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BACTERIAL FLAGELLINS: MEDIATORS OF PATHOGENICITY AND HOST IMMUNE RESPONSES IN MUCOSA

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ABSTRACT

Flagella contribute to virulence of pathogenic bacteria through chemotaxis, adhesion to and invasion of host surfaces. Flagellin is the structural protein that forms the major portion of flagellar filaments. Thus, flagellin is constituted of a conserved domain widespread in bacterial species dedicated to filament polymerization. Conversely, mammalian hosts detect the conserved domain on flagellin monomers through the Toll-like receptor (TLR) 5 and trigger pro-inflammatory and adaptive immune responses. This review describes the relation among flagellin molecular structure, bacterial virulence, and host defences with special emphasis on mucosal tissues.

- 88 words -

Teaser: Mucosal pathogens use flagella for invasion of host surfaces whereas the host detects and induces defences to pathogen through Toll-like receptor 5 that detects flagellin, the subunit of flagellum.

Motile bacteria display complex surface organelles, known as flagella, which are up to 15 μm long. Flagellar activity is coupled to chemotaxis machinery that senses environmental chemical and physical information and orchestrates migration for bacterial growth and survival [1]. Three parts are distinguished in the flagellar structure: a basal body that functions as a motor dependent on proton motive force, a torsion hook and a helical hollow filament (herein referred to as flagellum) [1]. Bacterial motility alternates a run lasting a few seconds and a tumble lasting a fraction of second. During a run, the motor rotates counterclockwise the left-handed helical flagellar filament, which forms a bundle and propels the cell. A tumble is caused by quick reversal of the motor rotation, which switches to a right-handed filament. Here, we summarize the structural and functional organization of the flagellum of the enteropathogenic bacteria *Salmonella enterica* serovar Typhimurium (Fig. 1). The flagellar filament is composed of as many as 20,000 subunits of a unique protein, flagellin (FliC, 495 amino acids, accession number AAL20871). Flagellin is secreted through the central channel of the growing filament in order to be assembled at the distal end in a helical structure [1,2]. A capping structure promotes the polymerization at the tip and prevents release of subunits in the bacterial environment. Depending on bacterial species, flagellins have molecular masses ranging from 28 to 80 kDa [3]. Sequence alignment shows conserved termini regions (about 170 N- and 100 C-terminal residues) flanking a central region, which is hypervariable both in residue composition and size. The N- and C-terminal chains of flagellin form packed α-helices structures, which constitute D0 and D1 domains, positioned in the filament core [2] (Fig. 1). The variable region of flagellin is exposed as a β-sheet folded structure (D2 and D3 domains) on the filament outer surface. Monomers of flagellin in solution are compactly folded as in flagellum except for the most terminal regions that are disordered [4].

Flagella are essential structures in the pathogenic potential of bacteria by providing motility

or increasing adhesion. On the other hand, recent findings highlight a major role of flagellin

monomer in the detection of microbes by the host and in the induction of immune responses.

Contribution of flagella to pathogenicity

The prerequisite event for any infection is the encounter of pathogenic bacteria with the target tissue. Plants, insects and mammals are all dealing with flagellated pathogens. Mucosal surfaces, especially epithelia, are the main sites of mammal host-pathogen interactions. Pathogenic bacteria specifically produce flagella to promote colonization and invasion of mucosa [5-7]. In mucosa, the flagellar structure is required for motility, adhesion, invasion or secretion of virulence factors.

Motility and pathogenesis

The glycocalyx and mucus layer associated to epithelium form inevitable physical and chemical obstacles for pathogens. Similarly, dynamic processes like upward flow of mucus of the bronchial epithelia or peristaltism in the intestine, have to be counterbalanced by pathogens to achieve colonization. In the host, motility combined to chemotaxis allow the fine tuned access of pathogens to target mucosal tissues (Fig. 2). Motility functions of *Helicobacter pylori* and *Pseudomonas aeruginosa* are crucial for infection of stomach and lung, respectively [8,9]. Colonization of intestinal mucosa by *Vibrio cholerae* strictly requires motility [7]. Colonization of rabbit appendix by *S. enterica* serovar Typhimurium depends also on accessibility and motility [10]. Since motility increases the occurrence of host-pathogen interactions, this feature contributes to the main role of the flagellum in pathogenesis.

Role of flagella in adhesion to and invasion of mucosal surfaces

Flagella can participate in the occupancy of a specific niche acting as an adhesin (Fig. 2). Crude flagella from the opportunistic pathogen *Clostridium difficile* bind to cecal mucus of germ-free mice [11]. In addition, non-flagellated *C. difficile* associate 10-fold lower with the cecal tissue than a flagellated strain, highlighting the role *in vivo* of flagella in adherence to mucus. In cystic fibrosis, *P. aeruginosa* colonizes the airway lumen at several μm from the

surface. Noticeably, *P. aeruginosa* flagellin binds mucin Muc1, an abundant component of airway mucus [12]. Furthermore, enteropathogenic *Escherichia coli* adhere to the intestinal mucosa or to tissue culture cells via flagellum-dependent mechanism [13]. The hypervariable region of flagellin (D2-D3) is likely bearing the adhesin-like properties (Figs. 1 and 2). The importance of flagella for the invasion of epithelial cells has been reported for several bacteria like *Yersinia enterocolitica* [5,14].

Flagellum as a secretion system for toxins

The machinery for flagellum biosynthesis is the paradigm of type III secretion system (TTSS) [1]. In pathogenic bacteria, TTSS serve as molecular syringes required for export and injection of virulence factors into the cytosol of host cells [15]. Accordingly, pathogens hijack cytosolic pathways to colonize or kill the host cells. The flagellar TTSS can be an additional mechanism for export of virulence factors (Fig. 2). In *Y. enterocolitica*, flagellum mediates secretion of several extracellular toxins including the phospholipase YplA [16].

Coordinated expression of virulence genes and genes involved in flagellum synthesis

The transcription of about 50 flagellar genes is hierarchically controlled by environmental conditions via the master regulator operon *flhDC* [17]. In *Salmonella*, both flagellar genes and loci encoding virulence factors are part of regulons dependent on FlhD-FlhC and two-component sensor kinase and response regulator such as PhoP-PhoQ or BarA-SirA [17,18]. In *V. cholerae*, the ToxR regulatory system coordinates the transcription of motility genes and specific virulence genes in response to environmental conditions [7]. In contrast, the BvgAS system of *Bordetella bronchiseptica* represses flagellum gene transcription while it activates the expression of virulence factors [19]. In general, expression of flagella is likely switched off once mucosal pathogenic bacteria disseminate into deeper tissues [6].

Mucosal pro-inflammatory responses to flagellin

Colonization of mucosa is restricted by renewal of epithelial cells, barrier function of the intercellular junctions, andthe production of antimicrobial molecules such as lysozyme and defensins, which clear microbes [20]. Since pathogens are equipped to breach the mucosal barriers, hosts need to respond rapidly to circumvent dissemination of bacteria. These transient defences, classified as pro-inflammatory response or inducible innate immunity are stimulated by mucosal sentinel cells. Whereas macrophages, monocytes and dendritic cells (DC) are the main sentinels in systemic compartment, epithelial cells represent the first and major cell type encountered by microorganisms in mucosa [20]. The sensing of pathogens is achieved by pattern-recognition receptors (PRR) that detect conserved microbe-associated molecular patterns (MAMP) [21]. The Toll-like receptor (TLR) family plays a key role in intra- and extra-cellular detection of MAMPs, like bacterial lipopolysaccharide or LPS (TLR4), lipopeptides (TLR2), peptidoglycan (TLR2-TLR6), or unmethylated CpG DNA (TLR9) [21]. TLRs are transmembrane proteins that function as homo- or hetero-dimers [21]. TLRs are organized in three functional domains: an extracellular leucine-rich repeat (LRR) module involved in MAMP recognition, a membrane spanning motif and a Toll/Interleukin 1 receptor domain (TIR) required for transmission of stimulus to adaptor molecules such as MyD88 [21]. TLR signalling activates nuclear factor (NF)–κB and mitogen-activated protein kinase (MAPK) pathways that in turn modulate transcription of genes encoding immune mediators [21].

Flagellins present features of MAMPs, i.e. sequence conservation and wide distribution among bacteria (Box 1). In mammals, TLR5 is involved in detection of flagellin [22] (Figs. 1 and 3b). Differences in human and mouse TLR5 sequences mediate the species-specific recognition of flagellins [23]. MyD88 is an essential adaptor molecule for TLR5 since responses to flagellin are totally abolished in MyD88-knockout animals [22,24]. TLR5

stimulates transcription of pro-inflammatory genes dependent on NF-κB and MAPKs, namely p38, JNK, and ERK1-2 [22,25,26].

Flagellin monomers mediate mucosal pro-inflammatory responses

Low concentrations of flagellin ($ED_{50} \sim 10\text{-}50 \times 10^{-12}$ M) triggers pro-inflammatory signalling in sentinel cells [27,28]. Monocytes and macrophages produce pro-inflammatory cytokines TNF-α and IL-6, and/or nitric oxide [27,29]. Systemic injection of flagellin induces similar effects in mice [22,30]. In epithelial cells, the flagellins from *S. enterica* serovar Typhimurium, Dublin, and Enteritidis, enteropathogenic *E. coli*, and *P. aeruginosa* stimulate the polarized secretion of interleukin (IL)-8 (also named CXCL8), the chemokine essential for recruitment of neutrophils and macrophages at the sites of injury [25,28,30-33]. Upon flagellin stimulation, epithelial cells also up-regulate production of inducible nitric oxide synthase (iNOS) and nitric oxide, matrilysin (MMP-7), human β-defensin 2 (h-βD2), and chemokines like CXCL2 (MIP-2α) [26,30,32]. In mucosal tissues, these factors participate in anti-microbial activity, in the recruitment of professional killer as well as antigen-presenting phagocytes, and in the production of inflammatory mediators that set up the platform for phagocyte activation. Although virulence factors such as invasins were initially found to be required in activation of pro-inflammatory response by pathogenic bacteria in epithelial cells, recent studies showed that this response is fully recapitulated by flagellin [25,26].

Epithelial cells of the gut and the airways produce TLR5 *in vitro* and *in vivo* [34-36]. TLR5 expression was initially found on the basolateral surface of epithelial cells and dedicated to detection of invasive bacteria such as *S. enterica* serovars [35]. This distribution was confirmed *in vivo* [30]. However, flagellin also activates apical signalling in epithelial cells, indicating that TLR5 is present in the luminal compartment [25,32]. Therefore, TLR5 is able to detect both extracellular-luminal and invasive flagellated pathogenic bacteria.

Relation between flagellin structure and immunostimulatory activity

The hypervariable domain of flagellins from pathogenic *E. coli* and *Salmonella* species is not required for TLR5 signalling [23,37]. This is consistent with stimulatory activity of *Listeria monocytogenes* flagellin that lacks variable region [22]. Monomers of flagellin induce TLR5 signalling whereas filamentous flagella do not [23]. Indeed, the signalling moiety of flagellin is hidden in the flagellum but becomes accessible in the monomer (Figs. 1 and 3b). Most mutations in the conserved D1 domain (residues 44-129 and 406-454 of *S. typhimurium*) reduce recognition of flagellin by TLR5 and have a profound effect on motility [23,37]. The hydrophobic motif 88-97 in *S. typhimurium* and conserved in 377 public sequences was proposed to interact with TLR5 [38]. TLR5 activation is independent of any post-translational modification of flagellin [23,39]. Interestingly, mechanisms developed by bacteria to escape from TLR5 detection can depend on flagellin sequence (Box 2). Together, TLR5 detects a specific conformation of flagellin domain D1, which is required for flagellum formation and function and is exposed only in monomer.

TLR5 is unique since the detected pattern is a protein. TLR5-flagellin complexes can be isolated suggesting direct protein-protein interactions [23,40]. Both LRR regions 1-407 and 386-636 of human TLR5 mediate binding of flagellin in contrast to 1-386 [40]. Thus, Mizel *et al.* proposed that the peptide 386-407 on TLR5 is a major flagellin binding site [40]. A putative flagellin-binding site (positions 552-561) in human TLR5 has also been proposed from bioinformatics analysis [38]. However, the two sites do not correlate with any function in signalling [35,40]. Remarkably, a natural truncated form of TLR5 (1-392) acts as a dominant negative mutant on wild type TLR5 [41]. In conclusion, further investigations are needed to decipher the molecular interactions of TLR5 with flagellin.

Another striking finding on TLR5 is the recognition of a monomer ligand. The simplest hypothesis is that detection of one monomer by one receptor results in signal transduction.

Such interaction has been observed with recombinant LRR regions of TLR5 and flagellin [40]. However, signalling function of TLR5 requires dimerization [22]. We can therefore speculate that TLR5 homodimers bind (1) two monomers in symmetric arrangement, or (2) one monomer in an asymmetric form. TLRs usually detect MAMPs formed of repetitive motifs like peptidoglycan. Such MAMPs aggregate many TLRs and thereby concentrate signalling machinery in focal complexes. In contrast, TLR5 combined to flagellin are likely to form individual signalling units. Such specificity might account for the effect of flagellin on adaptive immune responses [24].

Recent studies proposed that TLR2, TLR4, and gangliosides including asialo-GM1 cooperate with TLR5 as receptors for binding flagellin and/or signalling [42,43]. However, their contribution needs to be addressed to rule out the possible effects of other bacterial components in the preparations.

Delivery of flagellin monomer in mucosa

The mechanisms by which flagellin is released by bacteria during tissue colonisation are crucial for TLR5-mediated responses. Although flagellin is usually assembled in the flagellum, leakage and/or uncapping account for the *in vitro* secretion of flagellin [44] (Fig. 3a). Flagellin might be released in mucosa by secretion as observed *in vitro* (Fig. 3a). Flagellin delivery could be part of bacterial- and/or host-directed activities (Fig. 3a). For example, *Caulobacter crescentus* ejects its flagellum when this organelle is no longer required for the bacterial life cycle [45]. Alternatively, flagella could be sheared from bacterial surfaces by host proteases or detergents such as bile salts or surfactants.

Modulation of mucosal adaptive immune response by flagellin

PRRs translate the pathogen-derived component in a specific "identity card". Thus, appropriate antibodies and T cell responses are elicited to eradicate the pathogen. DCs are the key antigen-presenting cells that control the induction of adaptive immunity [46]. Immature DCs are resident sentinel cells in tissues that are specialized in antigen capture and that can be activated by MAMPs. Upon activation, DCs migrate to draining lymphoid tissues where they present antigens to naïve T cells and provide signals for the development of appropriate CD4^{+} T helper (Th) responses [46]. CD4^{+} T cells committed to Th2 phenotype produce IL-4 and promote antibody responses whereas Th1 cells promote cell-mediated mechanism via interferon-γ (IFN-γ□□□□□□□□□□ Here, we will describe the studies that explored the TLR5-mediated effect of flagellin on adaptive immunity (Fig. 4).

TLR5: the paradigm of TLRs promoting Th2 and regulatory responses

The pioneer studies conducted with flagellin isolated from *Salmonella adelaide* showed that flagellin is a potent stimulator of antibody responses, a hallmark of Th2 responses (for review see [47]) (Fig. 4a). However, immune deviation towards delayed-type hypersensitivity, a hallmark of Th1 responses was observed when flagellin structure was disturbed. Thus, this seminal work was instrumental in the elaboration of the paradigm of Th1-Th2 cells [47]. PRR-mediated DC activation can now provide a molecular clue. Recent works indicate that the primary signalling events, which initiate Th2-type responses are mediated by TLR5. First, TLR5-stimulatory activity similarly to antibody-promoting properties is lost when mutations perturb the conserved domain of flagellin [23,37] (Fig. 4a). Although flagellin was initially reported to stimulate IFN-γ production in mice [39], we recently established that flagellin promotes the development of Th2-biased (IL-4 producing T cells) and antibody responses [24] (Fig. 4b). Flagellin induces maturation of TLR5-espressing DCs and the up-regulation of co-stimulatory molecules and antigen-presenting capacity in a MyD88-dependent manner

[24]. TLR5 signalling in DCs likely involves production of Th2-promoting or Th1-suppressing signals. In mouse or human DCs, flagellin does not enhance the production of IL-12 p70, a key role cytokine in development of Th1 cells, that could favour Th2 development [24,48]. Alternative mechanism for Th2-biased responses could be a moderate T cell-mediated suppression by TLR5-stimulated DCs, a process involving IL-6-dependent blockade of regulatory T cells (CD4$^+$CD25$^+$) [49]. Finally, TLR5 signaling challenge the dogma that TLR activation promotes Th1 immunity whereas Th2 responses are elicited by other PRRs [46].

Parish *et al.* observed that the immune deviation depends on flagellin dose, a phenomenon called high- and low-dose antibody tolerance and this might also correlate with the TLR5-stimulatory activity [47]. At low doses, there is no effective level of TLR5 agonist whereas at stimulation with increasing doses of TLR5 agonist, DCs first and then regulatory CD4+ T cells might directly be activated. High doses of TLR ligands might directly stimulate the suppressive functions of regulatory T cells (CD4$^+$CD25$^+$) on CD4$^+$ T cell responses in a TLR-dependent manner [50]. Interestingly, regulatory T cells do express TLR5 [50].

TLR5-dependent mucosal adaptive responses: DC recruitment by epithelial cells

Stimulation of mucosal immunity requires activation and antigen uptake by lamina propria DCs [20,51]. In Peyer's patches, bacteria are transported by M cells from lumen to lamina propria where DCs are activated and promote imprinting of gut-specific T cells [51,52]. Although pathogens target such sites, they represent only a minor part of mucosa. DCs with membrane protrusions extending into the lumen might directly interact with bacteria throughout the mucosa [53]. The direct activation of DCs within mucosa by flagellin might participate in Th2 differentiation that favours secretory antibody responses (Fig. 4b).

Flagellin-mediated stimulation of epithelial cells is probably decisive for mucosal immune responses. Flagellin triggers the transient expression of the DC-specific chemokine CCL20

(also known as MIP-3α or LARC) [32] (Fig. 4c). Interestingly, *ccl20* expression is constitutive in Peyer's patch epithelium and associated to the sub-epithelial positioning of DCs producing CCR6, the CCL20-specific receptor [54]. CCR6-deficient mice have impaired mucosal adaptive immune responses [54]. Flagellin-treated epithelial cells attract immature DC in a CCL20-dependent manner [32]. At the same time, flagellin-stimulated epithelial cells secrete IL-8 and recruit neutrophils that provide appropriate pro-inflammatory signals for DC maturation [28]. *ccl20* transcription is restricted to mucosal epithelium by mechanisms involving the epithelium-specific transcriptional factor ESE-1 and NF-κB [54-56]. Remarkably, lymphotoxin-β activates NF-κB2 (RelB-p52) and sustained *ccl20* expression that can account for constitutive CCL20 production in Peyer's patch epithelium. On the other hand, TLR5 causes NF-κB1 (p50-p65) activation and a short-lived expression of CCL20 in epithelial cells [55,56]. It is tempting to enlarge this analogy to TLR-mediated epithelial "education" of DCs to imprint the mucosal responses as described for Peyer's patch DCs (Fig. 4c) [52].

The characterization of processes occurring after mucosal TLR activation in mucosa will be essential to decipher how adaptive immune system handle antigens and to develop new strategies for mucosal vaccination.

Conclusions

The widespread distribution of flagellin in bacteria and its use during pathogenesis result in elaborated mechanisms for recognition by eukaryotes. Now, the crystal structure of flagellar filament and flagellin provide outstanding information to investigate the contribution of the various domains of the molecule to the pathogenic and immunological processes. Combining this approach with genetically engineered animals, including mice conditionally deficient for TLR5 will help to decipher the role of mucosal and especially epithelial TLR5 in the host protection against flagellated bacteria. Recent findings emphasize the association of particular TLR5 polymorphisms in humans with the incidence of *L. pneumophila* infection [41], thereby providing new opportunities to control the human mucosal innate-adaptive immune systems.

Boxes

Box1: Conservation of flagellin detection systems in plants and mammals

Similarly to mammals, plants detect MAMPs from pathogenic microorganisms, called general elicitors, via PRRs and then elicit a defence response. Like fungal cell wall constituents (e.g. chitin, ergosterol, and glucans), flagellin monomers of *Pseudomonas syringae* activate the production of reactive oxygen species, ion fluxes, and ethylene in plants. A highly conserved linear motif of 22 residues close to the N-terminus of flagellin ("QRLSTGSRINSAKDDAAGLQIA" for *P. syringae*) is recognized by FLS2, the flagellin sensitive locus 2 product [57] (Figs. 1 and 3b). FLS2 is a membrane-spanning protein composed of a detection LRR domain and an intracellular signalling module, which functions as a serine-threonine kinase. Stimulation of FLS2 triggers rapid protein phosphorylation, the activation of MAPK cascade and the induction of defence-related genes. Since LRRs are not homologous in FLS2 and TLR5 and flagellin-detected motifs are different, the LRR structure has likely been selected independently in plants and mammals to serve as MAMP detection domain for flagellin of harmful flagellated bacteria.

Box2: Bacterial strategies to escape to flagellin-specific host immune responses

Activation of TLRs causes immune responses deleterious for any micro-organisms. Although some flagellated pathogenic bacteria cope with defences induced by TLR5-flagellin, others pathogenic as well as non-pathogenic bacteria use various strategies to prevent or hijack this activation (see also [20]). Flagellated bacteria can produce flagellin lacking pro-inflammatory properties. The flagellins from *H. pylori* FlaA and FlaB (accession numbers NP-207936 and NP-206915) preserve motility properties but not stimulating activity for human TLR5 [58]. Interestingly, the primary sequences of signalling D1 domain from *Helicobacter* flagellins are

poorly homologous to TLR5-stimulatory flagellins, especially to the region 88-97 of *S. typhimurium* flagellin [38]. The control can also operate at the level of flagellin expression. Flagellated bacteria when cultivated *in vitro* might not express flagellin in the host environment or only in a defined sequential virulence process. Alternatively, flagellin can be tightly enclosed in a highly stable flagellum or covered by a membranous sheath to prevent any release of monomers in the surrounding environment like in *V. cholerae* and *H. pylori.* Finally, factors produced by bacteria can down-regulate the pro-inflammatory signalling in response to their own or to PAMPs, including flagellin. In order to prevent nuclear activation of NF-κB-dependent genes, avirulent *Salmonella pullorum* blocks the degradation of IκB-α, the cytosol-sequestering molecule of NF-κB and commensal *Bacteroides thetaiotamicron* stimulates the nucleus-cytosol shuttling of RelA (a subunit of NF-κB) [59,60]. Nevertheless, the *in vivo* contribution of these various mechanisms remains to be established.

FIGURE LEGENDS

Figure 1. Structure and organization of flagellum and flagellin. Flagella show a hook (dark green) and a filament, referred to as flagellum (yellow). Schematic transversal and longitudinal views of the flagellum and ribbon diagram of the Cα backbone of flagellin from *Salmonella enterica* serovar Typhimurium. A colour code represents the flagellin domains: the terminal α-helix chains (D0, purple), the central α-helix chains (D1, blue), and the hypervariable region with β-sheets (D2 and D3, yellow). The concentric circles on the end-on view show the organization of domains within the flagellum. The α-helix regions (purple and blue), necessary for filament architecture and motility functions, are embedded in the flagellum inner core. Flagellin monomer is the molecular pattern detected by innate receptors. In the monomer, flagellin D0 terminal chains are totally disordered whereas the D1, D2, and D3 domains remain compactly folded. Both terminal regions of D1 domain (blue rectangle) are required for Toll-like receptor 5 (TLR5) signalling in mammals, suggesting that a flagellin conformation is detected by TLR5. In plants, a linear motif in the N-terminus (purple circle) is required for defences mediated by flagellin sensitive receptor (FLS2).

Figure 2. Contribution of flagellum to bacterial pathogenesis in mucosa. (1) Motility combined to chemotaxis participate in the penetration and colonization of specific niches in mucosa. (2) Bacterial adhesion to mucus or to epithelial cell surface assisted by flagella. The outer hypervariable domain of flagellin is likely involved in this function, suggesting a dependence on serotype. (3) Upon bacterial penetration of mucus, virulence factors (black dots) can be specifically exported by the flagellar secretion system to poison the host or hijack machinery of epithelial cells (hatched). (4) Flagella promote bacterial invasion during infection.

Figure 3. Mucosal pro-inflammatory responses to flagellin monomers. (a) Models of flagellin monomer delivery in mucosal tissue. Some bacterial species secrete flagellin monomers (red dots) in culture due to inefficient capping or flagellum break. This release might spontaneously occur *in vivo* or be controlled by host proteases and/or detergents shearing or uncapping flagella. When expression of flagellar components is already turned off, removal of flagella might occur and deliver intact filament into environment. A bacterial-regulated removal of flagellum might take place in mucosa. Alternatively, host factors can cause flagellum break. The conversion of filament into flagellin monomers demands specific physico-chemical conditions that can be encountered in the mucosa. (b) Flagellin-specific pattern-recognition receptors and signal transduction pathways. Detection of flagellin monomer is performed by Toll-like receptor 5 (TLR5) in mammals and flagellin sensitive receptor (FLS2) in plants (*Arabidopsis*). Both transmembrane receptors are formed by extracellular leucine-rich repeats that detect distinct regions of flagellin monomers. TLR5 requires the universal TLR-specific adaptor molecule MyD88 that transfers the stimulus from Toll-Interleukin 1 receptor domain (TIR) to downstream molecules of the signalling cascade like IRAK1, TRAF6, IKK, and IκB-α molecules. Finally, the signalling results in activation of nuclear factor (NF)–κB and mitogen-activated protein kinases (MAPK) that turns on transcription of genes involved in innate and adaptive immunity. Here are reported some well-characterized factors that are upregulated upon flagellin stimulation of epithelial cells. In plants, flagellin activates the serine-threonine kinase (K) domain of FLS2 that induces a phosphorylation cascade of MAPKs and expression of plant defence mechanisms. Interleukin 8 (IL-8); Nitric oxide synthase (iNOS); Matrilysin (MMP-7); human β-defensin 2 (h-βD2)

Figure 4. Flagellin-specific regulation of adaptive immunity. (**a**) Correlation between Toll-

like receptor 5 (TLR5)-stimulatory activity and flagellin structure. *Left panel*: The model of Parish considers *S. adelaide* flagellin as a potent antigen that stimulates antibody responses in rats. By disturbing flagellin structure, antibody response is impaired but delayed-type hypersensitivity that reflects Th1 response is stimulated. In these experiments, animals were primed with native (orange bar) or modified flagellin molecules that imprint the immune response, boosted with native flagellin and then assayed for antibody or delayed-type hypersensitivity. *Right panel*: TLR5-stimulatory activity is lost when mutations and/or insertions disturb the conserved domain of flagellin as presented schematically by the decrease in nuclear factor (NF)–κB induction. (**b**) Flagellin-dependent activation of dendritic cells (DC). Mucosal DCs sending membrane protrusions in lumen are in direct contact with the bacteria. M cells (yellow) are specialized epithelial cells intestinal Peyer's patch that transport and deliver luminal bacteria to subepithelial DCs. These DCs can directly be stimulated by flagellin released by bacteria. Flagellin induces DC maturation in a TLR5-, MyD88-, and NF-κB-dependent manner. In this model, TLR5 signalling stimulates DC to shape Th2-biased immunity via production of Th2-promoting or Th1-suppressing factors. Such a mechanism in combination with epithelial environment imprints DCs and lymphocytes with mucosal-specific immunity program. (**c**) The flagellin-specific epithelial pathway. The following scenario might support an activity of flagellin on adaptive immunity controlled by epithelium: (1) flagellin monomers released from flagellated bacteria activate nearby epithelial cells. TLR5 signalling can be induced either on apical or basolateral side of epithelial cells; (2) for a short period, epithelial cells produce CCL20 and pro-inflammatory mediators that recruit immature DCs and neutrophils, respectively, and factors that shape recruited DC in a mucosal-type DC (3) transient histological changes occurs in epithelia facilitating uptake of luminal bacteria by recruited DCs; (4) DC maturation in the stimulated mucosa is followed by migration and presentation of bacteria-derived antigens in draining

lymphoid structures, thereby stimulating mucosa-specific responses. Capture of bacteria might result from additional changes on epithelial cells dependent or not on TLR5. These changes are illustrated on the right panel: (top) the disruption of epithelial barrier provide a portal of entry for bacteria, (middle) the recruited DCs displace tight junctions and send membrane protrusions in the lumen, and (bottom) the epithelial cells become transiently capable to transport particles like do M cells of Peyer's patch.

Acknowledgments

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