Received: 28 June 2019 | Revised: 2 August 2019 | Accepted: 12 August 2019
DOI: 10.1111/cmi.13097

SPECIAL ISSUE - RESEARCH ARTICLE

WILEY

Microbiology and immunology: An ideal partnership for a tango at the gut surface—A tribute to Philippe Sansonetti

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Funding information
Program Investissement Avenir, Grant/Award Numbers: ANR-10-IAHU-01 and ANR-10-LabX 6201; Université de Paris; H2020 European Research Council, Grant/Award Number: ERC-2013-AdG 339407-IMMUNOBIOTA; Fondation Princesse Grace; Université de Paris; Institut national de la santé et de la recherche médicale

Abstract

Over the past 20 years, the highly dynamic interactions that take place between hosts and the gut microbiota have emerged as a major determinant in health and disease. The complexity of the gut microbiota represents, however, a considerable challenge, and reductionist approaches are indispensable to define the contribution of individual bacteria to host responses and to dissect molecular mechanisms. In this tribute to Philippe Sansonetti, I would like to show how rewarding collaborations with microbiologists have guided our team of immunologists in the study of host–microbiota interactions and, thanks to the use of controlled colonisation experiments in gnotobiotic mice, toward the demonstration that segmented filamentous bacteria (SFB) are indispensable to drive the post-natal maturation of the gut immune barrier in mice. The work led with Philippe Sansonetti to set up in vitro culture conditions has been one important milestone that laid the ground for in-depth characterization of the molecular attributes of this unusual symbiont. Recent suggestions that SFB may be present in the human microbiota encourage further cross-fertilising interactions between microbiologists and immunologists to define whether results from mice can be translated to humans and, if so, how SFB may be used to promote human intestinal defences against enteropathogens. Nurturing the competences to pursue this inspiring project is one legacy of Philippe Sansonetti.

KEYWORDS

E. coli K12, gut barrier, host–microbiota interactions, intestinal immunity, radiative evolution, segmented filamentous bacterium

1 | INTRODUCTION

Following avenues opened by Robert Koch and Louis Pasteur at the end of the 19th century, microbiologists have succeeded in identifying many disease-causing microbes. They next made remarkable progresses in characterizing the cellular and molecular mechanisms that govern dual interactions between individual pathogens and their hosts and also in developing antibiotics and vaccines that have radically transformed epidemiology and prognosis of infectious diseases. Along this line, work led by Philippe Sansonetti has been instrumental to unravel the multiple mechanisms evolved by *Shigella* to hijack host defences and to establish its niche of replication (Schnupf & Sansonetti, 2019) as well as to develop a vaccinal approach against a murderous bacterium causing millions of deaths in young children in developing countries. After demonstrating in 1982 that the virulence of *Shigella flexneri* depends on a large plasmid of approximately 220 kb that encodes for a type III secretion system (Sansonetti, Kopecko, & Formal, 1982), Philippe and his collaborators developed numerous in vitro and in vivo approaches to dissect how the different functions encoded by this plasmid enable the interactions of *Shigella* with the intestinal barrier, opening the new discipline of cellular microbiology. They notably uncovered how *Shigella* can propagate within and between epithelial cells by hijacking the host actin cytoskeleton (Bernardini, Mounier, d'Hauteville, Coquis-Rondon, & Sansonetti, 1989). They showed how *Shigella* induces the death of Peyer's patch macrophages, which release IL-1β and IL-18, and how these two cytokines, in turn, induce a host inflammatory response that initially promotes bacterial invasion but is ultimately indispensable to clear infection (Sansonetti et al., 2000; Zychlinsky, Prevost, & Sansonetti, 1992). Another set of important studies led by Philippe with Stéphane Girardin and Dana Philpott established how the recognition of a tripeptide derived from bacterial peptidoglycan by a novel intracellular receptor, the nucleotide oligomerisation domain receptor 1, enables mammalian cells to react upon invasion by *Shigella* and more generally upon invasion by pathogenic strains of Gram-negative bacteria (Girardin et al., 2001; Girardin et al., 2003). Eager to translate his work into tools to fight against *Shigella*, Philippe has pursued for many years with his collaborators, and especially with Armelle Phalipon, the quest for the best vaccinal strategy against *S. flexneri*. Their efforts are now close to succeeding with the development of synthetic functional mimics of the O-antigen that can drive a potent protective IgG response (Phalipon et al., 2009; van der Put et al., 2016). Given the capacity of *Shigella* to acquire and accumulate multiple antibiotic resistances (Njamkepo et al., 2016), this vaccine remains more than ever indispensable. Over the past 15 years, Philippe and his collaborators have progressively extended their expertise to the field of host–microbiota interactions, with notably the goal to understand how the gut can aggravate or, on the contrary protect, against stunting and environmental enteropathy, a devastating condition that increases susceptibility to infections and impairs the development of young children in developing countries (Vonaesch et al., 2018; Vonaesch et al., 2018). The interest of Philippe into host–microbiota provided our team with the chance to set up the very rewarding collaboration that is discussed below to study an unusual symbiont, segmented filamentous bacteria (SFB).

A novel era in microbiology opened at the end of the 20th century with the discovery by Carl Woese of a sequence-based phylogenetic framework for the identification of microbes and with the rapid development of sequencing technologies (Pace, Sapp, & Goldenfeld, 2012). Limitations of microbial cell culture were overcome, and beyond the study of the small number of bacteria that were culturable, it became possible to explore the structure and dynamics of multiple microbial communities in diverse ecosystems (Gibbons & Gilbert, 2015). The microbial communities that assemble at our body surfaces and notably the gut microbiota, which is by far the largest of these communities, became the centre of considerable attention (Davenport et al., 2017; Dominguez-Bello, Godoy-Vitorino, Knight, & Blaser, 2019). A new field of research for microbiologists and immunologists opened at the gut mucosal surface. In a perspective published in *Cell and Host Microbe* in 2008, Thierry Pédron and Philippe Sansonetti thus highlighted the change of paradigm in the host–pathogen relationship and outlined the "ménage à trois" that takes place between pathogens, commensals, and the host immune system at the gut surface (Pedron & Sansonetti, 2008). The barrier effect of the microbiota, initially described in the 1950s (Miller, Bohnhoff, & Rifkind, 1956), received renewed attention, leading microbiologists to identify the multiple mechanisms evolved by symbiotic bacteria to protect their niche and to restrain the access of pathogens to their hosts (reviewed in: Schnupf, Gaboriau-Routhiau, & Cerf-Bensussan, 2018; Sorbara & Pamer, 2019). Simultaneously, immunologists realised that the immune system may not only have evolved under the selective pressure of highly virulent pathogenic microbes (Shultz & Sackton, 2019), but also to cope with the complex communities of symbiotic bacteria that colonise body surfaces after birth (Lee & Mazmanian, 2010; McFall-Ngai, 2007). As highlighted in a recent review by Philippe and his collaborators, new paradigms based on in-depth knowledge of intestinal ecology are now necessary to apprehend the role of microbes in health and disease and to revisit Koch's postulates (Vonaesch, Anderson, & Sansonetti, 2018).

Here, I would like to outline how cooperation with microbiologists has guided our team of mucosal immunologists toward the study of host–microbiota interactions and how the use of gnotobiotic mice led us to embark on the quest for bacteria able to stimulate the post-natal maturation of the gut immune barrier. Once the SFB were identified as a key taxon, Philippe Sansonetti provided us the chance to initiate cellular microbiological approaches to culture this unusual symbiont and to characterize its intimate relationship with the intestinal epithelium. Strikingly, SFB proved to be not only a strong inducer of gut innate and adaptive immune responses, but its presence was shown to greatly enhance the barrier effect of the gut microbiota against enteropathogens, raising considerable interest by both immunologists and microbiologists for this unusual commensal bacterium. This tribute to Philippe Sansonetti will thus be an opportunity to summarise present results and to discuss perspectives in SFB research.

2 | LESSONS FROM *Escherichia coli* ADAPTATION TO INTESTINAL LIFE

Two circumstances led our team to step into the field of host–microbiota interactions. Valérie Gaboriau-Routhiau joined the group, bringing skills in gnotoxeny learned from Pierre Raibaud and Robert Ducluzeau, two pioneers in microbial ecology at the National Institute of Research in Agronomy in Jouy-en-Josas (Raibaud et al., 1980). Simultaneously, microbiologists headed by François Taddei, requested our help to delineate how *Escherichia coli K12 MG1655*, a commensal strain that had been isolated 50 years ago from human faeces and had acclimated to test tubes, could readapt to the life in the mouse intestine. Using strains that differed by their spontaneous rate of mutation, Antoine Giraud and François Taddei had shown that adaptive mutations promoted gut colonisation by *E. coli K12* (Giraud et al., 2001).

The obvious next steps were to define the nature of the mutations and to identify the constraints that drove selection of the mutant stains in the mouse intestine. After colonisation of previously germ-free mice, we observed a rapid genetic diversification of *E. coli K12* with the systematic successive selection of mutations in the *EnvZ/OmpR* operon, in the flagellar *flhDC* operon, and in *malT*, the transcriptional activator of the maltose regulon (De Paepe et al., 2011; Giraud et al., 2008). Strikingly, the gain of fitness conferred by the first two types of mutations was associated with loss in motility and flagella expression (De Paepe et al., 2011; Giraud et al., 2008). Given the potent agonist effect of the flagellin derived from *E. coli K12* on Toll-like receptor 5 (TLR5) and the downstream activation of the NF-κB cascade (Bambou et al., 2004), a tantalising hypothesis for immunologists was that the two first mutations reflected the selective pressure exerted by the host immune system and enabled *E. coli* to escape destruction by an NF-κB-induced inflammatory response. To our surprise, however, we found no transcriptomic evidence of NF-κB activation in the intestine of *E. coli*-monocolonised mice even at very early time points (Giraud et al., 2008). Moreover, the same radiative evolution was observed in *MyD88*-deficient mice that cannot activate NF-κB upon TLR5 ligation (De Paepe et al., 2011). In contrast, determination of the fitness advantages of the selected mutations in controlled in vitro experiments showed that the selective forces that drove *E. coli* diversification in the mouse gut were the osmotic stress induced by bile acids and the competition for nutrients (De Paepe et al., 2011; Giraud et al., 2008). Overall, these results indicated that the trade-off between stress resistance and nutritional competence can generate sympatric diversification of the gut microbiota independently of host immune responses. They also left us with the unexpected finding that colonisation of adult mice by a single commensal bacterium such as *E. coli* K12 was not sufficient to trigger a significant inflammatory response in the mouse ileum. Among all immune responses tested, only a moderate increase of the concentration of SIgA could be detected (see below).

3 | LESSONS FROM COLONISATION EXPERIMENTS IN GNOTOBIOTIC MICE

Observation in *E. coli*-monocolonised mice contrasted with published evidence that mouse colonisation by a complex microbiota induces a broad spectrum of immune responses in the intestine. Such responses result in a state of tightly regulated physiological inflammation that is now known to be indispensable to confine the microbiota within the intestinal lumen while maintaining intestinal homeostasis (Cerf-Bensussan & Gaboriau-Routhiau, 2010; Hooper & Macpherson, 2010). Taking advantage of our access to gnotoxeny, a powerful tool to explore the impact of microbial colonisation on the hosts (Macpherson & McCoy, 2015; Skelly, Sato, Kearney, & Honda, 2019), we first confirmed that colonisation of adult germ-free mice by a complex microbiota induced the coordinated maturation of both proinflammatory and regulatory immune responses. A robust transcriptomic response combining both innate (*Nos2*, *Reg3*, *Il1b*, and *Il12p40*) and adaptive (*Ifng*, *Il10*, *Gzb*, *Foxp3*, and *Il17*) signals was induced in the ileum; and a strong expansion of T cells, including $CD4^+$ T cells producing IL-10, IL-13, interferon gamma, and IL-17, was evidenced in the *lamina propria* of colonised mice compared with germ-free mice (Gaboriau-Routhiau et al., 2009). To our surprise, however, monocolonisation by a variety of culturable bacteria as well as colonisation by a complex microbiota derived from human faeces or from the culturable fraction of the mouse microbiota failed to recapitulate the full spectrum of responses induced by a complete pathogen-free mouse microbiota (Gaboriau-Routhiau et al., 2009). In keeping with observations in *E. coli*-monocolonised mice, all mice developed an IgA response. In addition, some mice colonised by the culturable fraction of the mouse microbiota showed increased transcription of IL-10 and FOXP3, suggesting expansion or induction of regulatory T cells. The latter result is in keeping with numerous studies, which have demonstrated that a diverse spectrum of gut symbionts, and notably anaerobic *Clostridium* species, which metabolise dietary fibres into short-chain fatty acids, can induce regulatory T cells in the gut mucosa (Arpaia et al., 2013; Furusawa et al., 2013; Skelly, Sato, Kearney, & Honda, 2019; Smith et al., 2013). Unexpectedly, however, only colonisation by the spore-enriched fraction of the mouse microbiota was able to generate proinflammatory innate and adaptive immune responses measurable by transcriptomic analysis of intestinal biopsies. This fraction of the mouse microbiota was notably indispensable to recapitulate the strong TH17 response induced by the complete microbiota in C3H/HeN mice (Gaboriau-Routhiau et al., 2009). Because spore-forming gut bacteria are enriched in strict anaerobes difficult or impossible to culture, we inferred that one or several unculturable bacterial taxa present in the mouse but not in the human microbiota were indispensable for driving the full-blown maturation of homeostatic gut immune responses (Gaboriau-Routhiau et al., 2009). Several clues led us to select SFB as a likely candidate. SFB are spore-forming Clostridia-related bacteria that colonise the mouse intestine at time of weaning concurrently to the initiation of the post-natal maturation of the gut immune barrier (Davis & Savage, 1974). SFB display tight adherence to ileal epithelial cells (Chase & Erlandsen, 1976; Davis & Savage, 1974; Ferguson & Birch-Andersen, 1979). Although SFB have been observed in many vertebrates, attachment has been shown to be species specific, suggesting that SFB species have coevolved with their respective hosts (Tannock, Miller, & Savage, 1984). This property of SFB seemed to us germane to why the microbiota from mice but not from humans induced a sizeable immune response. Furthermore, a potent inducing effect of SFB on the intestinal IgA response (Klaasen et al., 1993; Talham, Jiang, Bos, & Cebra, 1999) and on the expansion and activation of $CD8^+$ intraepithelial T cells (Umesaki, Okada, Matsumoto, Imaoka, & Setoyama, 1995) had been demonstrated in SFB-monocolonised mice that were obtained in the late 1990s by two groups in the Netherlands (Klaasen, Koopman, Van den Brink, Van Wezel, & Beynen, 1991) and in Japan (Umesaki et al., 1995). Thanks to Jan Snel, who provided access to the SFB strain maintained in monocolonised mice in the Netherlands, we confirmed our hypothesis and demonstrated that monocolonisation of C3H/HeN mice by SFB induced a broad spectrum of innate and adaptive immune responses (Gaboriau-Routhiau et al., 2009). SFB monocolonisation notably recapitulated the proinflammatory innate signals and the strong TH17 response induced by the complete microbiota (Gaboriau-Routhiau et al., 2009). The role of SFB as a potent inducer of the intestinal homeostatic TH17 response was simultaneously demonstrated by Ivan Ivanov and Dan Littman in C57BL/6 mice using the SFB strain maintained in monocolonised mice in Japan (Ivanov et al., 2009). Littman and coworkers also showed that SFB colonisation induced the production of IL-22, a cytokine that, alike IL-17, stimulates the production of microbicidal peptides by the gut epithelium (Ivanov et al., 2009). During colonisation by SFB, IL-22 is, however, mainly produced by type 3 innate lymphoid cells (ILC3) in response to IL-23-dependent signals (Sano et al., 2015). A recent work further indicates that activation of IL-22-producing ILC3 by SFB is maximal very early after colonisation and in $Rag^{-/-}$ mice, which lack adaptive immunity, but that it decreases at later time points in immunocompetent mice with the development of the SFB-induced adaptive TH17 response (Mao et al., 2018). Confirming and extending previous work led by the group of John Cebra in the 1990s (Talham et al., 1999), we observed that SFB is a potent inducer of gut-associated lymphoid tissues (Lecuyer et al., 2014). SFB not only can stimulate the development of Peyer's patches but also, in the absence of Peyer's patches, can drive the development of cryptopatch-derived lymphoid follicles and induce de novo formation of gut tertiary lymphoid tissue (Lecuyer et al., 2014). The absence of IgA and SFB-specific TH17 responses in SFB-monocolonised mice, in which the formation of Peyer's patches and inducible gut-associated lymphoid tissue has been inhibited (Lecuyer et al., 2014), suggests that the outstanding role of SFB in driving gut adaptive immune responses largely depends on its strong impact on the development of gut-associated lymphoid tissues where these responses are initiated (Figure 1).

Recent work suggests that other bacteria present in the gut microbiota, and notably in the human gut microbiota, can induce intestinal $CD4^+$ TH17 (Atarashi et al., 2015; Tan et al., 2016), $CD4^+$ TH1 responses (Atarashi et al., 2017), or $CD8^+$ TH1 responses (Tanoue et al., 2019) in the mouse intestine. In two studies, a consortium of several strains was, however, necessary to observe the induction of TH17 cells or of $CD8^+$ TH1 T cells (Atarashi et al., 2015; Tanoue et al., 2019). In another study, the *Klebsiella* strains that induced intestinal $CD4^+$ TH1 responses were isolated from the saliva and enriched in the microbiota of patients with inflammatory bowel diseases, suggesting that they may not be the symbionts that activate steady-state responses (Atarashi et al., 2017). A fourth study identified a strain of *Bifidobacterium adolescentis* that could induce the accumulation of gut CD4+ TH17 cells in monocolonised mice. Yet this strain failed to stimulate the complete spectrum of innate and adaptive responses induced by SFB and notably did not induce the development of gut-associated lymphoid tissue (Tan et al., 2016). Thus, up to now, SFB remains unique in its capacity to launch the full maturation of the mouse gut immune barrier without inducing any intestinal pathology. Ileal colonisation by SFB decreases, however, after 2 months of life, when gut immune responses induced by colonisation reach a plateau. In SFB-monocolonised mice, the decreased colonisation is accompanied by a reduction in the intensity of gut homeostatic immune responses (reviewed in Schnupf, Gaboriau-Routhiau, & Cerf-Bensussan, 2013). This is not the case in mice colonised by SFB in the context of a complex microbiota, indicating that other gut bacteria can later in life maintain the homeostatic immune responses initiated by SFB (Chung et al., 2012). Besides its outstanding immunostimulatory functions, SFB has attracted much interest due to its protective role against colonisation by enteropathogens, such as *Salmonella* Typhimurium or *Citrobacter rodentium* (reviewed in Schnupf et al., 2018). In contrast with the stimulation of gut immune responses, which can be initiated by SFB independently of the presence of other bacteria, the barrier effect of SFB was shown to require the additional presence of a complex microbiota (Chung et al., 2012). The exact role of SFB is not well delineated, but a mechanism of cooperation between SFB and fucosylase-producing Bacteroidetes has been proposed to account for SFB-enhanced protection against *C. rodentium* (Pickard et al., 2014). Accordingly, SFB activates, via an IL-23-dependent mechanism, the production of IL-22 by ILC3. In turn, IL-22 stimulates epithelial expression of fucosyl-transferase 2, which can decorate glycocalyx proteins with fucosyl residues. These

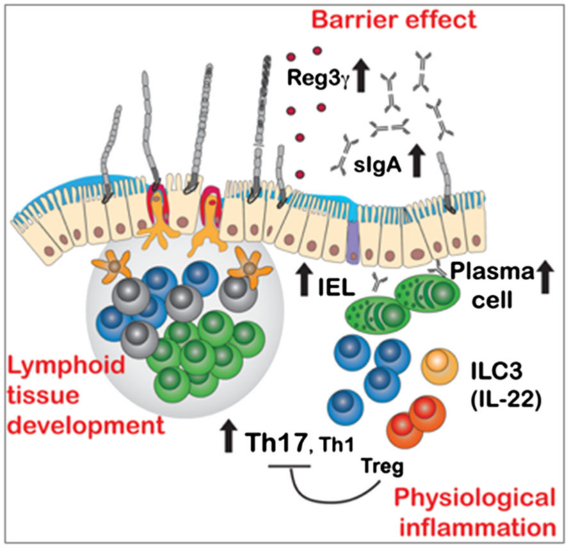

FIGURE 1 Activation of gut innate and adaptive homeostatic immune responses by segmented filamentous bacteria (SFB). SFB stimulate epithelial innate defences both directly and indirectly by activating the production of IL-22 by type 3 innate lymphoid cells and of IL-17 by T cells. IL-22 notably induced Reg3γ epithelial expression. SFB simultaneously stimulate the development of Peyer's patches and of inducible gut-associated lymphoid tissue where they initiate adaptive IgA and specific T-cell responses. SFB induce notably a strong expansion of ROR-γt-expressing T cells, the majority of which differentiate into TH17 cells and a minority into FOXP3 regulatory T cells (Treg) in C57BL/6 mice. As a result, SFB promote a state of physiological inflammation, which licenses TH17 responses against itself and other commensal bacteria and which sustains the intestinal barrier. The presence of SFB enhances the barrier effect of the microbiota. As discussed in the text, the exact mechanism of the protection provided by SFB is not fully elucidated but may involve the induction of IL-22. Scheme provided by the courtesy of P. Schnupf

residues are released by bacterial fucosylases into the intestinal lumen where they can influence *C. rodentium* metabolism and reduce expression of virulence genes (Pickard et al., 2014). Whereas many studies converge to stress the protective role of SFB at the gut surface, some others have indicated that SFB colonisation may aggravate several mouse models of arthritis or of experimental autoimmune encephalitis (EAE), notably through the induction of intestinal TH17 cells that may migrate to the periphery (reviewed in Schnupf, Gaboriau-Routhiau, Sansonetti, & Cerf-Bensussan, 2017; and Luu et al., 2019). Along the same line, it was suggested that the induction of TH17 cells in response to SFB colonisation can aggravate the neurodevelopmental and behaviour disorders observed in the offspring of pregnant mice treated by Poly-IC, a model used to mimic the putative predisposing role of ante-natal viral infections on the development of autism spectrum disorders (Kim et al., 2017; Shin Yim et al., 2017). Recent work failed, however, to confirm an aggravating role of SFB colonisation in the model of EAE induced by myelin oligodendrocyte peptide (MOG) and complete Freund adjuvant and noted that the frequency of TH17 cells in the spinal cord was not modified by the presence of SFB (Omenetti et al., 2019). The reasons for the difference remain uncertain. The aggravating role of SFB on EAE was observed in two studies that compared the induction of EAE between mice monocolonised by SFB and germ-free mice (Ohnmacht et al., 2015). As the latter mice do not have fully developed lymphoid tissue and fail to generate efficient immune responses in periphery, they may not be able to develop EAE. In contrast, a third recent study used mice with a relatively diverse microbial flora, and MOG/complete Freund adjuvant immunisation induced potent proinflammatory MOG-specific Th17 cell responses independently of the presence of SFB in the microbiota (Omenetti et al., 2019). Monocolonisation by SFB may partially replace the adjuvant effect of the microbiota on peripheral immune responses and enable de novo generation of MOG-specific TH17 cells upon immunisation. Of note, however, Omenetti et al. used the SFB strain isolated initially in the Netherlands, whereas most other studies have used the strain isolated in Japan. As the two SFB strains show some genetic variations, it cannot be completely excluded that they possess slightly different immunostimulatory properties (Bolotin et al., 2014). Overall, these results raise the question of the molecular mechanisms underlying the unusual immunostimulatory properties of SFB.

4 | SFB: LESSONS FROM MICROBIOLOGY

Complete sequences of the genomes of mouse and rat SFB that were published in 2011 have positioned SFB as a unique clade of Clostridiaceae that is most closely related to Type I Clostridia, but differing from other commensal clostridial strains by the reduced size of their genome (approximately 1.5–1.6 instead of 2.7–3 Mb), and by the evidence of numerous auxotrophic needs (Kuwahara et al., 2011; Pamp, Harrington, Quake, Relman, & Blainey, 2012; Prakash et al., 2011; Sczesnak et al., 2011). SFB lack notably the enzymatic pathways that are necessary for the synthesis of most amino acids and for de novo synthesis of nucleotides. Description of the SFB life cycle that was inferred from electron microscopy studies performed in the late 1970s further suggests that SFB may derive some anabolic resources from host epithelial cells (Chase & Erlandsen, 1976; Ferguson & Birch-Andersen, 1979). These studies notably revealed that SFB life cycle starts by the attachment of teardrop-shaped unicellular SFB to host epithelial cells. Attachment is followed by elongation and septation, resulting in the formation of long filaments (50–80 μm), which differentiate while remaining bound to the epithelium. Differentiation is associated with the appearance of spherical forespore-like inclusions, which convert into two teardrop-shaped intracellular offsprings (IOs) that can be encapsulated into spores or released from the free end of the filaments to start a new cycle (Chase & Erlandsen, 1976). On the basis of these observations, we decided in 2012 to try to demonstrate definitively that SFB growth depends on its intimate contacts with epithelial cells by setting in vitro culture conditions that mimicked its replication niche. This goal was achieved thanks to Philippe Sansonetti and Pamela Schnupf, then a postdoctoral scientist in his group. Genome analysis predicted SFB to be an obligate anaerobe with a complete glycolysis pathway but lacking most components required for aerobic respiration (Kuwahara et al., 2011; Pamp, Harrington, Quake, Relman, & Blainey, 2012; Prakash et al., 2011; Sczesnak et al., 2011). Yet SFB develops in the small intestine, which is not a strict anaerobe environment, notably at the epithelial surface where oxygen can diffuse from the rich capillary network irrigating the villi (Marteyn et al., 2010; Marteyn, Scorza, Sansonetti, & Tang, 2011). It was, therefore, likely that SFB should tolerate some oxygen in order to attach to ileal epithelial cells. The presence of genes encoding two catalases and a peroxidase in the SFB genome further suggested that the bacterium was equipped to resist oxidative stress (Kuwahara et al., 2011). Accordingly, it was decided to attempt coculture of SFB with epithelial cells in a hypoxic chamber at low concentrations of oxygen. After over 2 years of strenuous efforts, Pamela Schnupf succeeded in establishing all steps necessary to purify SFB IOs from SFB-monoassociated mice and to obtain within 4 days the growth of numerous long filaments that released newly formed IOs, overall recapitulating in vitro the life cycle of SFB that had been described in vivo (Schnupf et al., 2015). She observed that epithelial cells cocultured with SFB displayed a transcriptomic programme largely overlapping that induced during in vivo colonisation. With Valérie Gaboriau-Routhiau, she further demonstrated that both IOs and filaments derived from in vitro culture could successfully colonise germ-free mice and recapitulate the induction of SIgA and TH17 intestinal responses (Schnupf et al., 2015). In keeping with our initial hypotheses, successful in vitro growth of SFB was strictly dependent on the presence of alive epithelial cells, was optimal at 2.0% of oxygen, and also depended on SFB IOs being grown in direct contact with the apical surface of epithelial cells. SFB in vitro growth also required the addition of iron and that of several as yet unidentified components present in brain–heart infusion and yeast/peptone/casein media (Schnupf et al., 2015). Despite this remarkable success, in vitro culture of SFB remains challenging,

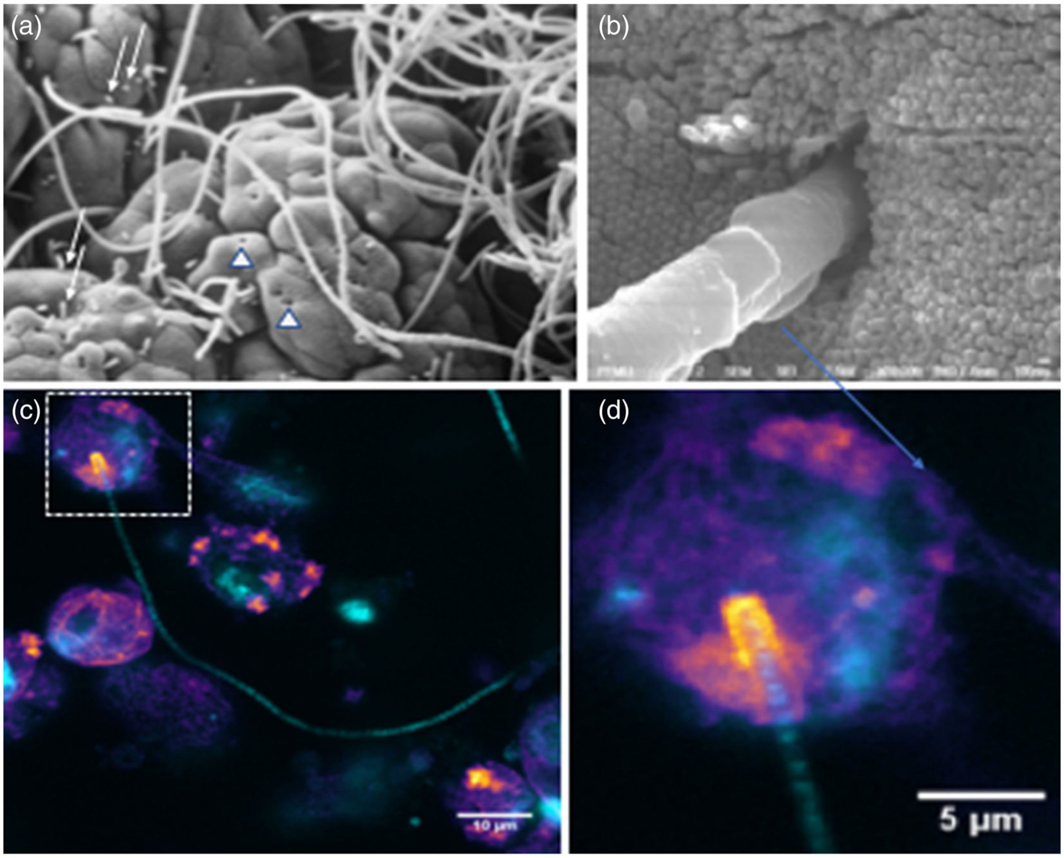

FIGURE 2 In vivo and in vitro evidence of segmented filamentous bacteria (SFB) attachment to epithelial cells. (a,b) Scanning electron microscopy of the ileal mucosa in SFB-monocolonised mice. (a) Short (likely unicellular) forms of SFB (arrows) attached to epithelial cells and long filaments of SFB attached or not to epithelium (arrowhead) are visible. Small holes at the epithelial surface reveal the previous attachment of SFB that have likely been removed during sample processing (asterisk). (b) In vivo attachment of SFB to the epithelial surface of an enterocyte. Photographs provided by the courtesy of V. Gaboriau-Routhiau. (c,d) Confocal imaging of an SFB filament obtained 2 days after culture of unicellular SFB on the murine epithelial cell line mICcl2. One long segmented filament (stained in cyan by 4,6-diamidino-2-phenylindole) is attached to an epithelial cell with a nucleus also stained in cyan by DAPI. Gradient colouring (ranging from purple for dim phalloidin staining to yellow for intense staining) shows the characteristic accumulation of polymerised actin at the site of attachment of SFB to the epithelial cell. Photographs provided by the courtesy of I. Nkamba and P. Schnupf

and in vitro passage of SFB is not used for long-term propagation. The in vitro growth system is, however, a promising advance to try to establish SFB transformation methods. As the in vitro system also recapitulates SFB attachment, including actin recruitment (Figure 2c, d), it may be used to interrogate the intriguing SFB–epithelial cell interaction and to screen for the putative receptors that underlie adhesion and trigger downstream signalling.

5 | MECHANISMS OF SFB-INDUCED TH17 RESPONSES: LESSONS FROM IN VIVO STUDIES

For now, several studies have used in vivo approaches to further characterize the molecular mechanisms, which underlie the immunostimulatory properties of SFB and notably its remarkable TH17-inducing activity. Yang et al. screened a whole-genome shot gun library of SFB with T cell hybridomas carrying T-cell receptors expressed by lamina propria SFB-specific TH17 cells and identified two putative secreted or surface SFB proteins (SFBNYU-003340 and SFBNYU-004940) specifically targeted by the latter cells (Yang et al., 2014). They next compared the induction of T cells specific for SFBNYU-003340 in mice orally infected with SFB or with *Listeria monocytogenes* engineered to express SFBNYU-003340. Strikingly, specific CD4+ T cells expanded in both groups of mice, but they differentiated into ROR-γt$^+$, presumably TH17 cells, only in mice colonised by SFB, indicating that the nature of the T-cell responses induced by SFB can be uncoupled from specific T-cell recognition (Yang et al., 2014). Overall, these data suggest that SFB delivers (a) signals that create a microenvironment permissive for the differentiation of TH17 cells. In keeping with this hypothesis, we observed that mice monocolonised by the commensal strain *E. coli K12* do not develop TH17 cell responses, whereas TH17 responses specific of this bacterium can be detected in mice colonised by a complex microbiota containing both SFB and *E. coli* (Lecuyer et al., 2014).

SFB may use several nonexclusive mechanisms to shape gut immune responses. A first mechanism may involve the production of flagellin(s). Indeed, SFB harbour a full set of flagella genes in their genome (Kuwahara et al., 2011; Pamp et al., 2012; Prakash et al., 2011; Sczesnak et al., 2011), and recombinant SFB flagellins are TLR5 stimulatory (Chen, Yin, Wang, Wang, & Xiang, 2017). Moreover, a recent work led by Pamela Schnupf has demonstrated SFB flagellation at the single-cell stage during both in vivo and in vitro SFB growth conditions (unpublished). Whereas flagella-mediated motility may enable IOs to reach their replicative niche at the epithelial surface, flagellated SFB IOs may simultaneously stimulate TLR5 or the NLR family CARD domain-containing protein 4 inflammasome in the intestinal epithelial cells, as well as, perhaps, in antigen-presenting cells if they can be reached by IOs. Of note, however, mice lacking MyD88, an adaptor indispensable for TLR5 signalling, develop normal TH17 response after colonisation by a complex microbiota (Ivanov et al., 2009), or monocolonisation by SFB (Gaboriau-Routhiau, unpublished observations), indicating that TLR5 is dispensable for this response.

One hallmark of SFB is its strong host-specific attachment of ileal epithelial cells (Figure 2a,b). By analogy with the attachment-dependent induction of TH17 cells by the enteropathogen *C. rodentium*, Atarashi et al., therefore, suggested that the TH17-inducing capacity of SFB requires epithelial attachment (Atarashi et al., 2015). Accordingly, they showed that, although the SFB isolated from mouse or rat intestine could comparably colonise germ-free rats and mice, gut TH17 responses were only induced in autologous hosts in parallel with SFB attachment to ileal epithelial cells. This result contrasted with a comparable induction of IL-22-producing ILC3 in autologous and heterologous hosts (Atarashi et al., 2015). SFB attachment was associated with an epithelial transcriptomic response and notably with the induction of mRNA encoding serum amyloid A (SAA), a protein that can act on $CD11c^+$ dendritic cells to promote TH17 differentiation from naive T cells and, of DUOX2, an epithelial enzyme that, via the production of reactive oxygen species, may also foster TH17 differentiation. Atarashi et al. further suggested that the actin reorganisation that is associated with the attachment of SFB to epithelial cells could promote SAA transcription (Atarashi et al., 2015). A very recent study also links the induction of TH17 cells with SFB-induced actin rearrangement in epithelial cells (Ladinsky et al., 2019). This study used electron tomography to analyse the synapse between SFB and epithelial cells and demonstrated that proteins, including the SFBNYU-03340 immunogenic protein that is targeted by the TH17 response (Yang et al., 2014), was transferred into epithelial cells trough adhesion-triggered endocytosis. Endocytosis of vesicles budding from the surface of SFB was independent of clathrin but required dynamin and activation of the cell division control protein 42 (Cdc42), a small GTPase of the Rho family, which plays a key role in actin cytoskeleton dynamics and vesicular trafficking (Ladinsky et al., 2019). Moreover, selective inactivation of Cdc42 in epithelial cells impaired SFB adhesion-triggered endocytosis and simultaneously reduced the induction of TH17 cells by SFB, especially those displaying specificity for SFBNYU-03340 (Ladinsky et al., 2019). Surprisingly, however, inactivation of Cdc42 in epithelial cells only partially impaired the epithelial transcriptomic response induced upon colonisation by SFB, and notably, it did not affect the transcription of genes encoding SAAs or DUOX2. It also failed to impair the IgA response induced by SFB (Ladinsky et al., 2019). Further studies are, therefore, necessary to delineate the exact role of adhesion-induced endocytosis and epithelial actin rearrangement in driving the spectrum of immune responses stimulated by SFB. Of note, adhesion-triggered endocytosis was not observed with other commensal or pathogenic strains, including strains that induce TH17 responses, suggesting that this mode of communication with epithelial cells is specific to SFB (Ladinsky et al., 2019). TH17 cells elicited by SFB may thus differ from those induced by pathogens. Accordingly, recent work showed that the homeostatic TH17 cells induced by SFB differ from the proinflammatory TH17 cells induced by *C. rodentium* by distinctive metabolic and transcriptomic programmes (Omenetti et al., 2019). Finally, it is interesting that the capacity of SFB to induce an intestinal TH17 response varies between mouse strains. Thus, in BALB/c mice, monocolonisation by SFB induced the expansion of ROR-γt^+ T cells in the gut lamina propria, but the latter cells did not express IL-17 unless mice were treated with IL-1β. Accordingly, this cytokine was strongly induced upon colonisation by SFB in LP $CD11c^+$ cells in C57BL/6 but not in BALB/c mice (Atarashi et al., 2015). It is now known that ROR-γt^+ T cells induced in response to intestinal colonisation can differentiate alternatively into ROR-γt^+ TH17 cells in the presence of proinflammatory cytokines or into ROR-γt^+ $FOXP3^+$ Tregs in the presence of butyrate or retinoic acid (Ohnmacht et al., 2015). Dietary factors that affect the production of short-chain acids in the intestinal tract can thus influence the differentiation of SFB-induced ROR-γt^+ T cells toward a TH17 or a Treg fate (Al Nabhani et al., 2019; Luu et al., 2019; Ohnmacht et al., 2015). Whether and how the mouse genetic background may influence SFB-induced signals and thereby modify the balance between different T cell subsets remain to be investigated.

6 | CONCLUSION AND PERSPECTIVE OUTLOOK

Over the past 20 years, it has become clear that the gut microbiota has a considerable impact on multiple host metabolic and immune pathways, whereas, conversely, environmental factors, lifestyle habits and host responses can modify the intestinal ecosystem and influence the composition and the metabolism of intestinal bacteria (Rook, Backhed, Levin, McFall-Ngai, & McLean, 2017). The highly dynamic interactions that take place between hosts and the gut microbiota have thus emerged as a major determinant in health and disease, raising multiple questions at the crossroads between microbiology, ecology, and host physiology. This review illustrates how cross-fertilising interactions between microbiologists and immunologists can help to dissect this complex cross-talk. The outstanding role of one single symbiont in orchestrating the maturation of the gut immune barrier in mice was not anticipated. This finding is, however, not completely surprising as most intestinal symbionts possess a core genome that allows their growth embedded within the mucus, a lifestyle that restricts direct contacts with the host surface and thereby minimises the activation of the immune system. In contrast, SFB have a reduced genome and a complex life cycle that requires their intimate contact with the epithelium for growth. SFB lifestyle is thus closer to that of enteropathogens than that of commensals. Yet if there is now compelling evidence that SFB adherence to epithelial cells contributes to the robust activation of the host immune system, it does not result in epithelial damage, indicating that SFB and its hosts have evolved a unique trade-off. The biology behind this unusual partnership remains largely enigmatic. Work is needed to identify the host receptors and signalling pathways that are used by SFB to launch self-limited immune responses as well as to define how these homeostatic responses may limit colonisation by SFB and/or participate to the barrier effect of the microbiota against pathogens. On the bacterial side, elegant studies have already identified surface proteins and immunodominant epitopes that are specifically targeted by host T cells (Yang et al., 2014). Yet much remains to be learned concerning the SFB receptors and molecular pattern motifs that mediate adherence and trigger host proinflammatory responses. Improvement of SFB culture and method(s) to genetically manipulated SFB remains to be established and will be instrumental to address adequately these questions. A crucial question also concerns the translation to humans of the outstanding role of SFB that has been established in laboratory rodents. Although SFB have been identified in the microbiota of many vertebrates, their presence in the human microbiota has remained debated due to the difficulty to identify relevant 16S DNA in human faeces (Sczesnak et al., 2011). SFB-related 16S DNA sequences have, however, been detected in the human faeces of Chinese children and, more recently, of U.S. children (Chen et al., 2017; Yin et al., 2013). The presence of SFB in the colonic lumen was confirmed using in situ hybridization and mass spectrometry detection of SFB-derived peptides (Chen et al., 2017). Interestingly, SIgA concentrations in the colonic fluid and ileal expression of immune transcripts and, notably IL-17 mRNA, were significantly increased in children with colonic fluid positive for SFB-16S DNA (Chen et al., 2017). These exciting results encourage cooperation between microbiologists and immunologists to further characterize the putative human SFB, to define whether and how it may contribute to a healthy and robust gut immune barrier in humans, and if so, to learn how to use it to boost gut defences against pathogens. Nurturing the competences necessary to pursue this inspiring project is one important legacy of Philippe Sansonetti.

ACKNOWLEDGEMENTS

The laboratory of Intestinal Immunity is supported by Institut national de la santé et de la recherche médicale (INSERM), Université de Paris, Fondation Princesse Grace, and ANR-10-LabX 6201. N. C.-B. is the recipient of ERC-2013-AdG 339407-IMMUNOBIOTA (H2020 European Research Council). Institut Imagine is supported by the programme "Investissement d'Avenir" ANR-10-IAHU-01. N. C.-B. thanks all the members of the laboratory and the collaborators who have contributed over the years to the work on host–microbiota interactions. She is particularly grateful to V. Gaboriau-Routhiau and P. Schnupf for the work led together, for their helpful comments on the manuscript, and for their generous gifts of figures. She thanks also I. Nkamba for providing the image of in vitro culture of SFB.

CONFLICT OF INTEREST

The author discloses no conflict of interest.

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How to cite this article: Cerf-Bensussan N. Microbiology and immunology: An ideal partnership for a tango at the gut surface—A tribute to Philippe Sansonetti. *Cellular Microbiology*. 2019;21:e13097. https://doi.org/10.1111/cmi.13097