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Review

Akkermansia muciniphila and its role in regulating host functions

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ABSTRACT

Akkermansia muciniphila is an intestinal bacterium that was isolated a decade ago from a human fecal sample. Its specialization in mucin degradation makes it a key organism at the mucosal interface between the lumen and host cells. Although it was isolated quite recently, it has rapidly raised significant interest as *A. muciniphila* is the only cultivated intestinal representative of the Verrucomicrobia, one of the few phyla in the human gut that can be easily detected in phylogenetic and metagenome analyses. There has also been a growing interest in *A. muciniphila*, due to its association with health in animals and humans. Notably, reduced levels of *A. muciniphila* have been observed in patients with inflammatory bowel diseases (mainly ulcerative colitis) and metabolic disorders, which suggests it may have potential anti-inflammatory properties. The aims of this review are to summarize the existing data on the intestinal distribution of *A. muciniphila* in health and disease, to provide insight into its ecology and its role in founding microbial networks at the mucosal interface, as well as to discuss recent research on its role in regulating host functions that are disturbed in various diseases, with a specific focus on metabolic disorders in both animals and humans.

Contents

Abbreviations: DSS, dextran sulfate sodium; FISH, fluorescent *in situ* hybridization; HFD, high fat diet; FODMAP, fermentable oligosaccharides disaccharides; monosaccharides and polyols IBD, inflammatory bowel disease; LPS, Lipopolysaccharide; MGS, metagenomic species; qPCR, quantitative PCR; IgA, immunoglobulin A; T-RFLP, terminal-restriction fragment length polymorphism; T2D, type 2 diabetes.

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1. Culturability of the human gut microbiota

The human intestine is home to more than a thousand microbial species. A recent review pointed out that over 1000 microorganisms, belonging to Bacteria, Archaea and Eukarya, have been obtained in pure cultures [1]. In 1950, the study of intestinal bacteria was revolutionized by the development of an array of techniques for culturing strict anaerobes by Robert Hungate [2]. Prior to this, mostly only aerobic or facultative anaerobic bacteria could be isolated from intestinal samples. The use of strict anaerobic conditions according to the Hungate approach enabled the extensive characterization of the major intestinal microbes in the 1970s. Cultivation of most intestinal bacteria has been carried out using rich media, or semi-defined media with targeted carbon sources. In the late 1970s, Carl Woese discovered a third domain of life, Archaea, using a proposed universal phylogenetic marker, the 16S rRNA gene, that can be used as a signature of prokaryotic species [3]. This and the subsequent molecular revolution based on rapid sequencing methods have drastically changed the perception of microbial ecology, allowing for a more representative description of various ecosystems, and circumventing the need to cultivate bacteria in order to describe the community of a specific niche [3]. This has also emphasized that most of the sequences returned from profiling human intestinal microbiota samples are derived from microbes that have not yet been cultivated. In parallel, although there has been a decline in new cultivation approaches, there is an obvious renewal of interest in cultivating gut microbes. Indeed, obtaining bacteria in pure culture is complementary to molecular approaches since they provide information (e.g. physiology, interaction with host and other bacteria) that molecular approaches do not. However, the direct use of genome sequencing from intestinal samples to characterize as yet uncultivated microorganisms, can also provide information on their genetic capacity to use specific nutrients [4,5]. As a major fraction of the gut microbial ecosystem has not yet been cultured, it is often regarded as being refractory to cultivation in the laboratory. Although that is probably true for some microbes that are either too dependent on the host or on other bacteria to grow, the use of defined medium combined with novel isolation strategies (such as culturomics) has nevertheless, led to the successful isolation of an increasing number of intestinal bacterial species [6–8]. A recent example of an organism that was refractory to *in vitro* isolation is *Candidatus arthromitus* (also known as segmented filamentous bacteria, or SFB) that is found abundantly in the intestinal tract of mice although not, or not all, in humans. Using a strategy that combines an SFB–host cell co-culturing system, SFB was first isolated in pure culture in 2015 [9]. Some examples of currently uncultivable bacteria from human microbiota that are frequently detected in human samples by sequencing technologies include members of the Candidate TM7 phylum and Cyanobacteria [5], as well as some genera of Clostridiales such as *Oscillospira*, neither of which have been obtained in pure culture, although indications for the sequence of their genomes have been obtained. A species that was successfully isolated is *Akkermansia muciniphila* (Fig. 1). Interestingly it was, and still is, the first intestinal microbial isolate of the phylum Verrucomicrobia. With its isolation came the awareness that this phylum is represented in the intestine. It was originally isolated from a fecal sample from a healthy Caucasian female in a specific medium that contained purified mucin as the sole carbon source, using the most probable number approach [10]. Mucin was chosen as a selective carbon source since it was hypothesized that microbes capable of utilizing these host-produced glycans as carbon sources are those that are located at the interface between the luminal bacteria and the host, a prediction that materialized with the discovery of *A. muciniphila*.

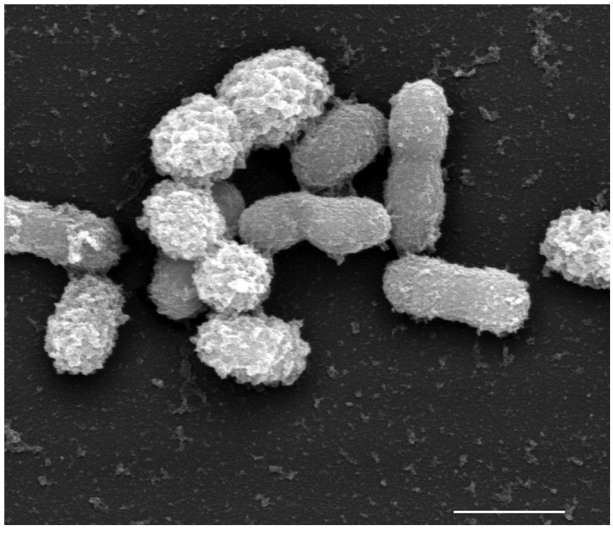

Fig. 1. Scanning electronic micrograph of *Akkermansia muciniphila* ATCC BAA-835 (Bar represents 1 μm.

2. Ecology of *A. muciniphila* in the intestine

2.1. Abundance in human samples

Once isolated, it was important to quantify the amount of *A. muciniphila* cells within human stool samples in order to evaluate whether it is commonly present. It was originally determined that *A. muciniphila* accounted for more than 1% of the total microbiota using fluorescent *in situ* hybridization (FISH) and quantitative PCR (qPCR) [11,12]. Notably, at that time, FISH was also commonly used to quantify major bacterial taxa. Interestingly, it was observed that *Akkermansia* spp. could not be targeted by the classical EUB-338 I universal bacterial probe. Later, the wider availability of 16S rRNA gene sequencing allowed for the detection of the genus *Akkermansia* in a large number of studies. When the

Table 1
Overview of clinical studies (observational or interventional) related to metabolic disorder in which a differential *Akkermansia* abundance was observed.

Target population	Study (observational or intervention)	Groups (number of individuals)	Microbiota analysis approach	Samples analysed time	*Akkermansia* population	References
Obese women	Observational	Obese women (n = 53)	Whole shotgun metagenomic	Stool One time point	Negatively associated with markers for insulin resistance or dyslipidaemia	[96]
Elite athletes	Observational	Elite athletes high BMI (n = 40) Healthy males Low BMI ≤25 (n = 23) Healthy males high BMI (n = 23)	16S sequencing	Stool One time point	Higher proportions in athletes and in low BMI control group	[122]
Infants of overweight and normal-weight mothers	Observational	Lean (n = 16) Overweight mothers (n = 26) Infants (1, 6 months)	qPCR FISH-FCM	Stool Infants (1, 6 months)	Decreased prevalence in infants of normal-weight mothers and of mothers with normal weight gain during pregnancy	[98]
Lean and overweight lactating women	Observational	Lean women (n = 34) Overweight women (n = 22)	qPCR	Breast milk (after delivery, 1 and 6 months later)	Trend towards increased prevalence in breast milk (1 month after delivery) from overweight mothers	[37]
Overweight and obese adults	6-week calorie restriction (CR) and 6-week follow up	Overweight (n = 11) Obese (n = 38)	qPCR Metagenomic	Stool Baseline (T0) After CR (T = 6 weeks) After weight stabilisation (T = 12 weeks)	At baseline, *A. muciniphila* MGS was inversely related to fasting glucose, waist-to-hip ratio, and subcutaneous adipocyte diameter Subjects with higher level of *A. muciniphila* at baseline had greater improvement in insulin sensitivity markers and other clinical parameters after CR	[15]
Lean, overweight and obese adults	Observational	Lean (n = 10) Overweight (n = 10) Obese (n = 10)	16S sequencing	Stool One time point	*Akkermansia* negatively correlated with BMI	[95]
Lean, overweight and obese children(4–5 years)	Observational	Lean (n = 20) Overweight, obese (n = 20)	qPCR, T-RFLP	Stool One time point	Decrease in obese/overweight children	[92]
Obese women	8-week of impact of 4 g of Ephedra sinica extract/day	Obese women (n = 7)	16S sequencing	Stool 2 samples/subject (before and after Ephedra sinica extract intake)	Positive association of *Akkermansia* with weight loss	[123]
T2D and healthy individuals	Observational	T2D (n = 71) Healthy controls (n = 74)	Whole shotgun metagenomic	Stool One time point	Increase in T2D	[99]
Overweight individuals	1-week fasting program and 6-week probiotic intervention	Overweight adults (n = 13)	qPCR	Stool Before fasting (T1) During fasting (T2) After 6-week probiotic intervention (T3)	Increase between T1 and T3	[124]
Obese individuals	16-week weight reduction diet	Obese individuals (n = 33)	qPCR	Stool Before, during and after intervention	Increase after weight reduction	[94]
Normal weight and overweight pregnant women (24 weeks)	Observational	Normal weight (n = 34) Overweight (n = 16)	qPCR	Stool One time point	No difference between normal and overweight Decrease in excessive weight gain	[97]
Adult women	Observational	Lean (n = 17) Obese (n = 50)	qPCR	Stool One time point	Trend to increase prevalence in lean individuals	[93]

(*continued on next page*)

Table 1 (*continued*)

Target population	Study (observational or intervention)	Groups (number of individuals)	Microbiota analysis approach	Samples analysed time	*Akkermansia* population	References
Lean, morbidly obese post-gastric-bypass surgery human subjects	Gastric bypass	Normal weight (n = 3) Morbidly obese(n = 3) Post-gastric-bypass surgery (n = 3)	16S sequencing	Stool One time point	Increase after bariatric surgery Low in obese	[85]
Normal glucose tolerance (NGT), Prediabetes (PD) and newly diagnosed T2D subjects	Observational	NGT (n = 44) Pre-DM (n = 64) T2D (n = 13)	16S sequencing	Stool One time point	Decrease in Pre-DM and T2D	[125]

qPCR: Quantitative PCR, FISH-FCM: Fluorescent *in Situ* Hybridisation coupled with flow cytometry, FODMAP: Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols, MGS: Metagenomic Species, T-RFLP: Terminal-Restriction Fragment Length Polymorphism, T2D: Type 2 Diabetes.

human gut microbiota was proposed to segregate into three distinct bacterial communities or enterotypes, *Akkermansia* spp. were found to occur in the enterotype in which *Ruminococcus* or Clostridiales were the main drivers [13]. Using a correlation network, *Akkermansia* was found to be positively associated with *Gordonibacter* as well as Ruminococcaceae, and negatively associated with *Prevotella* [13]. In two independent clinical studies (Danish and French cohorts), *A. muciniphila* was present in greater abundance in subjects with a high metagenome richness [14,15]. Moreover, in the French cohort, correlation networks of metagenomic species (MGS) including that of *Akkermansia*, were determined. Several MGS positively correlated with *Akkermansia*, including *Methanobrevibacter smithii*, the most abundant and prevalent methane producer, as well as members of the family Ruminococcaceae [15]. This could indicate that these bacteria have similarnutritional requirements or that they engage in cross-feeding.

In mono-colonised mice, *Akkermansia* is more prevalent in the colon than in the ileum [16]. This is supported by studies in conventional mice [17]. In an *in vitro* model involving three fermenters to mimic the human colonic environment, *Akkermansia* was not detected in the microbiota of the ascending colon, while it was detected in the transverse and descending colon compartments, with highest abundance in the transverse compartment [18,19].

2.2. Ecological advantage of intestinal mucus

Intestinal mucus is composed of an inner layer devoid of bacteria and a thicker outer layer with commensal bacteria [20]. Its major components, mucins, are a source of nutrients for intestinal bacteria since it is composed of amino acids and oligosaccharides. Some bacteria possess the enzymatic machinery necessary for the breakdown of the mucins' oligosaccharide chains, which in turn, release fucose, galactose, N-acetylglucosamine, N-acetylgalactosamine, sialic acid, sulfate, and also di-sacharides and small oligosaccharides that can be further metabolized by the resident microbiota. Mucin degradation by commensal bacteria has been reviewed elsewhere [21,22]. Elegant experiments with nano-SIMS (stable isotope mass spectrometry) in combination with FISH showed that *Akkermansia* is among the first utilizers of labeled mucus on the mouse mucosa [23]. Mucin degradation offers an ecological advantage to bacteria that are dependent on dietary nutrients. Indeed, in the absence of dietary glycans, host-derived mucins represent a constant source of nutrients. This advantage would explain the diverse ecological habitats of *Akkermansia* spp. When we screened the databases available for the presence of *Akkermansia* species it became apparent that this organism is commonly found in the intestines all over the animal kingdom [24]. Indeed, *A.* spp. have been detected in the intestines of various animals including rodents (references Table S1), but also donkeys [25], rabbits [26], Syrian hamsters [27] pythons [28], horses [29,30], pigs [31] and rock ptarmigans [32] amongst others. An interesting finding has been that its relative abundance increases in humans, mice, hamsters and snakes under conditions of caloric restriction (Table 1 and S1). This supports the suggestion of the existence of mucosal communities driven by *A. muciniphila* [24]. While these experiments do not exclude the possibility that there are other mucus utilizers, it is relevant to note that mucosal analysis showed *A. muciniphila* to be abundant in biopsies of healthy subjects but reduced in those of patients with Inflammatory Bowel Disease (IBD), who in contrast, had increased amounts of *Ruminococcus gnavus* [33]. Comparative biochemical analysis of the sialidases of these bacteria revealed marked differences consistent with a selfish phenotype of *R. gnavus*, while the *A. muciniphila* lifestyle was compatible with a phenotype that stimulates tropic chains [34].

3. Development of *A. muciniphila* during the human life span

The development of the intestinal *A. muciniphila* community with age has been investigated in several studies with subjects of different nationalities. In an early study, its development over a wide age-range was determined in infants (1, 6 and 12 months of age), adults (25–35 years of age) and the elderly (80–82 years of age) in a Finnish population. *A. muciniphila* was found to colonize the intestinal tract in early life and to achieve a level close to that observed in adults within a year [12,35]. *A. muciniphila* was more abundant in adults than in Finnish children less than one year old, as determined by qPCR and FISH. In the same study, the *A. muciniphila* levels were found to decline in elderly subjects [12]. In another study that compared the abundance of selected bacteria in breast-fed Finnish and German infants at 6 months of age [36], the presence of bacteria resembling *A. muciniphila* was also detected at a very low concentration in breast milk (<3 log number of gene copies/ml of breast milk) [37] and in the microbiota of human breast tissue [38]. This could be attributed to the presence in breast milk of oligosaccharides that resemble mucin in composition and structure [21]. However, studies have indicated that *A. muciniphila* levels were higher in formula-fed as compared to breast-fed infants [39,40].

A detailed comparison was made between the fecal microbiota of Italian centenarians (99–104 years), elderly (63–76 years) and young adults (25–40 years) using qPCR and a phylogenetic array approach. Notably, there was less *A. muciniphila* present in young

adults compared to elderly subjects [41]. However, a slightly lower abundance of *Akkermansia* was observed in Chinese centenarians (100–108 years) compared to Chinese elderly subjects (80–92 years) [42]. These studies suggest that the evolution of *Akkermansia* communities with age may differ between populations and this warrants further investigation. However, care should be taken to interpret all the results of these different studies and populations as it has been shown that the levels of *Bifidobacterium* and *Bacteroides* spp., and to some extent also *A. muciniphila* levels, correlate with the fucosylation status, suggesting that the genotype of the host determines the colonization of mucus [43]. Moreover, the abundance of *A. muciniphila* in the human intestine may depend on body mass, mucus thickness and the immune status of the host. These factors are likely to change during a life time and also during or immediately prior to the onset of disease. Such changes might be expected to alter the *A. muciniphila* levels. Some studies in mice reported a decreased relative abundance of *Akkermansia* with age. In aging leptin deficient obese mice (16 versus 8-week old mice), the level of *Akkermansia* decreased as glucose tolerance declined [44]. Interestingly, in immunodeficient Rag1−/− mice (lacking mature lymphocytes), the abundance of *Akkermansia* also decreased with age [45]. However, it should be emphasized that the translation of results from mouse models is continuously challenged [46] and it is has now been well established that the human and mouse microbiome only share small fraction of common metagenomic information [4].

4. Modulation of *Akkermansia* spp. Following dietary or pharmaceutical interventions

4.1. Modulation by diet

Various nutritional interventions, mainly in animal models, have been reported to affect the levels of *Akkermansia* spp in the intestine. In some cases, the administration of specific dietary components improved host health and also increased the *Akkermansia* population. Such compounds include polyphenols [19,47,48], fructo-oligosaccharide [49,50], conjugated linoleic acid [51], oat bran [52], type 2 resistant starch from high-amylose maize [53], fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAP) [54], whole grain barley [55] and polyamines [56,57], red pitaya [58] *Bifidobacterium animalis subsp lactis* LMG P-28149 [59], maize-derived non-digestible feruloylated oligo- and polysaccharides [60], amongst others. Interestingly, medicinal herbs (*Flos Lonicera* and fermented *Rhizoma Atractylodis Macrocephalae*) as well as fungi (*Ganoderma lucidum*) that are traditionally considered in Asian culture as medicine [61–63], also increased the proportion of *Akkermansia* with a concomitant beneficial effect on host metabolism. *Akkermansia* spp. were also reported to be increased upon consumption of pectin or guar gum only in rats fed a High Fat Diet (HFD) [64] or barley malt [55], but decreased in HFD supplemented with barley malt [55]. However, a decrease in the level of *Akkermansia* was observed in rats fed a HFD enriched in pectin or guar gum [64]. Several studies have reported a decrease in abundance of *Akkermansia* following HFD [65–67]. Recently, a diet enriched in heme (the pigment of red meat) was shown to increase the abundance of *Akkermansia* in mice [17,24]. Various reports describe the impact of pharmaceutical treatments on the intestinal level of *Akkermansia*. Metformin is a common antidiabetic drug and was found to increase the levels of *Akkermansia* spp. populations in mice both on a HFD and normal diet, while the growth of *A. muciniphila* was stimulated in Brain Heart Infusion broth supplemented with metformin [67,68]. Another widely used drug, the proton pump inhibitor omeprazole, was shown to decrease the abundance of *Akkermansia* spp. in mice [69]. However, this has not been noted in a recent human study where the effect of proton pump inhibitors had some impact on bacteria other than *Akkermansia* [70].

4.2. Modulation by antibiotics

A major known stressor of the intestinal microbiota is the exposure to antibiotics. Although the impact on the microbiota is antibiotic-dependent, in both animals and humans, *Akkermansia* spp. was found to bloom after exposure to specific antibiotics. However, many studies are only based on 16S rRNA sequencing data and these do not provide a quantitative approach and hence it is not always clear whether *Akkermansia* spp. increase in number, or remain stable while the other members of the gut microbiota are severely impacted. An example is a recent study reporting that the use of tylosin boosted the relative abundance of *Akkermansia* in young mice [71]. In a more comprehensive study, on the basis of 16S rRNA gene sequencing, an enormously increased level of *Akkermansia* spp. (up to 80%) was reported in two subjects treated with broad-spectrum antibiotic therapy [72]. As the subjects did not have gastrointestinal disorders, this indicates that these extreme numbers have no harmful effect on the human host. This unusual observation led to a further exploration of the absolute amount of *Akkermansia* present in the stool of these individuals by FISH, which confirmed the increase in relative and absolute abundance of *Akkermansia* in these adults. Although the *Akkermansia* strain responsible was not isolated in pure culture, its deduced genome based on metagenome analysis of an *Akkermansia*-enriched stool sample revealed high identity to the cultured strain, *A. muciniphila* Muc^T [73]. The cultivated *A. muciniphila* strain Muc^T culture appeared to be resistant to vancomycin and metronidazole [72]. Higher abundance of *Akkermansia*, together with lactic acid bacteria and other bacteria with unusual cell-envelopes, was also observed in the microbiota of adult volunteers exposed to therapeutic doses of vancomycin [74]. Similarly, an increase of *Akkermansia* was observed in one patient, reaching 6% of total bacteria, after 6 days of consumption of a β-lactam. However, the level of *Akkermansia* declined at the end of the 14- day consumption period [75,76]. The apparent vancomycin-resistance of *A. muciniphila* explains why vancomycin treatment resulted in an enrichment of *Akkermansia* in a NOD mouse model for type-1 diabetes [77].

5. *Akkermansia* in health: the case of metabolic disorder

Several studies reported a reduction in the abundance of *A. muciniphila* in various disorders and diseases in humans. The majority of these include intestinal diseases, such as IBD, as well as extra-intestinal diseases, such as autism, atopy or obesity and related diseases. In this section we will focus primarily on *Akkermansia* and metabolic disorder as most studies have addressed associations with this widely spreading aberration.

"Metabolic disorder" encompasses a variety of clinical manifestations, including central obesity and impaired fasting glucose, and ultimately increases the risk of developing T2D or cardiovascular diseases. In experimental models of obesity, increased intestinal permeability leads to metabolic endotoxemia, a migration of Gram-negative derived lipopolysaccharide (LPS) from the intestine into the circulation [78]. A great variety of association studies involving the intestinal microbiota and metabolic syndrome have been reported, with some contradictory results (for a review see [79]). Few studies showed an effect of fecal transplantations in germ-free or conventional animals [80,81]. Moreover, in a pioneering human fecal microbiota transplantation study, individuals with metabolic syndrome were infused with microbiota from a lean donor or their own microbiota. After 6 weeks, an improvement in insulin

sensitivity was observed only in the lean donor treatment group and this was associated with the increase of a phylotype related to the butyrate producer *Eubacterium hallii* in the small intestinal mucosa [82]. This study supports the therapeutic potential of manipulating the gut microbiota for treatment of metabolic syndrome, and more specifically, for promoting insulin homeostasis in human.

5.1. Insight from animal studies

Recently, it was established that the intestinal *Akkermansia* abundance decreased in various knock-out or diet-induced mouse models that develop obesity or other signs of metabolic disorder. No less than 25 studies are reported (Table S1). These include studies that reported a lower abundance of *Akkermansia* in mice that were leptin deficient [49,66] or fed a HFD [66,71,83] and which were all obese or had T2D-like symptoms. Moreover, induced weight loss through gastric bypass surgery has been shown to lead to increased fecal numbers of *Alistipes* spp., Proteobacteria and Verrucomicrobia in the mouse and this was associated with immediate amelioration of glucose metabolism [84]. This is reminiscent of an earlier bypass study in obese humans where increased levels of *Akkermansia* among others, were reported in fecal samples [85]. While these studies are indicative of an association with *Akkermansia*, only a limited number of interventions have been described and provide direct support for the role of *A. muciniphila* in preventing metabolic disorders. In a seminal study, it was found that the daily administration of live cells of the *A. muciniphila* type strain (10^8 CFU/day) for four weeks could counteract the deleterious metabolic features of a HFD diet in mice [66]. Remarkably, this administration of *A. muciniphila* prevented not only weight gain, but also restored epithelial integrity (mucus thickness) that was disturbed by HFD treatment, counteracted metabolic endotoxemia (serum LPS), and improved the metabolic profile [66]. This impact was only observed when viable *A. muciniphila* was administered, suggesting that metabolically active bacteria were required. The positive impact of *A. muciniphila* on metabolic syndrome features was subsequently reproduced in independent studies in various parts of the world using different experimental designs. In a metabolic feeding study, mice were first fed with a HFD for four weeks, followed by daily gavage of *A. muciniphila* (4.10^8 cfu/day) for six weeks, resulting in improved glucose tolerance and metabolic endotoxemia. However, a daily dose greater than 4.10^7 live cells of *A. muciniphila* is necessary to improve the impaired glucose tolerance [67]. Of great interest was the additional observation that this *A. muciniphila* treatment also increased the number of T-reg and globlet cells in the gut, in line with observed immune signaling and increased mucus production. In another recent study, a mouse strain prone to obesity (AxB19) was used to investigate the impact of *A. muciniphila* inoculation on metabolic parameters [86]. Male AxB19 mice were fed with viable or heat killed *A. muciniphila* for one week followed by the high fat and high sucrose diet for 4-weeks, with concomitant *A. muciniphila* gavage (10^9 cfu/day). After five weeks of gavage, a significant decrease of various metabolic disorder parameters was observed, including body weight, body fat and insulin resistance among others [86]. Finally, Chevalier et al. inoculated live *A. muciniphila* (2.10^8 cfu/day) into germ-free mice conventionalized with microbiota from cold-exposed mice for 21 days. Notably the co-inoculation of *A. muciniphila* with the cold microbiota prevented the transferable increase in intestinal glucose absorption that was observed when the inoculum consisted of the cold-microbiota alone [87].

Overall, these observational and interventional studies suggest a role for *A. muciniphila* in the improvement of glucose-insulin homeostasis, reduction of fat accumulation and body weight, and— importantly, this was reproduced by different laboratories and with different experimental designs. While the mouse studies are convincing, some recent rat studies reported conflicting data. An increased abundance of *Akkermansia* was found in rats on a HFD supplemented with barley compared to the HFD control, while a low fat diet supplemented with barley led to a lower abundance of *Akkermansia* [88]. Similarly, an increased abundance of *Akkermansia* was reported in rats on a HFD [89–91]. The different outcomes are likely the effects of environmental and genetic factors. Moreover, these animal model experiments call for human trials with *A. muciniphila* to further study causality in a real life situation.

5.2. Insights from clinical studies

In clinical studies (Table 1), the abundance of *Akkermansia* is generally decreased in individuals with metabolic impairments, such as obese children [92] and adults (trend) [93]. Others show negative correlations between *Akkermansia* spp. and markers of metabolic disorder [94,95]. Variability between studies can be due to several factors including microbiota, host andmucin production amongst others. In a recent study, using quantitative metagenomics, *A. muciniphila* was found to be negatively associated with serum total and LDL cholesterol in obese Danish women. Moreover, a negative correlation was observed between dietary fat intake and its fecal abundance [96]. In a large observational study of a Danish cohort that consisted of obese and lean individuals, subjects with a high metagenome richness were found to be healthier than individuals with low metagenome richness. Furthermore, *A. muciniphila* was present in greater abundance in the former group [14]. Recently, these findings were reproduced in a French cohort consisting only of obese and overweight individuals [15]. The relationship between gut microbiota, metabolic syndrome and diet intake before and after a 6-week calorie restriction intervention was also investigated [15]. *A. muciniphila* MGS negatively correlated with fasting glucose, waist-to-hip ratio, and subcutaneous adipocyte diameter. Interestingly, individuals who harbored more *A. muciniphila* at baseline exhibited a better metabolic profile including improved insulin sensitivity [15]. In addition, *Akkermansia* spp. were less prevalent in women who gained more weight during pregnancy in a Spanish cohort [97]. Surprisingly, in a Finnish cohort, infants of overweight mothers harbored *Akkermansia* more frequently than infants of normal-weight mothers [98]. While a large study of Chinese subjects found that *Akkermansia* was more abundant in T2D patients compared to healthy controls [99], the antidiabetic drug metformin was recently reported as a confounding factor [100] and it has been shown that *A. muciniphila* can be stimulated by metformin [68]. Therefore, the high abundance of *Akkermansia* in these individuals could be an indirect effect of taking the drug. Support for this explanation stems from another study with Chinese adults where *Akkermansia* was found to be lower in pre-diabetic and newly diagnosed T2D patients [85]. Thus, it is tempting to speculate that metformin may also have an indirect effect on host metabolism via *A. muciniphila*, which could open new avenues to understanding the potential beneficial effect of metformin in T2D.

6. Impact of *Akkermansia* on barrier function, immune response and gut microbiota: insights from preclinical models

6.1. Barrier integrity

Compromised barrier function is the basis for many diseases

varying from IBD to metabolic syndrome. One of the mouse models for IBD that targets intestinal barrier function include mice treated with dextran sulfate sodium (DSS). In some studies *Akkermansia* was found to increase markedly after DSS treatment [101–104], while in other studies this was not observed [105]. One could speculate that the thickness of the mucus layer and the observed low-grade inflammation in the DSS mice may negatively influence *A. muciniphila* colonization. Support for the beneficial effect of *Akkermansia* on colitis derives from the observation that extracellular vesicles from *A. muciniphila* were found to protect against the DSS-induced phenotype [105]. In most human studies a depletion of *A. muciniphila* is observed in IBD mucosa and in fecal samples from ulcerative colitis patients [33,106]. However, some other studies do not show this effect, but a technical bias cannot be excluded as the *A. muciniphila* may have a special spatial location in fecal samples [107].

The intestinal barrier plays a crucial role by spatially protecting the intestinal cells from the luminal bacteria through the turnover of mucus (synthesis and shedding), production of secretory immunoglobulin A (IgA) via immune exclusion, and secretion of antimicrobial peptides and proteins, mostly in the ileum, by Paneth cells [108]. Intestinal mucus is composed of an inner layer devoid of bacteria and a thicker outer layer with commensal bacteria [20]. Its major components, mucins, are a source of nutrients for intestinal bacteria since it is composed of amino acids and oligosaccharides. Some bacteria possess the enzymatic machinery necessary for the breakdown of the mucins' oligosaccharide chains, which in turn release fucose, galactose, N-acetylglucosamine, N-acetylgalactosamine, sialic acid, sulfate, and also di-sacharides and small oligosaccharides that can be further metabolized by the resident microbiota. Mucin degradation by commensal bacteria has been reviewed elsewhere [21,22]. As *Akkermansia* is specialized in mucin degradation, it is expected that its variation in the intestine might be associated with the amount of mucin present, although other bacteria are also involved in mucin degradation [21,22]. In rodents on a diet with or without prebiotics (arabinoxylans, inulin), *Akkermansia* abundance (as measured by FISH) positively correlated with the level of mucins in the cecum [109]. Earle et al., using imaging methods, observed a bloom of the *Akkermansia* population in mice following a depletion of microbiota-accessible carbohydrates, resulting in a thinner mucus layer in the distal colon [110]. Recently, a study described the enrichment of *Akkermansia* in mice on a heme-enriched diet, and its depletion in mice fed with heme and a cocktail of broad-spectrum antibiotics (ampicillin, neomycin and metronidazole). The reduction of *Akkermansia* abundance following antibiotic treatment was accompanied by a reduction in the expression of the gene Muc2 encoding the major mucin of the colonic mucus in colonic tissues, as well as reduced mucolysis [111]. Indeed, besides being able to degrade mucins, *Akkermansia* was also found to simulate mucin production [67,68]. Hence, *Akkermansia* has not only the capacity to degrade mucins, but also to stimulate mucin synthesis, illustrative of an autocatalytic process. Regardless of whether its capacity to degrade mucins or stimulate their production depends on mucus thickness, the impact of

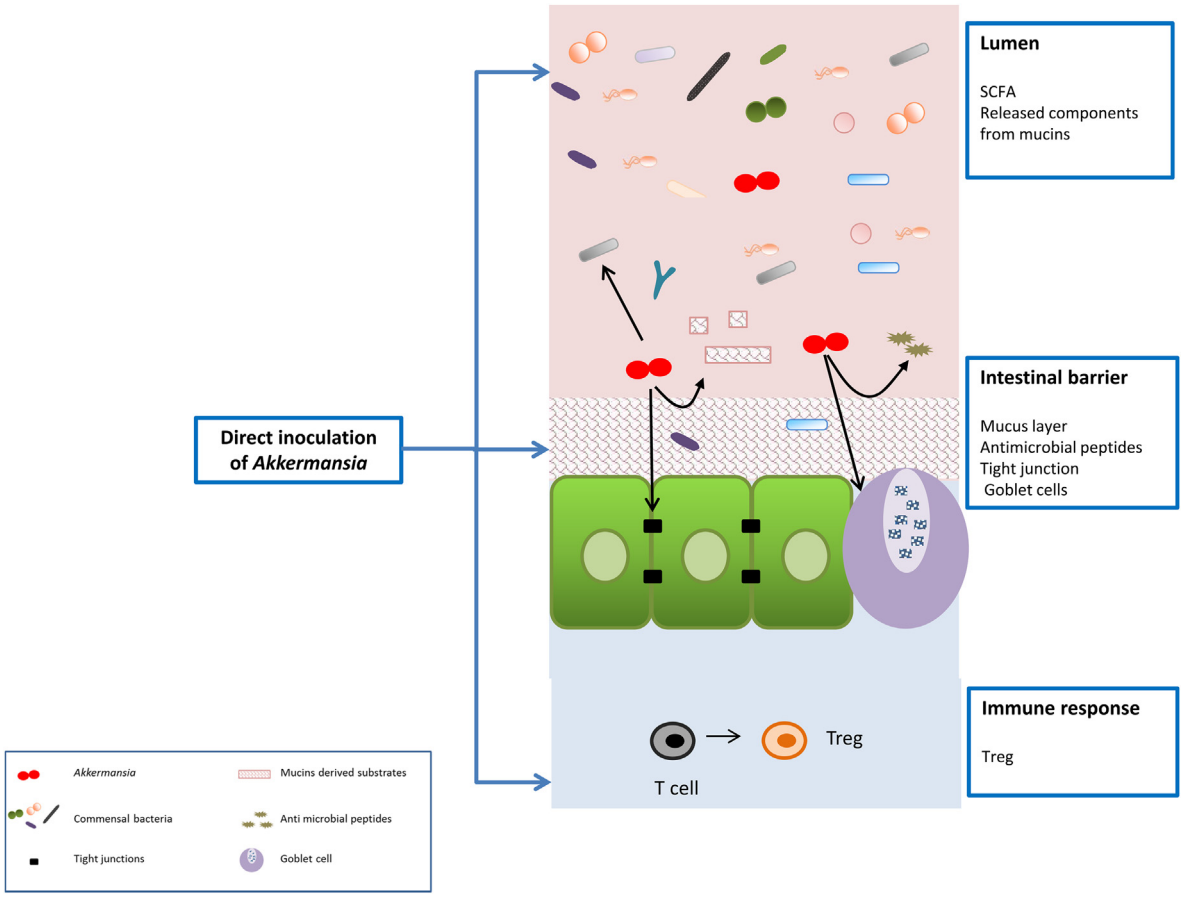

Fig. 2. Schematic representation of the interaction of *Akkermansia* with the microbiota and its host. *A. muciniphila* may impact the resident microbiota by suppling growth factors released from mucin degradation. *A. muciniphila* interacts with its host by strengthening the intestinal barrier, or by modulating mucin turnover and immune responses.

the resident microbiota on mucin turnover in the gut merits further investigation.

6.2. Immune response

The first inoculation study with *A. muciniphila* involved a 7-day follow-up after a single inoculation of germ-free (GF) mice [16]. Subsequently, the host's transcriptional responses were determined in the small and large intestine. Although the normal behavior of a bacterium cannot be accurately mimicked in GF models, in which competition for niche and substrates is lacking, the study nevertheless highlighted the preferential niche of *A. muciniphila* in the colon, and the potential communication with host, with emphasis on immune signaling. Recently, the impact of *A. muciniphila* metabolites on the transcriptome of mouse ileal organoids was determined, with SCFA, notably propionate, as controls [112]. Both propionate alone, and metabolites produced from the grown culture, could induce pathways involved in lipid metabolism, growth, and immune response, confirming the previous experiment [22]. In addition to the mucus layer, antimicrobial peptides and the immune system are factors that maintain homeostasis. Mice fed a HFD with daily gavage of *Akkermansia*, showed enhanced production of the antimicrobial peptide Reg3γ in the colon, and slightly in the ileum [66]. This was not observed in the group fed with heat-killed *Akkermansia*. Secretory IgA is essential to maintain the luminal bacteria under control, and to keep the homeostasis at mucosal surfaces (see [113–115] for recent reviews). Recently, the microbiota was studied from discordant twins for Kwashiorkhor, a form of malnutrition that results from protein deficiency [116]. It was hypothesized that IgA would target the microbiotas of the undernourished and the healthy twin differently. To test this hypothesis, viable bacteria were isolated from a pair of discordant Malawian twins. Interestingly, *Akkermansia* was the major IgA-targeted bacterium in the healthy twin child, while members of Enterobacteriaceae were the major IgA-targeted bacteria in the undernourished child. Direct inoculation of live *A. muciniphila* with one other selected bacterium, *Clostridium scindens* prevented lethality in mice, and reduced the sloughing of epithelial cells compared to mice colonized with a consortia of IgA-targeted bacteria from the undernourished child, and fed with a diet mimicking the Malawian diet [116]. Interestingly, no increase of IgA was observed following a 4-week *A. muciniphila* gavage with HFD [66]. These results indicate that *A. muciniphila* is actively communicating to the host immune system and is in line with earlier findings that its administration stimulated the proliferation of anti-inflammatory regulatory T cells in mice [67].

Recently, Reunanen and colleagues examined the effect of live *A. muciniphila* on *in vitro* colonic cell lines (HT-29 and Caco2). Interestingly, *A. muciniphila* was able to adhere to the epithelium and strengthen the intestinal barrier. Notably, it induced a weak pro-inflammatory response (measured by IL-8 release) compared to a strain of *E. coli* K12 [117]. The intimate relationship of *A. muciniphila* and the host immune system is exemplified in the recent study from Zhang, where mice mutated in gene Rag1 (Rag1 −/−), which lack mature lymphocytes, exhibit a notable increase of *Akkermansia* according to the results of 16S sequencing [45]. It is also worth mentioning that *A. muciniphila* has been associated with increased inflammation in a mouse model of a simplified microbiota with a *Salmonella typhimurium* infection [118]. The unexpected outcome from these experiments may relate to the use of GF mice that have a strongly compromised mucus barrier function prior to microbial colonization. Remarkably, it was found that *A. muciniphila* did not bind as effectively to mucus as a very active mucus-binder such as the probiotic *Lactobacillus rhamnosus* GG that is decorated with a mucus binding protein [117,119]. Another recent study, however, showed mucus binding of the type strain of *A. muciniphila* as well as isolates from healthy and IBD patients, indicating that experimental conditions may determine the strength of the binding [120].

6.3. Resident gut microbiota

The potential modulation of the resident gut microbiota following *A. muciniphila* intake has also been investigated in three of the four mouse studies described [66,86,87] in which *A. muciniphila* cells were gavaged into mice. While no significant modulation of the gut microbiota was detected after daily gavage of 10^8 cfu *Akkermansia*/day for four weeks [66], it was reported in two studies that some shift occurred between the two major phyla, Bacteroidetes (increased) and Firmicutes (decreased) following a 5-week gavage of 10^9 cfu *Akkermansia*/day [86], and following inoculation of live *A. muciniphila* (2.10^8 cfu/day) for 21 days in germ-free mice conventionalized with microbiota from cold-exposed mice [87]. Notably an increase of *Bifidobacterium* was reported. Apart from the different doses used in studies, other variations included the methodology used, the culturing of cells and other factors. As discussed above, it is likely that administration of *Akkermansia* impacts the mucosal microbiota and its networks. In conclusion, all of these studies highlight that *A. muciniphila* communicates with host, exhibits potential anti-inflammatory responses, promotes barrier integrity and potentially modulates resident gut microbiota (Fig. 2). However, additional work is needed to understand the association of *Akkermansia* and inflammation in some disturbed conditions, notably in humans.

7. Perspectives

In summary, overall there are consistent findings related to decreased abundance of *Akkermansia* in metabolic disorder in both preclinical and clinical studies. The few interventional studies have reported a beneficial impact on host metabolism, strongly indicating a direct involvement of *Akkermansia*. However, some opposing effects have been reported, suggesting that further research should be carried out to investigate the relationship between *Akkermansia* and host metabolism. The presence of *A. muciniphila* in the upper intestine could be of importance for health in later life, because the microbial colonization of the mucosal layer by *A. muciniphila* can enable associations with other beneficial microbes, leading to a stable mucosal colonization throughout life. Moreover, a host with no or very low levels of *A. muciniphila* might therefore have a strong and specific response toward *A. muciniphila* derivatives given that they can be quite different from other microbial signatures in the gut. Possibly, the strong effects of *A. muciniphila* treatment are due to its phylogenetic distinctness within the intestinal microbiota. As this *A. muciniphila* is the only cultivated representative of a whole phylum, it has many proteins that are typically and solely expressed by this organism, with no homologues within the microbiome. A total of eight different species in the genus *Akkermansia* have been identified [121], supporting the unexplored *Akkermansia* diversity within the human gut microbiome. To date, only one *A. muciniphila* strain, the type strain (Muc^T), is available in public culture collections. However, new isolates from humans and other animals are to be reported and may indicate how this unique mucolytic symbiont has adapted to its host.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.micpath.2016.02.005.

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