitman and Wagner 2008; Shendure and Ji 2008; Peterson et al. 2009; Walter and Ley 2011; Ding and Schloss 2014). Applying those techniques led to the discovery of the enormous impact of complex microbial communities that are involved in human health and physiology (Cho and Blaser 2012; Clemente et al. 2012). The establishment of large-scale projects such as the Human Microbiome Project (Turnbaugh et al. 2007; Peterson et al. 2009) and MetaHIT (Qin et al. 2010) revealed that each individual’s microbiome is unique and does not only depend on the genetic background or demographic and life history characteristics of the host, but also on individual’s recent interactions with its environment such as diet or medications (Walter and Ley 2011). Besides, microbial communities found in different body sites show huge differences including distinct community types as
well as interindividual variability (Costello et al. 2009). Generally, these communities are known to interact with the host’s immune system, which in turn is shaping the microbial community composition by various innate and adaptive defense mechanisms (Majewski et al. 2000; Ley, Peterson and Gordon 2006; Cho and Blaser 2012; Hooper, Littman and Macpherson 2012; Belkaid and Hand 2014). The resulting established microbiota promotes humans’ physiology as well as normal development and function of the mucosal immune system (O’Hara and Shanahan 2006; Chow et al. 2010). The identification of factors affecting one’s community type profile is crucial, since microbiota’s composition has been shown to be associated with predisposition to diseases (Ding and Schloss 2014).

Due to the fact that bacteria form the most abundant part of the human microbiota, their impact on human health and disease has been studied extensively in the recent years (Turnbaugh et al. 2007; Peterson et al. 2009; Qin et al. 2010; Walter and Ley 2011; Belkaid and Hand 2014). Thus, the acquired knowledge regarding the interdependency between bacterial communities and the human host far overcomes current information on the interactions between viral, eukaryotic and particularly archaeal communities and humans. However, to date it has not been successful to establish a causative relationship between a specific bacterium and the development of several severe diseases such as inflammatory bowel disease (IBD), periodontitis, cancer as well as diabetes and asthma (Ding and Schloss 2014). In this respect, it appears urgently required to consider the interplay between all present members of the microbiota and consequently include community members of the two remaining domains of life in order to address this current research focus. Whereas viruses and eukaryotes are coming into this focus these days (Virgin, Wherry and Ahmed 2013), viruses and eukaryotes are coming into this focus these days (Virgin, Wherry and Ahmed 2013).

DISCOVERY, DESCRIPTION AND DISTRIBUTION OF ARCHAEA

More than 40 years ago, Carl Woese discovered the presence of two distinctly different groups within the prokaryotes by studying their relationships on the basis of their 16S ribosomal RNA (16S rRNA) gene sequences (Woese and Fox 1977). Though the overall morphology of archaea with regard to size, shape and cell organization resembles that of bacteria, the identified remarkable genetic and biochemical differences among those two prokaryotic groups encouraged Carl Woese and co-workers to propose a division of life into the three domains Eubacteria, Archaea and Archaeobacteria. In the following years, numerous studies identified unique characteristics shared by members of the domain Archaea as well as evidence for a closer evolutionary relationship to the domain of Eukarya. Thus, in order to underline its phylogenetic distance to Bacteria, the taxonomic order was generally revised and the domain Archaea was renamed in Archaea (Woese 1987; Woese, Kandler and Wheelis 1990). In general, archaea have many features more similar to their eukaryotic than bacterial counterparts (e.g. DNA replication and repair mechanisms, transcription and translation machinery) (Garrett and Klenk 2007). Furthermore, the ribosomal structure of archaea suggests a close relationship to eukaryotes (Woese, Kandler and Wheelis 1990; Allers and Mervarech 2005; Cavicchioli 2007). With respect to their ribosomal structure, the eocyte hypothesis even discusses whether Eukarya may have evolved as a sister group to the Crenarchaeota within the archaeal domain (Lake et al. 1984; Embley and Martin 2006). Recently, Williams et al. (2013) also obtained strong evidence that eukaryotes arose through symbiosis events between the two primary domains of life, Archaea and Bacteria. However, due to the use of different phylogenetic methods as well as the problems associated with phylogenetic reconstruction, this is still controversially discussed. Apart from their relationship to the eukaryotes, the biochemically unique-ness of archaea has to be highlighted, since members of this domain exhibit several unique features in biochemistry of their cell structures and their metabolic pathways (Schäfer, Engelhard and Muller 1999; Thauer and Shima 2006). In particular, the chemical composition of the archaeal cell envelope differs profoundly from that of bacteria and eukaryotes. For instance, the cell membrane is built up by glycerol-ether lipids composed of L-glycerols, whose stereochemistry is reverse to the D-glycerols found in bacteria and eukaryotes (Fig. 1B) (Koga et al. 1993; Koga and Morii 2005). Moreover, archaea are the only known organisms using isopenoid side chains for the synthesis of membrane phospholipids (Fig. 1B) (Koga and Morii 2007). In some species, the cell membrane forming phospholipid bilayer is replaced by a monolayer composed of tetraether lipids that enhance stability and rigidity to extreme conditions (Fig. 1B) (Hancock and Peeples 2002; Koga and Morii 2007). Additional unique biochemical components of archaea are their cell wall structures, which are highly diverse ranging from methanochondroin to surface-layer protein sacculi, and pseudomurein (Kandler and König 1998; Albers and Meyer 2011). Moreover, archaea have evolved unique metabolic capabilities in order to use several energy sources including sunlight (phototrophs) as well as organic (organotrophs) and anorganic (lithotrophs) compounds (Valentine 2007).

Considering the above-described biochemical as well as metabolic advantages, it is comprehensible that archaea are ubiquitous and exist in a broad variety of habitats. In detail, distinct archaeal species grow optimally in extreme environments with temperatures above 80°C (hyperthermophiles), very cold temperatures down to −20°C (psychrophiles) or with very high salinity (halophiles), but also in habitats with mild growth conditions such as sewages and soils (mesophiles) (Barns et al. 1996; DeLong and Pace 2001; Valentine 2007). These enormous adaptations to extreme environments and living conditions led to a general biotechnological interest in various archaeal species, particularly with respect to the identification of novel, technically useful, enzymes. Thus, several archaeal species have been cultivated and biochemically well characterized in the last 40 years (Breithaupt 2001; Schiraldi et al. 2002; Soppa 2006; Hess, Katzer and Antranikian 2008; Jenney and Adams 2008; Grogan 2009). However, the majority of archaeal species were detected...
by sequencing their 16S rRNA genes in environmental samples (Barns et al. 1996; Robertson et al. 2005) and only a few percent of them have been cultivated to date. This fact might be mainly due to the confined knowledge regarding the metabolic requirements of most archaeal species, which complicate laboratory cultivation. Based on 16S rRNA analysis, DNA sequences and biochemically well characterized isolates, the archaeal domain is currently divided into the five phyla Euryarchaeota, Crenarchaeota, Korarchaeota, Nanoarchaeota and Thaumarchaeota (Fig. 1A) (Spang et al. 2010). Recently, a superphylum including Aigarchaeota, Crenarchaeota, Korarchaeota and Thaumarchaeota has been proposed, which is hypothesized to be related to the origin of eukaryotes by the authors (Guy and Ettema 2011).

Based on the initial assumption that archaea occur only in extreme environments, their potential impact on multicellular eukaryotes regarding physiology or pathogenicity was not considered for many years (Conway de Macario and Macario 2009). However, members of the Archaea currently have been shown to appear frequently and in high numbers as part of the commensal microbiota found in insects and mammals including
Figure 2. Overview of archaeal species found as a part of the human microbiome at different sites. Various archaeal species have been shown to inhabit distinct human body ecosystems (indicated in orange), such as the intestine, the oral cavity, the vagina and most recently the skin. The majority of the so far detected archaea in humans, particularly from the gut and mouth, represent members of the Methanobacteriaceae, of which *Methanobrevibacter* and *Methanosphaera* (phase contrast micrographs on the right) are the most abundant ones.

**Archaea as a part of the human microbiome**

In the early 1980s, Miller et al. (1982) discovered and characterized the first archaeon from human feces, *Methanobrevibacter smithii*, and thereby initiated a slow but continuous interest in archaea that are associated with the human body (Cavicchioli et al. 2003; Eckburg et al. 2005; Conway de Macario and Macario 2009; Dridi et al. 2009; Horz and Conrads 2010; Oxley et al. 2010; Blais-Lecours et al. 2011; Dridi, Raoult and Drancourt 2011; Lurie-Weinberger, Peeri and Gophna 2011; Aminov 2013; Probst, Auerbach and Moissl-Eichinger 2013; Gaci et al. 2014; Saengkerdsub and Ricke 2014). Overall, it became clear that several members of the archaeal domain are frequently detected in the intestine and oral cavity of humans; however, their putative role in humans’ health and disease remained and is yet unclear; the current status will be discussed in the upcoming sections.

Humans (excellently reviewed by Dridi, Raoult and Drancourt 2011). Thus, particularly the physiological role of archaea as commensalistic microorganisms in humans has come into the focus of numerous studies (DeLong 1998; Cavicchioli et al. 2003; Eckburg, Lepp and Relman 2003; Cavicchioli et al. 2003; Rieu-Lesme, Delbès and Sollelis 2005; Robertson et al. 2005; Fricke et al. 2006; Samuel and Gordon 2006; Vianna et al. 2006; Samuel et al. 2007; Mihajlovski, Alric and Brugère 2008; Conway de Macario and Macario 2009; Dridi et al. 2009; Horz and Conrads 2010; Oxley et al. 2010; Blais-Lecours et al. 2011; Dridi, Raoult and Drancourt 2011; Lurie-Weinberger, Peeri and Gophna 2011; Aminov 2013; Probst, Auerbach and Moissl-Eichinger 2013; Gaci et al. 2014; Saengkerdsub and Ricke 2014). Overall, it became clear that several members of the archaeal domain are frequently detected in the intestine and oral cavity of humans; however, their putative role in humans’ health and disease remained and is yet unclear; the current status will be discussed in the upcoming sections.

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Archaea in the oral cavity

The oral microbiome is one of the most diverse of the human body with more than 700 bacterial species that have the potential to colonize the oral cavity, though the individual species number in human subjects is much lower (Aas et al. 2005; Paster et al. 2006; Costello et al. 2009; Cho and Blaser 2012; Langfeldt et al. 2014). In general, the majority of oral microorganisms grows as structurally complex consortia of different species adhering to surfaces mainly forming so-called biofilms (dental plaque). Biofilms develop under a range of diverse conditions including bacterial co-adhesion, nutrient limitation, as well as dramatic changes in physical parameters such as temperature, oxygen, nutrients and pH, probably explaining the overall diversity of oral biofilms (Socransky and Hafajee 2002; Marsh 2005). In addition to the huge variety of the oral microbiota, many more bacterial species pass through the oral cavity as transient community members. Most likely due to this fact, the oral cavity was found to be the least stable microbial ecosystem associated with the human body (Costello et al. 2009; Walter and Ley 2011).

In contrast to the overall huge diversity of the oral microbiome, the variety of archaea found in the oral cavity appears to be very low (Hörz and Conrads 2011). Methanobrevibacter oralis is the most abundant archaeal species within the oral cavity (Fig. 2). Notably, detection of this species as well as some related strains was predominantly correlated with diverse periodontal infections during earlier studies (Kulik et al. 2001; Lepp et al. 2004; Vianna et al. 2006, 2008, 2009; Jiang et al. 2009; Li et al. 2009; Hörz and Conrads 2011) (Table 1). Applying a target-specific established qRT-PCR protocol, Bringuer et al. (2013) were the first identifying the presence of M. oralis also in 30% of healthy individuals. However, again the abundance of methanoarchaea was found to be increased in individuals suffering from periodontal disease (54%). With respect to this, Hörz and Conrads (2011) noted that M. oralis exhibits an extraordinary high positive predictive value for periodontitis, which is not reached for any bacterial species involved in this disease. Moreover, clinical symptoms indicating acute inflammation processes were found to be significantly enhanced, when M. oralis was found in association with bacteria within primary and secondary root canal infections (Jiang et al. 2009). Most interestingly, higher abundances of methanoarchaea were frequently observed in deep periodontal pockets and persistent root canal infections (Jiang et al. 2009; Li et al. 2009; Aşk et al. 2013). Furthermore, Hörz, Seyfarth and Conrads (2012) found additional evidence for a novel lineage of methanoarchaea phylogenetically related to the Thermoanaerobacteriales in 10% of human subgingival plaque samples from periodontitis patients. Later on, this lineage was found to be phylogenetically affiliated to the newly identified seventh order of methanoarchaea, namely the Methanomassiliicoccales (Borre et al. 2013b). In conclusion, there are increasing indications that methanoarchaeal species might be involved in the development of periodontal disease—at least indirectly by promoting the growth of pathogenic bacteria (Kulik et al. 2001; Vianna et al. 2006).

Archaea in the intestine

During the last decade, the development and subsequent widespread use of modern molecular approaches and sequencing techniques revealed the presence of trillions of microorganisms in the human intestine that form a complex ecological community (Whitman, Coleman and Wiebe 1998; Hopkins, Patrick and Ball 2001; Macpherson and Harris 2004; Abreu, Fukuta and Arditi 2005; Ley, Peterson and Gordon 2006; O’Hara and Shanahan 2006; Artis 2008; Lozupone et al. 2012). These studies demonstrated that the number and density of microorganisms in the individual sections of the gastrointestinal tract differ in terms of species level and cell numbers (Whitman, Coleman and Wiebe 1998; O’Hara and Shanahan 2006). In detail, microbial cell numbers rise from 10^2 to 10^12 cfu ml^-1 in the stomach up to 10^5 to 10^12 cfu ml^-1 in the large intestine, thereby achieving the highest densities described for any ecosystem (Eckburg et al. 2005; Walter and Ley 2011). Besides alterations of microbial communities along the length of the gastrointestinal tract, differences in microbial populations between the luminal and mucosa-associated microenvironments have been observed, particularly in respect to the higher ratio of anaerobic to aerobic microorganisms that was found in the lumen (Zoetendal et al. 2002; Eckburg et al. 2005; O’Hara and Shanahan 2006; McKenna et al. 2008; Hill and Artis 2010; Heinsen et al. 2014). So far, most studies dealing with the characterization of intestinal communities mainly focused on the bacterial composition, thereby identifying the Firmicutes and Bacteroidetes phyla as the major bacterial groups that present about 90% of all phylogenetic types in the human intestine (DeLong and Pace 2001; Hayashi, Sakamoto and Benno 2002; Wang et al. 2003; Hill and Artis 2010; Walter and Ley 2011; Robles Alonso and Guarner 2013). Although these bacterial species dominate the human gut microbiome, several members of the archaeal domain as well as viruses and fungi were also found to be stable components of this complex community (Whitman, Coleman and Wiebe 1998; O’Hara and Shanahan 2006). In this respect, M. smithii was not only the first archaeon isolated from human feces, but is also the most abundant archaeon found in nearly every individual’s intestine as has been shown in a representative study including about 700 feces samples (Dridi et al. 2009). Several studies demonstrated enhanced efficiency of fermentative polysaccharide degradation in the human intestine through methanogenesis by M. smithii, since the hydrogen partial pressure is effectively lowered by the consumption of hydrogen and carbon dioxide (Miller, Weaver and Wolin 1984; Samuel and Gordon 2006; Samuel et al. 2007). Thus, M. smithii represents a crucial part of the gut microbiome regarding the overall metabolic physiology. Investigation of the genomes of several M. smithii fecal strains demonstrated genomic adaptations to the human gut ecosystem such as the production of surface glycans resembling those found in the gut mucosa and a regulated expression of adhesion-like proteins (Samuel et al. 2007). These adaptations are considered to be of bacterial origin, transmitted by interdomain lateral gene transfer (Lurie-Weinberger, Peeri and Gophna 2011) and support the hypothesis of M. smithii’s appearance as a commensal of the microbiota within the human intestine.

Besides M. smithii, an additional member of the Methanobacteriales, Methanospirillum hungatanae, has been detected in human stool samples (Fig. 2) (Lovley, Greening and Ferry 1984; Miller and Wolin 1985). In contrast to M. smithii, Me. hungatanae has a very restricted energy metabolism and generates methane exclusively by reducing methanol with molecular hydrogen (van de Wijngaard et al. 1991; Fricke et al. 2006). However, as observed for M. smithii as well as Me. hungatanae, the genome of Me. hungatanae exhibits several adaptations to the human intestinal habitat such as the genetic ability to synthesize cell-surface antigens (Fricke et al. 2006). Although M. smithii as well as Me. hungatanae were already isolated and cultivated in the early 1980s from the intestinal microbiota, it took nearly 30 years to cultivate another methanoarchaeal strain from human feces that was designated Methanomassiliicoccus luminyensis (Dridi et al. 2012a). In contrast to M. smithii and
Me. stadtmanae, this methanoarchaeon was found with higher prevalence in older individuals (Mihajlovski et al. 2010; Dridi et al. 2012b). Met. luminyensis has additionally come into the focus, since genomic studies indicated that this methanoarchaeon belongs to a new independent monophyletic group within the previously known methanoarchaeal order (Porrel et al. 2013b). The presence of such a new methanoarchaeal order that might be associated with the human intestine was already proposed earlier (Mihajlovski, Alric and Brugère 2008). The recent identification of the isolated and sequenced strains ‘Candidatus Methanomassiliicoccus intestinalis’ and ‘Candidatus Methanomethylophilus alveus’ from human stool samples now underlines the existence of this seventh order of methanoarchaea, which is most likely related to the order Thermoplasmatales based on 16S rRNA phylogenetic analysis (Porrel et al. 2012, 2013a, 2014). This new methanoarchaeal order was designated Methanomassiliicoccales (Lino et al. 2013). Though members of the Methanomassiliicoccales show a restricted, hydrogen-dependent methylotrrophic methanogenesis pathway, several studies indicate a broad environmental distribution, not only limited to the humans’ intestine (Paul et al. 2012). Most interestingly, members of this order also appear to be able to degrade methylated amines as has been shown for Met. luminyensis, and thus could be supportive in humans suffering from trimethylaminuria (Mackay et al. 2011; Brugère et al. 2014). Notably, the use of modern high-throughput sequencing approaches such as metagenomic studies as well as amplicon sequencing indicated the presence of additional community members from the eury- and crenarchaeal orders, such as Desulfurococcales, Crenarchaeales Sulfolobales, Thermoproteales, Archaeoglobales, Crenarchaeota, Methanobacteriales, Methanococcales, Methanopyrales, Thermococcales and Thermoplasmatales within the human intestine (Fig. 2) (Rieu-Lesme, Delbé and Sollelis 2005; Gill et al. 2006; Nam et al. 2008; Scanlan, Shanahan and Marchesi 2006; Oxley et al. 2010). In this respect, Oxley et al. (2010) found halophilic phylotypes in biopsy as well as fecal samples of IBD patients. In addition, they detected halohaloarchaeal sequence types in an aerobic enrichment culture of one biopsy sample. These findings suggest that, besides methanogens, members of the Halobacteriaceae might be associated with the human gastrointestinal mucosa. However, besides M. smithii,
**Met. luminyensis** and **Met. stadtmanae**, no further archaenal species originating from the human intestine could be cultivated and studied in pure culture to date. Nonetheless, the recent increasing knowledge on members of additional archaenal orders as part of the human intestine’s microbiome strongly supports evidence for higher archaenal diversity in the human intestine than has been considered hitherto.

**Archaea in other human body sites**

To our knowledge, there is one single study describing the occurrence of archaea in vaginal samples (Belay et al. 1990). Interestingly, in this study only samples from subjects suffering from bacterial vaginosis and not healthy ones were positive for methanoarchaea (**M. smithii**). Other studies, however, did not detect any methanoarchaea in samples from healthy or diseased females (DiGiulio et al. 2010), with the exception of one metagenomic study (Sundquist et al. 2007).

Though archaeal 16S rRNA gene sequences were found in earlier studies (Caporaso et al. 2011; Hulcr et al. 2012), the identification of archaea on human skin was not reported until 2013 by Probst, Auerbach and Moissl-Eichinger (2013). In this study, all investigated samples from 13 individual human torsos were found to be positive for archaea with an average abundance of 4.23% of the entire recovered microbiome. Most of the sequences belonged to the group of Thaumarchaeota (Fig. 2), whose occurrence was further confirmed by fluorescence in situ hybridization analysis. Since all cultured thaumarcheal species represent ammonia oxidizers and the authors also detected amoA genes within the samples (encoding for the key enzyme ammonium monoxygenase), they hypothesized a possible influence of the pH regulation of the human skin by Thaumarchaeota (Probst, Auerbach and Moissl-Eichinger 2013). However, further studies are urgently required to improve the knowledge regarding archaea associated with the human skin microbiota.

**RECENT DEVELOPMENTS TO DETECT AND QUANTIFY ARCHAEA ASSOCIATED TO HUMANS**

Regarding the high diversity of the domain Archaea and the astonishing high number of microorganisms associated with the human body, it appears hardly believable that only a few methanoarchaea as well as some thaumarcheal and halophilic species appear to be part of the human microbiome. Certainly, identification of archaea in medical samples is often hampered due to the challenging growth requirements as well as the specific staining and cell lysis protocols that have to be used for molecular identification. In addition, the unfavorable ratio of human and bacterial DNA to archaeal DNA complicates the analysis of archaea in these samples. However, all available classical and modern detection assays have been applied in order to uncover members of the human microbiota: cultivation, PCR-amplification of 16S rDNA or key genes, nested-PCR amplification, qRT-PCR as well as metagenomic analysis of high-throughput sequencing data sets (e.g. pyrosequencing or illumina sequencing techniques). Thus, in general it appears that the broad variety of detection assays used often fails to determine the abundance and diversity of archaea (Dridi 2012). However, the failure might rather originate from insufficient sample processing than of inadequate detection assays. Most likely the reason for the failure is that the DNA extraction protocols in most studies generally have been established and optimized for bacterial cell lysis. DNA isolation using those standardized protocols is often inefficient for archaenal cells due to the rigid cell walls, particularly of the Methanobacteriales **M. oralis**, **M. smithii** and **M. stadtmanae**. These cell walls are composed of an electron-dense pseudomurein layer and an additional second layer of heteropolysaccharides (Fig. 3) (Kandler and König 1998; König 2010). Thus, the extracted DNA does not necessarily reflect methanoarchaeal abundance in samples analyzed (Dridi et al. 2009; Dridi 2012). In this respect, Dridi et al. (2009) established an optimized detection system that includes an adapted

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**Figure 3.** Cell envelope of **M. smithii** and **M. stadtmanae**. Scanning electron (left) and transmission electron microscopy (right) photographs of **M. smithii** and **M. stadtmanae** are depicted in order to highlight the cell wall components. A detailed structure of the cell wall components including cytoplasmic membrane, pseudomurein and heteropolysaccharides is illustrated. Scanning electron microscopy has been performed in collaboration with M. Spinner and S. N. Gorb (Zoological Institute, University of Kiel). Transmission electron microscopy was done by T. Goldmann and T. Gutsmann (Clinical and Experimental Pathology, Biophysics, Research Center Borstel). The scale bar is 500 nm for each graphic.
protocol for efficient DNA isolation of the whole gut microbiome as well as an qRT-PCR approach with specific primers designed for M. smithii and Me. stadtmanae 16S rDNA as well as for the rpoB genes encoding for subunits of RNA polymerases. By using this detection system, 700 stool samples from individuals were analyzed and revealed the presence of M. smithii in at least 95.7% of all tested individuals, whereas Me. stadtmanae was less abundant (29.4%). However, these imposing numbers might be even higher, since both methanoarchaeal species have been shown to form biofilms under static conditions, implicating that the amount of methanoarchaea detected in stool samples might underestimate the number of methanoarchaea organized in a biofilm attached to the epithelium in the intestine (Bang et al. 2014a). Detection of archaea in clinical samples (e.g. biopsies) might additionally be complicated, since archaeal primers generated for PCR amplification often cross-react with human DNA due to the unfavorable ratio of human DNA versus microbial DNA as reported by Horz and Conrads (2011). We recently also observed this phenomenon of primer’s out-titration while evaluating archaeal abundance in human gut biopsy samples, which was overcome by applying a nested-PCR approach (Schilhabel, Bang, Schmitz, unpublished data). However, the application of this nested-PCR approach is only suitable for detecting the presence of archaeal species, but not for statistical relevant quantification regarding methanoarchaeal abundance within the human intestine. With respect to the overall archaeal abundance on human skin, Probst, Auerbach and Moissl-Eichinger (2013) recognized that several ‘universal’ archaeal 16S rDNA primers used in older studies do match only up to 50% of archaea. In addition, archaeal sequences are often removed from the datasets without further notice, due to the bacteria-focused filtering of sequence datasets in many microbiome studies.

Besides 16S rDNA and rpoB, PCR-based studies also used the mcrA gene (encoding a subunit of the key enzyme methyl-CoM reductase for methanogenesis) in order to determine overall methanoarchaeal diversity in humans (Luton et al. 2002; Eckburg et al. 2005; Scanlan, Shanahan and Marchesi 2008). However, though the key gene mcrA is present in all methanogenic species, subsequent studies demonstrated that mcrA-based primer pairs often fail to detect at least Me. stadtmanae (Dridi et al. 2009). In addition, using primers targeting the genes encoding enzymes unique for methanoarchaea will not allow identifying other archaeal orders associated with the human microbiota. Thus, by using different and partly not optimized primer pairs, many studies evaluating the overall archaeal diversity are neither comparable to each other nor do they reflect the actual archaeal abundance. In conclusion, sample preparation for DNA extraction and detection methods for archaea associated with the human body ecosystem have to be optimized and standardized in order to obtain a comprehensive overview of the archaeal diversity and quantity.

Another point one has to consider is the medical background of individuals that are sampled for subsequent microbiome analysis. As has been shown in several studies, antibiotic treatment can disturb the composition of the microbiota and can lead to a long-term decrease in microbial diversity (Jernberg et al. 2007; Dethlefsen et al. 2008; Knecht et al. 2014). Although most of the commonly used antibiotics, particularly those affecting bacterial cell wall components, are not effective against archaea, a few ones have been shown to inhibit also archaeal growth (Kheilaifa and Drancourt 2012). Particularly, metronidazole that is used to target anaerobic microorganisms during inflammatory diseases such as Crohn’s disease is highly effective against archaeal species (Ansorg et al. 2003; Dubreuil and Odou 2010; Kheilaifa and Drancourt 2012). Samples taken of individuals treated with archaeal-effective antibiotics might be negative for archaea due to this treatment. On the other hand, previous treatments with antibiotics targeting only bacteria might result in an increase of archaeal abundance. Overall, the effects of antibiotics on the detection and quantification of archaea might have been underestimated to date and need to be further elucidated.

In conclusion, detection and quantification methods of archaea associated with the human microbiome should be standardized with respect to sample processing (e.g. cell breakage), usage of specific primer sets (e.g. targeting 16S rDNA) and selection of human individuals. Moreover, there is strong evidence that archaeal abundance might alter with respect to the origin of samples taken (e.g. stool versus biopsy samples or antibiotic treatment of individuals).

ARCHAEA AND THE HUMAN IMMUNE SYSTEM

The human body with its stable temperature, its various epithelial surfaces and its large number of nutrients provides an environment with optimal growth conditions for mesophilic microorganisms. However, besides competition strategies for nutrients and niches of their habitat mates, all of these microorganisms have to cope with human defense mechanisms (Loetz, Ménard and Hornef 2007). These defense mechanisms are in general classified into innate and adaptive ones, which are the result of powerful and enduring selection (Janeway 1989; Cooper and Herrin 2010). Besides discrimination between ‘self’ and ‘non-self’, the ability to distinguish between beneficial and potential pathogenic microorganisms is the most important challenge of the host’s immune system (Lee and Mazmanian 2010). This ability is currently thought to be rather a feature enabled by the adaptive immune system, since both, commensal and pathogenic microorganisms, share molecular patterns that are recognized by the innate immune system. With respect to the recognition mechanisms, the evolutionary development of the adaptive immune system was most likely driven by the microbe itself (Lee and Mazmanian 2010)—and this microbiota includes not only bacteria, viruses and fungi, but also archaea. Thus, independent of which role human-associated archaeal species might play (commensalistic or pathogenic), one would expect that the human immune system is able to recognize and respond to those microorganisms. Unfortunately, only little work has been done regarding immune recognition of archaea by their human host, most probably due to the fact that no archaeal pathogen has been identified so far (Conway de Macario and Macario 2009). However, recent studies addressing this question obtained evidence for both—innate and adaptive—immune recognition and activation by human-associated archaea (Krishnan and Sproat 2008; Vianna et al. 2009; Blais-Lecours et al. 2011, 2014; Hirai et al. 2013; Bang et al. 2014b) and thus confirm this consideration.

Archaea and the innate immune system

The epithelia as the first line of human defense present a large surface for potential interaction between host and microbes, but also form the physical barrier separating those microorganisms from the underlying tissues. In addition, epithelia are able to recognize and respond to beneficial as well as pathogenic microorganisms (Loetz, Ménard and Hornef 2007). In this respect, we recently examined immune recognition by an epithelial cell line (Caco-2/BBe) of the gut-associated archaeal species M. smithii...
and *Me. stadtmanae*, particularly focusing on the expression and release of different proinflammatory cytokines (Bang et al. 2014b). This study revealed no apparent response of the epithelia to the tested archaeal strains, thereby indicating that these epithelia rather tolerate both—as has been shown for many bacterial commensals (Sansonetti 2004). However, an essential part of epithelial defense is the secretion of antimicrobial peptides (AMPs) (Zasloff 1992; Roman 1994; Andersson et al. 1995). AMPs exert their antimicrobial mechanism via interaction with the negatively charged membrane of microorganisms, thereby disturbing membrane integrity (Kagan and Sokolov 1994; Epand and Vogel 1999; Hancock and Chapple 1999; Lehrer and Ganz 1999). Previous results revealed not only regulation of various AMP’s expression in monocye-derived dendritic cells (moDCs) by *Me. stadtmanae* and *M. smithii*, but also methanoarchaeal susceptibility to a synthetic derivative of the human cathelicidin LL37 in low μM ranges (Bang et al. 2012). Thus, the release of AMPs by epithelium most likely is capable to influence not only bacteria of the microbiota but also archaea.

Interestingly, recent studies demonstrated severe proinflammatory response of peripheral blood mononuclear cells (PBMCs) as well as of moDCs to stimulation with *Me. stadtmanae*, whereas *M. smithii* induced only mild immune response (Bang et al. 2014b; Blais-Lecours et al. 2014). By recognition and binding of microbe-associated molecular patterns (MAMPs) on the surface of microorganisms, these and other immune cells recognize non-self-molecules by various membrane-bound, cytosolic or secreted receptors (pattern recognition receptors, RRRs) (Banchereau and Steinman 1998; Lipscomb and Masten 2002). After recognition of MAMPs, innate immune responses such as production of cytokines, initiation of the complement cascade or the release of AMPs are activated (Janeway 1989; Hart and McKenzie 1990; Lipscomb and Masten 2002). The specificity of various RRRs such as Toll-like receptors (TLRs) or NOD-like receptors (NLRs) for molecular microbial patterns as well as of the subsequent signal transduction cascades is in the focus of many studies (Gay and Gengloff 2007), but did not address archaea-associated molecular patterns. Very recently, the first comprehensive analysis regarding the specificity of TLRs and other PRRs potentially interacting with archaeal MAMPs was performed (Bang et al. 2014b). During this study, potential recognition by several common known TLRs and NLRs was evaluated: since the genome of *Me. stadtmanae* does not contain a coding DNA sequence (CDS) for flagellar proteins (Fricke et al. 2006) and the genome of *M. smithii* exhibits only two CDSs for putative flagellar proteins (Msm’0137, Msm’0662) (Samuel et al. 2007), recognition via TLR5, which is crucially involved in the recognition of bacterial flagellin, was not expected. Further, the unaffected activation of immune cells after heat-inactivation of methanoarchaeal cells that was obtained during earlier studies led to the assumption that neither methanoarchaeal DNA nor RNA is recognized by human immune cells. In fact, experiments using human embryonic kidney (HEK) 293 cells transfected with intracellular TLRs (TLR3, 7, 8 and 9) usually recognizing viral or bacterial DNA and/or RNA (Hemmi et al. 2000, 2002; Alexopoulou et al. 2001; Peng et al. 2005) did not result in the activation of immune responses, which was monitored by cytokine release (Bang et al. 2014b). Due to the unusual ether lipids of archaea (Fig. 1B), TLR2 was highly expected to bind lipid components in order to activate further signaling cascades (Takeuchi et al. 1999; Kataoka et al. 2006). This supposition was further enforced by several reports on the recruitment and activation of macrophages and DCs by archaeosomes in mice (Krishnan et al. 2007; Patel et al. 2007). Archaeosomes represent liposomes generated from archaeal ether polar lipids originated from various distinct archaeal strains such as *Thermoplasma acidophilum*, *M. smithii* or Halobacterium salinarum and are discussed as novel potential adjuvants, since they are able to induce CD8+ T-cell immunity (Krishnan et al. 2000, 2007; Krishnan and Sprott 2008). However, further studies also indicated that the membrane-bound receptors TLR2 as well as TLR2/1 and TLR2/6 were apparently not involved in recognition processes of *Me. stadtmanae* and *M. smithii*. Very recent studies showed that even the addition of purified lipid fractions of *Me. stadtmanae* and *M. smithii* did not lead to cytokine release by human immune cells (Bang, Heine and Schmitz, unpublished data), hence most likely excluding the unusual lipid moiety of the tested methanoarchaeal strains as archaea-associated molecular patterns. On the other hand, *Me. stadtmanae* and *M. smithii* exhibit very rigid cell walls composed of pseudomurein, whose chemical structure differs in the sugar moiety compared to murein, which is the most abundant cell wall component of bacteria. Bacterial murein of both Gram negatives and Gram positives is in part recognized via intracellular NOD1 and NOD2 receptors (Girardin et al. 2003a, b). Thus, those members of the NLR family were likewise expected to be involved in the recognition of archaeal pseudomurein (Fig. 3). However, experiments with HEK293 cells expressing NOD1 and 2 did not show recognition and activation by stimulation with *Me. stadtmanae* and *M. smithii*. In conclusion, the recognition of at least *Me. stadtmanae* and *M. smithii* does not appear to depend on common innate immune receptors such as the tested Toll-like (TLR2, 3, 4, 5, 7, 8, 9) or Nod-like (NOD1, 2) receptors, suggesting an alternative recognition mechanism that leads to cell activation of PBMCs as well as moDCs (Bang et al. 2014b). Identifying the potential human PRR(s) for archaea might also lead to the identification of involved archaea-associated molecular patterns and ultimately to a better understanding of the molecular crosstalk between archaea and their human hosts.

### Archaea and the adaptive immune system

Already in the early 1980s, evidence was obtained that methanoarchaeal species induce adaptive immune responses (Conway de Macario, Macario and Kandler 1982; Conway de Macario et al. 1983, 1984). In detail, monoclonal antibodies against several methanoarchaeal strains were detected in immunized rabbits, which revealed immunogenic properties in response to the methanoarchaeal pseudomurein glycan structures glucosamine, galactosamine, talosaminuronic acid and the C-terminal dipeptide γ-glutamyl-alanine. Besides, a later study reported on serum IgG against *M. smithii* and the closely related species *M. oralis* in sera of patients with periodontitis (Yamabe et al. 2008). These findings were approved by a recent study reporting on immunization of mice with cell lysates of *M. smithii* and *Me. stadtmanae* that revealed subsequent induction of antigen-specific IgGs in plasma with significant higher levels after treatment with *Me. stadtmanae* (Blais-Lecours et al. 2011). Moreover, this report demonstrated that compared to *M. smithii*, *Me. stadtmanae* induced a 3-fold higher accumulation of myeloid DCs in the airways of those mice. Most recently, one study confirmed these findings in humans by evaluating strain-specific serum IgGs in a healthy control group and a group of subjects suffering from IBD (Blais-Lecours et al. 2014). Here, the authors demonstrated that IgG levels specific for *M. smithii* were similar in both groups, although different amounts of *M. smithii* were detected in the respective stool samples. However, IgG levels specific for *Me. stadtmanae* were significantly enhanced in IBD patients with *Me. stadtmanae*-positive stool samples in

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Figure 4. Phagocytosis of methanoarchaeal cells leads to the activation of innate and adaptive immune responses. (A) moDCs were stimulated with methanoarchaeal cells for a period of 4 h in order to determine phagocytosis. Formed phagolysosomes in moDCs were stained with LysoTracker Red DND-99 during time of incubation, and cells were labeled with Hoechst for DAPI-staining. Scale bars indicate 5 μm. (B) Schematic simplification of moDC’s activation after phagocytosis of methanoarchaeal strains. After specific recognition of methanoarchaeal cells via yet not identified PRR(s), cells are phagocytized by moDCs. Thereafter, phagolysosomes are formed in order to degrade archaeal cells and presumably lead to activation of endosomal receptors. Subsequently, intracellular signaling cascades lead to the activation of MAPKs and transcription factors and finally result in the release of cytokines, antimicrobial peptides and the expression of modulatory surface molecules.

In conclusion, archaeal gut inhabitants appear to be able to activate antigen-specific adaptive immune responses as has been shown for many bacterial commensals. Besides evaluating these observations in more detail, particularly the impact of archaeal species on the differentiation of T- and B-cell populations has to be elucidated, since these populations are crucially involved in maintaining immune homeostasis and consequently in humans’ health and disease.

**ARCHAEA’S PUTATIVE INVOLVEMENT IN THE DEVELOPMENT OF DISEASES**

Based on the isolation of the first archaeal strains from human feces in the early 1980s, various reports addressed a direct correlation between the quantities of methanoarchaea in

comparision to IBD patients with *M. stadtmanae*-negative stool samples as well as in comparison to healthy control subjects with and without *M. stadtmanae* present in the stool. Since autoimmune diseases are suggested to be a result of inappropriate action of the adaptive immune system mediated by the gut microbiota, the authors proposed that *M. stadtmanae* might play a critical role during the establishment of chronic inflammatory diseases involving the human gut (Blais-Lecours et al. 2014).

Studying immune cell recognition and activation of *M. stadtmanae* and *M. smithii* with moDCs revealed phagocytosis of those strains (Fig. 4), which was further shown to be crucial for cellular activation by monitoring cytokine release (Bang et al. 2014b). In agreement, enhanced expression of the cell-surface receptors CD86 and CD197 on moDCs was obtained in the presence of *M. stadtmanae* and *M. smithii* demonstrating activation of adaptive immune responses, since expression of those cell-surface receptors is crucial for costimulatory signals that are involved in maturation of moDCs (Chen et al. 1994; Yanagihara et al. 1998). This finding might indicate that in the natural gut habitat, methanoarchaeal cells are phagocytosed by intestinal DCs as has been shown for commensal bacteria (Macpherson and Uhr 2004). Following phagocytosis, activated intestinal DCs are able to activate B- and T-cell responses within the draining lymph nodes that further result in adaptive immune responses such as selective IgA production by intestinal T cells (Fig. 4) (Steinman 2006; Coombs and Powrie 2008; Rescigno 2009, 2010).

In conclusion, archaeal gut inhabitants appear to be able to activate antigen-specific adaptive immune responses as has been shown for many bacterial commensals. Besides evaluating these observations in more detail, particularly the impact of archaeal species on the differentiation of T- and B-cell populations has to be elucidated, since these populations are crucially involved in maintaining immune homeostasis and consequently in humans’ health and disease.
the large intestine and the development of severe colon diseases (Table 1) (Haines et al. 1977; Karlin et al. 1982; Piquet et al. 1984; Weaver et al. 1986; Peled et al. 1987; Million et al. 2013). All of these studies based on breath-methane excretion experiments of numerous individuals and revealed conflicting data about the in- and decrease of methanoarchaea in severe gastrointestinal diseases. In this regard, a recent study demonstrated that breath-methane excretion was only found in 62% of individuals, though all tested subjects were positive for methanoarchaea using 16S rRNA gene-based PCR (Fernandes et al. 2013). Further, a positive correlation between the age of tested individuals and breath-methane concentration was found in this study. These interesting findings suggest that measuring exclusively breath-methane concentrations is not an appropriate method to study the overall methanoarchaeal abundance in health and disease, since it appears to be influenced by additional factors such as the age of individuals as well as by their diet. This assumption is further supported by the strikingly different findings on M. smithii’s abundance in obese patients during the last few years. In this respect, several studies proposed the involvement of M. smithii in the development of obesity (Zhang et al. 2009; Lee et al. 2011; Basseri et al. 2012; Mathur et al. 2013), of which some studies were also based on breath-methane studies (Basseri et al. 2012; Mathur et al. 2013). In addition, also a decrease of methanoarchaea in individuals with obesity or higher body mass index was observed (Schwitz et al. 2010; Million et al. 2012). Most interestingly, two studies also found that there is no significant correlation between the development of obesity and the abundance of methanoarchaea or particularly M. smithii, respectively (Fernandes et al. 2013; Million et al. 2013). Consequently, to date there is no conclusive evidence for the involvement of methanoarchaea in the development of obesity, high body mass index or anorexia. Thus, the inconsistent results of the above-mentioned studies that are summarized in Table 1 might be explained by external factors that disturb accuracy of the breath-methane measuring method. The investigation of Kim and co-workers points into the same direction, since they investigated whether there is a correlation between the degree of methane and the presence of M. smithii in IBD patients. Applying M. smithii-specific primers in a quantitative PCR of DNA originating from stool samples, they demonstrated that all IBD patients were positive for M. smithii, though not all patients were breath-methane-positive (Kim et al. 2012). This is a noteworthy observation, since there is increasing evidence that even the presence of methane in the human gut might be associated with decreased intestinal motility resulting in constipation-related diseases (Triantafyllou, Chang and Pimentel 2014).

A few years ago, a PCR-based study obtained evidence for decreased methanoarchaeal abundance in individuals suffering from gastrointestinal diseases (Scanlan, Shanahan and Marchesi 2008). However, primers specific to the methanoarchaeal mcrA gene were used in this study and, as mentioned above, these primers were later shown to be insufficient to detect Me. stadtmanae (Dréci 2012). In agreement with the mentioned methodological difficulties, the study of Scanlan et al. did not identify Me. stadtmanae in one of the 207 feces samples by PCR amplification using primers specific for Me. stadtmanae’s mcrA gene. A more recent study, using specific primer pairs for M. smithii and Me. stadtmanae, demonstrated a 3-fold increase of Me. stadtmanae’s abundance in individuals suffering from IBD compared to healthy controls, whereas M. smithii’s abundance did not differ between healthy and diseased individuals (Blais-Lecours et al. 2014). Though the total sample size was lower (29 diseased and 29 healthy individuals), this study indicated that Me. stadtmanae might be involved in pathologic conditions within the human intestine (Fig. 5). Based on the increased numbers and the high immunogenic potential of Me. stadtmanae (Bang and Schmitz 2014b; Blais-Lecours et al. 2014), it appears possible that this methanoarchaeal species has the capacity to trigger immune responses within the human intestine, whereas M. smithii appears to be a ubiquitous commensal gut inhabitant (Fig. 5). This fact would be in accordance with studies discussing that methanoarchaeal species, and in particular M. smithii, might represent metabolic key species within microbial consortia inhabiting the human body ecosystem (Rousk, Brookes and Baath 2009; Horz and Conrads 2011). Indeed, M. smithii’s metabolic function as a hydrogen consumer within the bacterial community is urgently required for the stability of the human gut ecosystem. In addition, M. smithii has been shown to be perfectly adapted to the human gut and its immune system and, most likely due to this fact, has been found to be a ubiquitous inhabitant of the human intestine, independently of the health status (Samuel et al. 2007; Dréci et al. 2009; Lurie-Weinberger, Peeri and Gophna 2011). Thus, overall the role of M. smithii in the commensal human intestinal microbiota appears to be the result of evolutionary co-adaptation of the human host and this archaeal species.

With respect to the health status of the oral cavity, it is noteworthy that the presence of M. oralis and M. orale-like species has been proposed to be linked to periodontal and endodontic diseases in nearly all reports evaluating archaeal diversity within this ecosystem (Table 1) (Kulik et al. 2001; Lepp et al. 2004; Vianna et al. 2006, 2008, 2009; Jiang et al. 2009; Li et al. 2009; Horz and Conrads 2011). Based on these findings, it was proposed that M. oralis is involved in the manifestation of periodontal disease e.g. by significantly lowering the redox potential in the microhabitats (Kulik et al. 2001; Vianna et al. 2006). In this respect, M. oralis appears to be able to promote the growth of pathogenic microbes and in conclusion is indirectly involved in pathogenicity (Vianna et al. 2008; Conway Macario and Macario 2009). The fact that there is only one study reporting on M. oralis’ presence in the oral cavity of healthy individuals to date (Bringuier et al. 2013) strongly supports this hypothesis. As mentioned before, the microbiota of the oral cavity was found to be less stable than the one of the gut (Costello et al. 2009) and thus, it is conceivable that colonization of dental plaque by high numbers of archaea is not as stable as it appears to be within the human gastrointestinal tract. However, during the establishment of microbial biofilms within the oral cavity, altered conditions might favor archaeal growth resulting in detectable high levels. Additional evidence comes from the observation that periodontal diseases are in general not caused by a single microbial species, but rather a consequence of microbial communities often existing as a consortium with synergistic interactions (Jenkins and Lamon 2005). Potential periodontal pathogens are known to attach through co-aggregation mechanisms such as adhesins to surface and build oral biofilms (Kolenbrander 2000, Kolenbrander et al. 2000). Comparative genomic studies of M. oralis’ very close relative M. smithii revealed regulated expression of adhesion-like proteins (Samuel et al. 2007) and thus, there are conclusive indications that M. oralis plays a role in the development and manifestation of periodontal disease. Based on a study by Yamabe et al. (2010), who identified one of the antigenic molecules of M. oralis as subunit of the group II chaperonins, a recent study examined human serum antibody response from patients with periodontitis and autoimmune disease to this molecule (Hirai et al. 2013). The authors obtained evidence for enhanced response of periodontitis and autoimmune disease patients to group II chaperonins by immunological detection and proposed
Figure 5. Potential interactions between methanoarchaea and the human immune system. Hypothetical model of the immunomodulatory abilities of archaeal species in humans is depicted. In healthy individuals (A), (methano)archaea are crucially involved in fermentation processes, tolerated by intestinal epithelial cells and able to induce adaptive immune responses. Besides, methanoarchaea are prone to the lytic effects of AMPs; however, they have the capability to regulate their release. (B) However, in case of tissue damage, some archaeal species such as *M. stadtmannae* appear to have the potential to be involved in inflammatory processes (directly or indirectly) leading to systemic immune responses. In contrast, commensal archaea such as *M. smithii* induce only mild immune responses.

A potential cross-reaction between human and archaeal group II chaperonins, which has already been described earlier (Yamabe et al. 2010). Since bacterial group I chaperonins have been shown to be highly antigenic molecules (Zügel and Kaufmann 1999), an involvement of the identified group II chaperonins in the development of periodontal and autoimmune diseases was proposed (Horz and Conrads 2011; Hirai et al. 2013).

In conclusion, although the number of studies on the presence and role of archaea in humans’ health and disease increased during the last decade, not a single archaeal pathogen has been discovered so far (Cavicchioli et al. 2003; Conway de Macario and Macario 2009; Gill and Brinkman 2011). Besides, it has been hypothesized that the development of numerous autoimmune, systemic and allergic diseases such as IBD,
diabetes or asthma might rather result from microbial dysbiosis than from the presence of a single pathogenic microorganism (Ding and Schloss 2014). In this respect, particularly the role of *Me. stadtmanae* in the development of autoimmune diseases of the human intestine such as IBD has to be considered, due to its enhanced abundance in IBD patients (see above), as well as its overall immunogenic potential (Bang et al. 2014b; Blais-Lecours et al. 2014). Thus, there is increasing evidence for a potential involvement of archaeal species in the development and manifestation of severe autoimmune and systemic diseases, which warrants future studies. Moreover, the involvement of *M. oralis* and *M. oralis*-like species in the manifestation of periodontal diseases has to be reflected in ongoing studies.

**CONCLUDING REMARKS**

The functional importance of the microbiota in humans’ health and disease has been confirmed in numerous studies and reports. Although archaea have been shown to be a stable part of the human-associated microbiota, a potential impact of archaeal strains on human immune homeostasis was rarely evaluated until now. However, recent studies strongly argue for a high immunogenic potential and a likely involvement of several archaeal species in the development of systemic diseases. Consequently, several issues have to be addressed in future studies regarding the overall impact of the human microbiota.

i. Most importantly, uniform and adequate detection methods for archaea associated with the human body have to be established in order to ensure reliable, comparable and approved evaluation of archaeal abundance in diverse human body sites.

ii. Recent reports on the inflammatory response of human immune cells to methanoregulae strongly argue for the presence of at least one specific archaea-associated PRR in humans, which has yet to be identified.

iii. The strikingly different immune response(s) to the most abundant archaeal gut inhabitants *Me. stadtmanae* and *M. smithii* might indicate diverse physiological roles of (methanoarchaea as part of the microbiota, which has to be confirmed.

iv. The role of archaeal species within the development of autoimmune disease has to be elucidated, since members of the domain Archaea are found in high abundances in the human intestine, the oral cavity and on human skin.

v. Based on the proposed high abundance, archaea may influence the overall human immune homeostasis to comparable extents as has been shown for bacteria and thus should be urgently included in all future studies regarding the human microbiota.

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