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# SEGMENTED FILAMENTOUS BACTERIA AND INCREASED RESISTANCE TO INFECTIONS

JOHANNES SNEL

Science2All, Wageningen, The Netherlands

## SUMMARY

Association of germfree mice with a complete intestinal microbiota promotes the development of the gut mucosal immune system, but this effect is not seen with individually cultured bacterial strains. *Clostridium*-related, segmented filamentous bacteria (SFB) are non-cultivable, commensal bacteria that strongly adhere to the epithelial cells of the ileum and of Peyer's patches. Their presence in mice stimulates formation of goblet cells, promotes intestinal transit, and results in MHC class II expression on the epithelium. Further, SFB stimulate IgA-producing cells in the lamina propria of the small intestine and activate T-cells, including T helper 17 (Th17) cells. As a consequence of this immunoactivation, adherence of SFB itself declines in time. Nevertheless, mice are better protected against enteropathogens such as salmonella, *Escherichia coli* and *Citrobacter rodentium*. Whether SFB are part of the normal human microbiota is still inconclusive despite several studies suggesting their presence. In conclusion, SFB are part of the microbiota of many species, and key players in the maturation of the murine immune system of the gut. They contribute to an enhanced resistance to enteropathogens.

## INTRODUCTION

Segmented filamentous bacteria (SFB) are Gram positive, sporeforming, yet non-cultivable bacteria that are related to clostridia. Intestinal spore-forming bacteria with a segmented filamentous appearance that attach to the intestinal wall were originally described in mice (*Hampton* and *Rosario*, 1965) and chickens (*Fuller* and *Turvey*, 1971), but have now been found in a wide range of animals. In mammals, they preferentially adhere to the epithelial cells of the ileum (see Figure 1) and of Peyer's patches, small lymphoid organs involved in antigen sampling from the intestinal lumen.

SFB do not have an official taxonomic name, as they cannot be cultured *in vitro*. Based on their 16S ribosomal RNA sequence, they were given the provisional status *Candidatus* and the genus name Arthromitus until *in vitro* culturing methods become available, and bacteria can be further characterized and added to culture collections. SFB are host specific: ileal bacterial preparations containing SFB did only lead to outgrowth in ex-germfree mice and rats when they were derived from the same species (*Tannock* et al., 1984). Because of this, Arthromitus can be divided into several species,

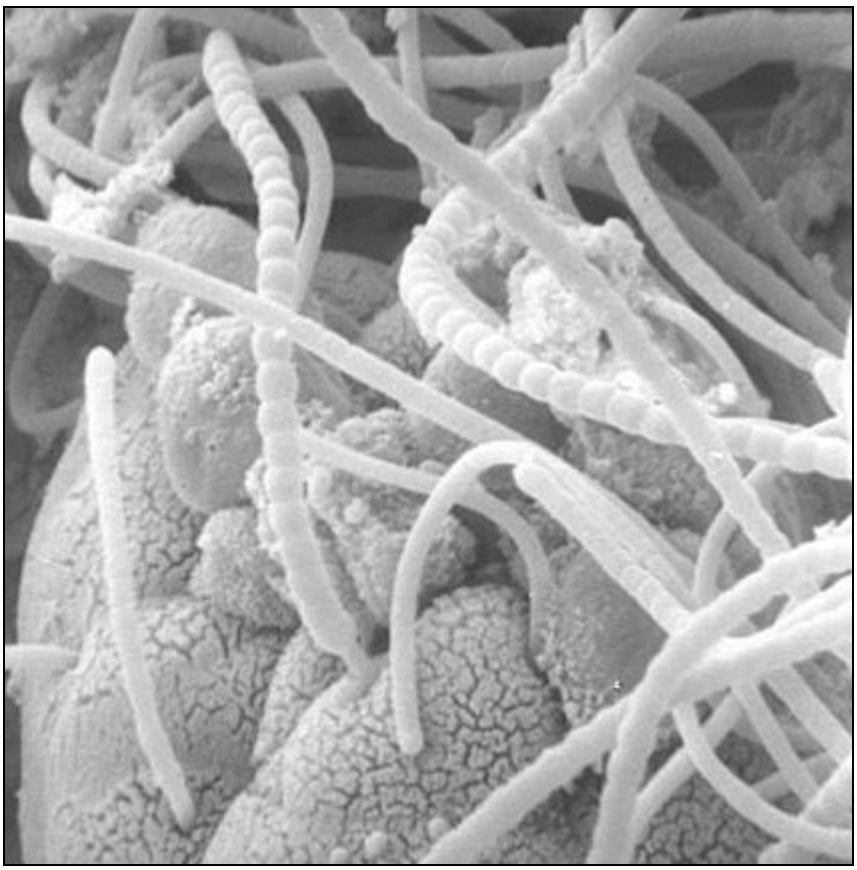

**Figure 1**: Scanning electron microscopic image of segmented filamentous bacteria in mice. Filaments are attached to epithelial cells without any signs of inflammation. Two morphotypes can be distinguished: smooth filaments and filaments with a beaded appearance.

also since small differences in 16S rRNA sequences are observed between these bacteria in different hosts (*Snel* et al., 1995). The species are designated *Candidatus* Arthromitus muris, *Candidatus* Arthromitus ratti and *Candidatus* Arthromitus galli for SFB from mouse, rat and chicken respectively.

The establishment of monocultures of SFB in ex-germfree mice (*Klaasen* et al., 1991b; *Umesaki* et al., 1995) has greatly facilitated research into these bacteria. Because of the intimate relationship with the host, several studies have suggested that SFB may increase the resistance of the host to infectious diseases. This may be by various mechanisms, including competitive exclusion (*Garland* et al., 1982; *Heczko* et al., 2000), immune activation (*Ivanov* et al., 2009), and stimulation of intestinal transit (*Snel* et al., 1996).

## ADHESION AND COMPETITIVE EXCLUSION

SFB adhere to the epithelial cells of the ileum with a special structure described as holdfast (Figure 2). The shape of this holdfast is variable from bean-, tear-

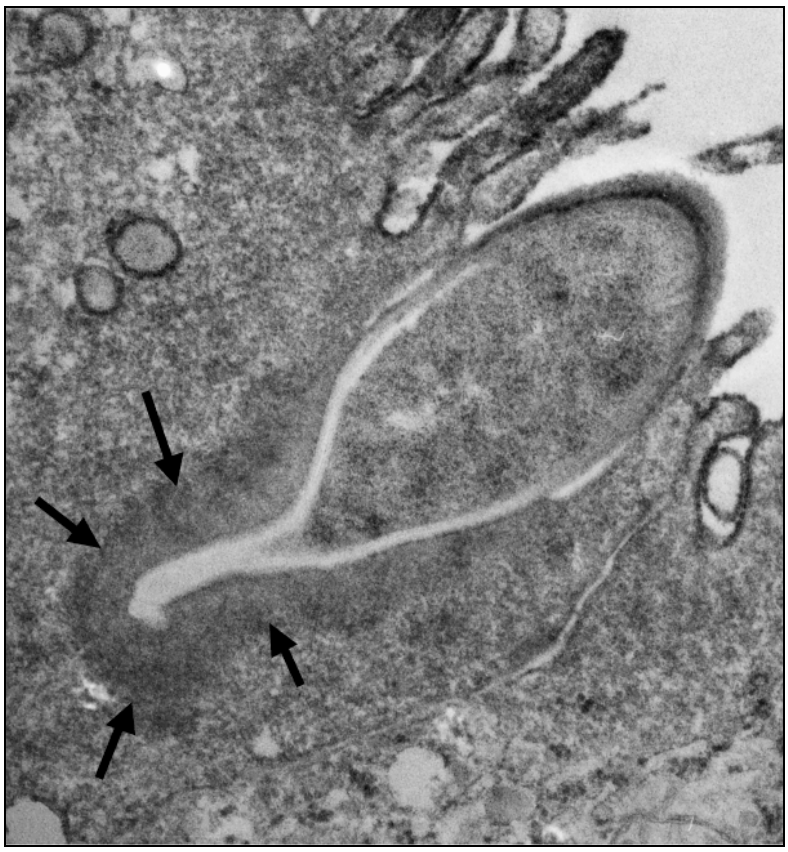

**Figure 2**: Transmission electron microscopic image of an epithelial cell containing an SFB holdfast. The brush border membrane of the epithelial cell is unaffected. The host cell has responded by accumulation of actin at the site of attachment (arrows).

drop-, to bulb-shaped (*Blumershine* and *Savage*, 1978). Using transmission electron microscopy, it is demonstrated that attachment of SFB causes an invagination of the plasma membrane and displacement of the microvilli at the site of attachment. The host cell responds to adhesion with the accumulation of polymerized actin, similar as seen after attachment of enteropathogenic *Escherichia coli* (*Jepson* et al., 1993), but despite intense association with the gut wall, SFB in mice generally do not possess pathogenic characteristics. In contrast to infection with pathogenic bacteria, the microvilli of the brush border of infected cells remain intact (*Jepson* et al., 1993).

During the weaning phase, peak levels of SFB are observed in the ileum. Colonization of mice starts around weaning at about 3 weeks of age, at which the animals shift their diets from milk to solid food (*Blumershine* and *Savage*, 1978; *Koopman* et al., 1987). Thereafter, levels gradually go down and hardly any adhering SFB are found in adult mice. In immunocompromised animals, the level of adhering SFB in the ileum remains high after weaning (*Snel* et al., 1998). Upon administration of the probiotic *Lactobacillus plantarum* to immunocompromised animals, the expan-

sion of SFB in the ileum of these mice was abolished suggesting direct competition between SFB and *Lactobacillus* (*Fuentes* et al., 2008).

Several electron microscopic studies in mice describe rod-shaped bacteria adhering to the SFB filaments in mice (*Klaasen* et al., 1992a; *Koopman* et al., 1987). The exact nature of these bacteria is unknown, although it is speculated that because of their morphology they may be *Lactobacillus* species (*Koopman* et al., 1987). Similar sub-colonization of SFB by rod-shaped bacteria is found in chickens (unpublished data). Although speculative, the combination of SFB with epibionts may even further strengthen the physical barrier for adherence of pathogenic bacteria. Alternatively, they may represent the direct competition between these bacteria.

## ATTACHMENT AND EPITHELIAL RESPONSES

Attachment is not restricted to regular epithelial cells of the ileum: SFB also adhere to the follicle associated epithelium of Peyer's patches in the ileum, specialized lymphoid organs as part of the mucosal immune system (*Jepson* et al., 1993; *Snel* et al., 1998). Within the follicle-associated epithelium, membranous cells (M-cells) are involved in the continuous sampling of antigens from the lumen. Although rarely seen, SFB are capable to adhere to M-cells of mice (*Jepson* et al., 1993; *Meyerholz* et al., 2002), and even extend from an M-cell into intimate association with an intraepithelial mononuclear cell (*Meyerholz* et al., 2002).

It is not surprising that the intimate relationship between SFB and the host results in a strong host response. Global transcriptomic analysis of terminal ileum tissues from healthy adult C3H/HeN germfree mice, conventional mice, and mice conventionalized with whole mouse faecal flora at adult age indicated that 45% of the genes induced by the microbiota could be assigned to immune response pathways (*Gaboriau-Routhiau* et al., 2009). This effect was observed at both 8 and 60 days post-colonization. Epithelial gene expression of a monoculture of SFB was compared to gene expression induced by the probiotic strains *Lactobacillus casei* Shirota and *Bifidobacterium breve* Yakult after 3 days of mono-association of germfree mice (*Shima* et al., 2008). Most pronounced effects were found in the ileum where SFB, far more than the two probiotics, differentially expressed 942 genes (478 more than 2-fold upregulated and 464 more than 2-fold downregulated compared to germfree animals) versus 362 for *Lactobacillus* (183 up and 179 down) and 264 for *Bifidobacterium* (75 up and 189 down). Surprisingly, the overlap in differentially expressed genes by these 3 strains was limited. It was found that, especially in the ileum, many of the SFB-upregulated genes belonged to the functional categories cell communication, defence and immunity, metabolism, and transport.

## IMPACT ON THE EPITHELIUM

Changes in gene expression are also reflected in phenotypical changes of the epithelium. In ex-germfree mice associated with SFB, fucosylation of asialo GM1 glycolipid occur in the small intestinal epithelial cells (*Umesaki* et al.,

1995). Using a genomics approach, it was demonstrated that in particular, alpha(1-2) fucosyltransferase was induced in the gut epithelium after mono-association with SFB, but not after mono-association with a *Lactobacillus* or *Bifidobacterium* strain (*Shima* et al., 2008). Another interesting gene induced by SFB is pancreatitis-associated protein (PAP or RegIIIγ). This gene encodes a C-type lectin, and is even found to be induced after mono-association of germfree severe combined immunodeficient (SCID) mice with SFB as an innate response to microbial colonization (*Keilbaugh* et al., 2005). A recent study described antimicrobial activity of the gene product, and suggested a role in gut homeostasis in order to maintain symbiotic host-microbe interactions (*Cash* et al., 2006).

Host epithelial cells in germfree mice are known for their low expression of major histocompatibility complex II (MHC-II) molecules on the apical surface, which is rapidly induced after conventionalization with a complete microbiota. After attachment, SFB can be phagocytised into the epithelial cells of the ileum and intracellularly processed by heterophagy (*Yamauchi* and *Snel*, 2000). Mono-association with SFB results in expression of MHC-II, a phenomenon that is not seen after mono-association with related spore-forming bacteria from the genus Clostridium (*Umesaki* et al., 1995; *Umesaki* et al., 1999).

## SFB AND INTESTINAL TRANSIT

One of the observed differences between germfree and conventional mice relates to the slower intestinal transit in the germfree animals that is rapidly increased after the introduction of a normal microbiota (*Abrams* and *Bishop*, 1967). It is suggested that this transit rate contributes to homeostasis of the microbiota in the small bowel (*Caenepeel* et al., 1989). In a study using germfree and mono-associated animals as well as animals with a complex microbiota with or without SFB, it was shown that SFB promotes intestinal transit (*Snel* et al., 1996). Twenty four hours after the addition of non-pathogenic *E. coli* TG1 to these animals, significantly lower numbers of these bacteria were found in the distal part of the small intestine of SFB-containing mice compared to germfree mice and mice associated with *Clostridium inocuum*. This suggests that the influence of SFB on intestinal transit may also contribute to an enhanced resistance to enteropathogens.

## IgA INDUCTION

It is known that SFB are abundantly colonizing the epithelium of mice only shortly after weaning (*Davis* and *Savage*, 1974; *Klaasen* et al., 1992a; *Snel* et al., 1998) and for about 10 days after hatching in chicks (*Yamauchi* et al., 1990). During this period, the mucosal immune system is strongly stimulated, resulting in the induction of high levels of IgA plasma cells in the gut lamina propria and secretory IgA in gut secretions (*Klaasen* et al., 1993b). Since SFB colonization is transiently abundant in immunocompetent mice whereas the bacteria persist in athymic nude mice (*Snel* et al., 1998) as well as in IgA-deficient mice (*Suzuki* et al., 2004), it is strongly suggested that it is

the induced immune response that leads to self-limiting colonization levels. A self-limiting response has also been reported for translocation of the Gram-negative bacterium *Morganella morganii*, the number of translocating bacteria begins to drop with the onset of a specific IgA response while colonization of the intestinal lumen is unaffected (*Shroff* et al., 1995).

## T-CELL RESPONSES

Colonization of SFB leads to an expansion of αβ-T-cell-receptor bearing intra-epithelial lymphocytes in the gastrointestinal tract (*Umesaki* et al., 1995). This was accompanied by an increase of cells with cytolytic activity. One recent study of the group of Littman demonstrated a unique role of SFB in T cell responses, particularly in respect to Th17 responses since a stimulation of IL17 and IL22 were described (*Ivanov* et al., 2009). Published in the same week, a study from the group of Cerf-Bensussan showed that not only Th17 was stimulated, but that SFB induced a broad spectrum of pro-inflammatory T helper 1 (Th1), Th17, and regulatory T cell responses (*Gaboriau-Routhiau* et al., 2009).

## SFB AND ENHANCED RESISTANCE TO INFECTIONS

Because of its non-pathogenic nature and the intimate relationship of SFB with the host, it has been speculated for years that these bacteria might increase host resistance against intestinal infections (*Glick* et al., 1978; *Porvaznik* et al., 1979). It was speculated that the high colonization level of SFB in weaning animals might competitively lead to reduced colonization levels of food-borne pathogens. Indeed, such an effect was observed in rats that were orally infected with *Salmonella enteritidis*: a reduction of surface colonization by these pathogens was found in the presence of SFB (*Garland* et al., 1982). Also rabbits that were infected with enteropathogenic E. coli O103 showed a negative correlation between presence of SFB and pathogen colonization (*Heczko* et al., 2000). In addition, mice mono-associated with SFB that were given *E. coli* had lower levels of coliforms in the small intestine that is likely due to increased intestinal transit (*Snel* et al., 1996). And mice with elevated Th17 activity due to the presence of SFB, and that were infected with *Citrobacter rodentium* had reduced levels of these enteropathogens in their ileum and colon. Last but not least, young adult mice infected with *S. typhimurium* had a prolonged survival when mono-colonized with SFB compared to germfree mice. Such a prolonged survival was not seen in mice mono-associated with *Clostridium innocuum,* and in preweaned mice with SFB but an immune system that is not fully mature (unpublished data).

Altogether, there seems substantial evidence that SFB enhances host resistance. Nevertheless, in another study, using either *S. enteritidis* or *Enterobacter cloacae* as the challenging micro-organisms, the presence of SFB did not lead to significantly reduced translocation of pathogens (*Klaasen* et al., 1992b). Since the animals were only 4-5 wks. of age with likely an immune system that was not fully mature, and since immune status of these

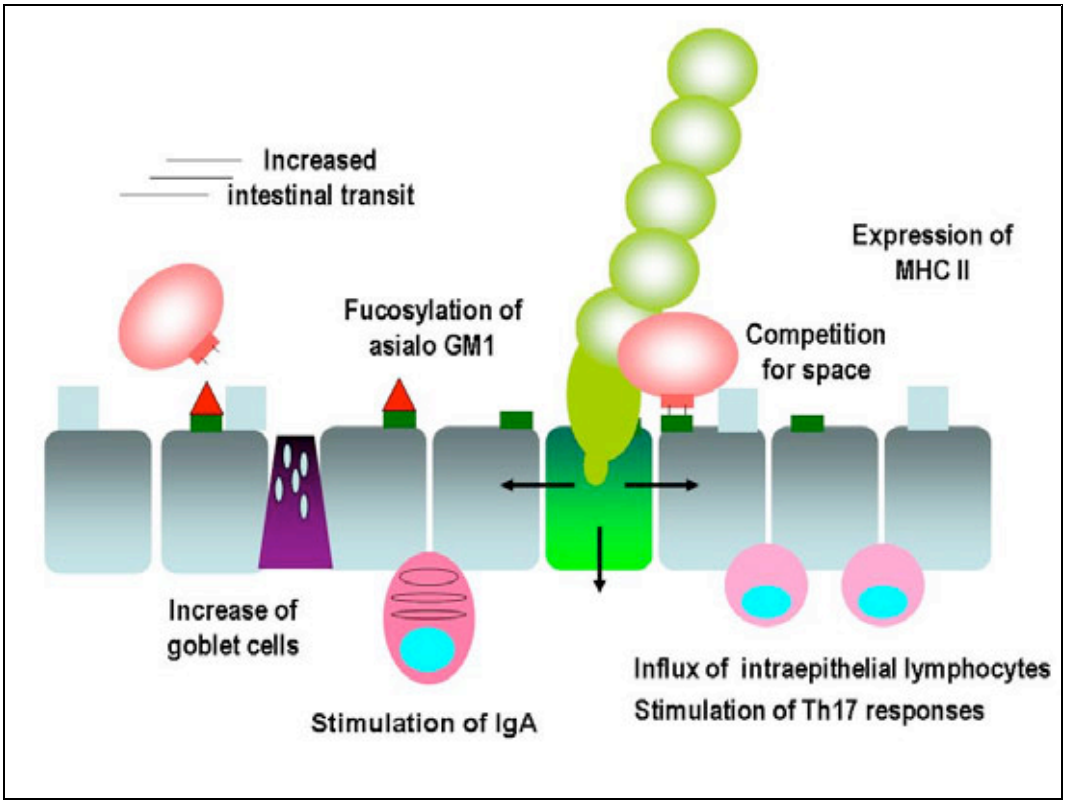


**Figure 3**: Schematic overview of the defence against enteropathogenic microorganisms. SFB contributes to competitive exclusion by high colonization levels during weaning and stimulates several aspects of immune maturation after weaning.

animals was not monitored, these results from this study are far from conclusive.

The influence of SFB on resistance to enteropathogens because of its high bacterial density during weaning and stimulation of the immune response post-weaning is schematically depicted in Figure 3.

## PRESENCE OF SFB IN HUMANS?

The demonstration of SFB in a wide range of host species suggests that these bacteria do not only affect immune maturation of mice but also of other vertebrates, including man. Whether SFB are present in humans is under debate. Although SFB are predominant in mice during weaning, bacteria with a filamentous morphology and adhering capacity have been described in a biopsy from a 67 years old patient undergoing surgery which is not even close to the age of weaning in man (*Klaasen* et al., 1993a). In another study, bacteria were associated with the mucosa of children with celiac disease, with both active and inactive disease, but not with controls (*Forsberg* et al., 2004). These bacteria were described as rod-shaped and were morphologi-

cally not comparable to SFB in mice. In both studies, the nature of these bacteria was not confirmed by e.g. analysis of the 16S rRNA gene sequence, which makes it doubtful that these bacteria were related to *Candidatus* Arthromitus.

The group of MacFarlane at the University of Dundee has studied the microbial composition of a continuous culture system of the human colonic microbiota using a range of phylogenetic DNA probes for fluorescent in situ hybridisation. This continuous culture system was inoculated with faecal material from a 30-year-old male. At various stages of the study, up to 16% of the microbiota reacted with an SFB-specific probe. Unfortunately, the full 16S rRNA sequence was not obtained, and therefore hybridisation may have been non-specific. Especially since so far, large-scale investigations of the human microbiome using P454 pyrosequencing or similar techniques have not revealed sequences with a homology to *Candidatus* Arthromitus.

At present, it is to early to conclude that SFB in humans are not found, especially since diet may have a strong impact on the presence of SFB (*Klaasen* et al., 1991a) and since SFB colonization in humans may be affected by age, like in mice. So far, to our knowledge, systematic studies that investigate the presence of SFB in humans have not been reported.

## CONCLUSIONS

Segmented filamentous bacteria are a group of bacteria that are found in the ileum of several animal species, including mammals, birds and fish. Here they have a profound effect on maturation of the immune system, intestinal transit and mucosal epithelium. This does not only affect colonization of SFB itself, but also that of various enteropathogenic microorganisms such as *Salmonella* and *E. coli*. In this way they contribute to enhanced resistance against these enteropathogens. Whether SFB are part of the normal microbiota of humans and play a role in human gut health is still unclear.

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