



Mucosal interplay among commensal and pathogenic bacteria: lessons from flagellin and Toll-like receptor 5.

Martin Rumbo, Clément Nempont, Jean-Pierre Kraehenbuhl, Jean-Claude Sirard

► To cite this version:

Martin Rumbo, Clément Nempont, Jean-Pierre Kraehenbuhl, Jean-Claude Sirard. Mucosal interplay among commensal and pathogenic bacteria: lessons from flagellin and Toll-like receptor 5.. FEBS Letters, 2006, 580 (12), pp.2976-84. 10.1016/j.febslet.2006.04.036 . inserm-00071278

HAL Id: inserm-00071278

<https://inserm.hal.science/inserm-00071278>

Submitted on 9 Oct 2007

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Mucosal interplay among commensal and pathogenic bacteria: lessons from Flagellin and Toll-like receptor 5

Martin RUMBO¹, Clément NEMPONT², Jean-Pierre KRAEHENBUHL³, and Jean-Claude SIRARD²

¹UNLP, Laboratorio de Investigaciones en el Sistema Inmune, Facultad de Ciencias Exactas, La Plata, Argentina

²INSERM, Equipe Avenir d'Immunité Anti-Microbienne des Muqueuses, U801, Institut Pasteur de Lille, Lille, France

³EuroVacc Foundation, Division of Immunology and Allergy, Centre Hospitalier Vaudois, CH 1011 Lausanne and Swiss Institute for Experimental Cancer Research, CH 1066 Epalinges, Switzerland

Correspondence: INSERM, Equipe Avenir d'Immunité Anti-Microbienne des Muqueuses, U801, Campus Institut Pasteur de Lille – Institut de Biologie, 1 rue du Pr. Calmette - BP 447, 59021 Lille Cedex, France. Telephone: +(33) 320871076. E-mail: Jean-Claude.Sirard@ibl.fr or Jean-Pierre.Kraehenbuhl@isrec.ch

Abstract **(96 words)**

Toll-like receptors (TLR) detect pathogen-associated molecular patterns (PAMP) and play a crucial role in triggering immunity. Due to their large surfaces in direct contact with the environment, mucosal tissues are the major sites of PAMP-TLR signalling. How innate and adaptive immunity are triggered through flagellin-TLR5 interaction is the main focus of the review. In view of recent reports on genetic polymorphism, we will summarize the impact of TLR5 on the susceptibility to mucosal infections and on various immuno-pathologies. Finally, the contribution of TLRs in the induction and maintenance of mucosal homeostasis and commensal discrimination is discussed.

Key Words: Toll-like receptor; Flagellin; Innate immunity; Epithelium; Pathogenic bacteria; Commensal flora

List of abbreviations: Dendritic cells: DC; Microbe-associated molecular pattern: MAMP; Pathogen-associated molecular pattern: PAMP; Toll-like receptor: TLR; Pattern-recognition receptor: PRR.

1. Introduction

Mucosal surfaces represent main interaction sites with environmental microorganisms and antigens. Tight control of microbial-host interactions is critical to the homeostasis of mucosal tissues. Colonization and invasion is continuously prevented by i/ the barrier function of the mucosal epithelia (tight junctions, glycocalyx and mucus), ii/ the continuous shedding of epithelial cells, and iii/ chemical factors, i.e. microbicidal peptides, bacteriostatic proteins and enzymes, which contribute to the neutralizing or the killing of microbes [1]. Pathogens that escape this first line of defence and invade the host are facing a second line of defence that consists of resident bone marrow-derived cells, i.e. granulocytes, monocytes and mast cells that also are capable to neutralize and kill the invading microorganisms. Conserved pathogen-associated molecular patterns (PAMP) and host pattern-recognition receptors (PRR) like the Toll-like receptors (TLR) play a crucial role in signalling danger and initiating the host innate responses. In contrast, the conserved molecular patterns expressed by the gut microbiota are essential in triggering TLR-dependent mucosal homeostasis [2-4]. Furthermore, self tissue-derived molecules produced in mucosa during infection or injury, such as hyaluronan break-down products, trigger TLR signalling and, therefore, contribute to protective response [5]. This constitutes, at the molecular level, one example of “danger signal” that extends TLR functionality to identification of self alarm signals [6].

Mucosal surfaces can face either a sterile environment as in the lower airways and the upper uro-genital tract or continuously face environmental microorganisms such as in the gut, the upper airways and the lower uro-genital tract. These features have a profound impact on how microbes are detected and the outcome of TLR signalling. In addition, mucosal tissues have developed strategies to tolerate commensals and eliminate pathogens. In mucosal tissues, pathogens trigger pro-inflammatory responses through TLR signalling characterized by the local release in the lumen of a broad spectrum of anti-microbial factors and in the lamina propria of chemokines that recruit professional phagocytes into mucosal tissues. Among the recruited cells, dendritic cells (DC) play a special role since they link innate to adaptive immunity by taking up

microbes at the recruited mucosal site and transporting them in draining lymph nodes where they can initiate adaptive immune responses. The first sentinel cells exposed to TLR ligands are the epithelial cells lining the mucosal surfaces. For instance, pneumocytes, bronchial epithelial cells, enterocytes or colonocytes express various sets of TLR and are activated by the appropriate microbial molecular patterns to generate a pro-inflammatory response. Microbes that are able to cross the epithelial barrier via specific transport mechanisms or upon injury stimulate resident sentinel cells expressing TLR such as macrophages, mast cells, granulocytes and DCs.

This review focuses on most recent findings on the contribution of TLR in mucosal immunity with special emphasis on Flagellin-TLR5 interaction.

2. Molecular features of Flagellin detection by TLR5

Flagellin is a structural protein of the flagellum, a surface filament required for bacterial motility. In pathogenic bacteria, flagella and chemotaxis machinery contribute to virulence by providing motility towards niches where infection can take place (as exemplified for *Vibrio cholerae* in [7]). There are also evidence that flagella are important for adhesion and invasion [8]. The size of bacterial flagellins ranges from 250 to 1250 amino-acids depending on the species [9]. The most studied molecule is the *S. typhimurium* Flagellin, a 494 amino-acid long protein, which is organized in two functional domains (Fig. 1A). The highly conserved 140 amino-terminal and 90 carboxy-terminal residues constitute a polymerization and motility-related domain, while the central part of the molecule is highly variable among *Salmonella* serovars and bacterial species and forms the domain exposed at the outer surface of the flagellum [9].

In mammals, the sensing of microbes is achieved by PRRs like TLRs [10]. TLRs are transmembrane proteins organized in an extracellular leucine-rich repeat (LRR) module, a membrane spanning motif and a Toll/Interleukin 1 receptor domain (TIR) required for signal transduction [10]. The 13 members of TLR family play a key role in extracellular and intravacuolar detection of PAMPs [11]; TLR5 is involved in detection of bacterial flagellins [12, 13]. Both human and mouse TLR5 recognize similar molecular determinants of flagellin from various bacteria but, like for the lipopolysaccharide (LPS)

detector TLR4, there are minor differences in the molecular interactions between the 2 species [14]. The hypervariable domain of flagellin is not required for TLR5 activation [15] [8]. The motif involved in signalling is formed by residues 89-96 of *S. typhimurium* flagellin [16, 17]. Interestingly, this moiety is hidden in the flagellum and becomes accessible only when flagellin is delivered as a monomer (Fig. 1A-B). The recent elucidation of the three dimensional structure of TLR3 may provide some clues on TLR5 function [18, 19]. TLR3 LRRs harbouring amino-acids insertions have been associated with the binding of cognate PAMPs. The extracellular domain of TLR5 is composed of 20 LRR with five LRR (7, 9, 14, 15 and 17) containing insertions. Deletion studies suggested that the flagellin-binding site in human TLR5 is located between residues 386 and 407 of LRR14 [20]. Deletion of TLR5 starting at LRR14 (392 N-terminal residues or TLR5_{392-stop}) results in the loss of responsiveness to flagellin [21]. Recently, the Naip5 pathway has been involved in the cytosolic detection of flagellin [22]. It will be important to define whether this is a mean for the host to detect intracellular flagellated bacteria.

The TLR5 cascade depends exclusively on the TIR adaptor molecule MyD88, a molecule essential for most TLRs (Fig. 1B) [12, 13, 23]. TLR5 signalling activates nuclear factor (NF)- κ B and mitogen-activated protein kinase (MAPK) pathways, i.e. p38, JNK, and ERK1/2, that regulate the transcription of genes encoding immune mediators [24-27]. Many pathogenic bacteria use flagella to establish their niche at the surface of mucosal tissues. Sensing flagellin by TLR5 may act as an early detection system that triggers a rapid host response [8, 28]. Detection of flagellin occur at the luminal surface of the epithelial cells covering mucosal tissues and not in intracellular compartments of infected host cell [13, 29-31]. How flagellated bacteria physiologically deliver monomeric flagellin is yet not known. Flagellin may be shed from the bacterial surface by host proteases or detergents such as bile salts or surfactants.

Phylogenically, vertebrate TLRs can be grouped in six families [11]. TLR5 belongs to a unique family and is expressed both in fishes, birds, rodents and primates. In plants a 22-mer peptide found in the proximal amino-terminal part of flagellin is recognized by the receptor FLS2 that shares structural and functional similarities with TLR [32]. Flagellin/FLS2 recognition takes place on the surface of plant cells and not in intracellular compartments, in parallel to what happens in mammalian mucosa.

3. Detection of pathogenic motile bacteria in the gut

Intestinal mucosa is specialized in nutrient and water intake from the lumen. This tissue is heavily colonized by a microbial flora that participates in digestive functions through degradation of some dietary substances. Bacterial metabolism also provides the host with compounds that influence intestinal gene expression thereby influencing the whole gut physiology [33]. The gut is also able to prevent colonization of pathogens without compromising the indigenous microflora. Remarkably, commensal as well as pathogenic microorganisms express similar conserved molecular patterns. Epithelial cells express several sentinel functions that makes them a central player in innate surveillance. In addition to the epithelial cells, various mucosal cell types express PRRs including TLRs. Thus, DCs and macrophages present in the lamina propria may be activated by translocated bacteria. Alternatively intraepithelial DCs may directly interact with luminal PAMPs via dendrites that reach the lumen between the epithelial cells [34]. While flagellin triggers pro-inflammatory signalling in sentinel cells such as macrophages, DCs and monocytes upon systemic challenge, less is known about the contribution of TLR5 signalling by these cells in the gut [23, 35, 36]. In contrast, the flagellin-TLR5 system plays a major role in intestinal innate immunity (Fig. 1C) [37, 38], since many pathogenic bacteria express flagella to colonize mucosa and likely release flagellin in the lumen [8, 28].

Gene profiling indicates that flagellin alone recapitulates the pro-inflammatory response triggered by the whole *Salmonella* [26, 39]. The expression pattern triggered by flagellin correspond to an archetypal TLR signature [40], with the up-regulation of i/ a broad-spectrum anti-microbial agents such as inducible nitric oxide synthase (iNOS), matrilysin (MMP-7), and human beta-defensin 2 (h- β D2) [8], ii/ chemokines (IL-8 or CXCL8) that recruit immune cells, i.e. neutrophils, macrophages and DCs, into the injured tissue, and iii/ cytokines that together with recruited DCs [41] provide an appropriate milieu to mount an adaptive immune response (Fig. 1C) [8, 37]. Flagellin triggers the transient epithelial expression of the DC-specific chemokine CCL20 (also known as MIP-3-alpha or LARC) that in turn attracts immature DCs [42]. Interestingly,

ccl20 expression in Peyer's patch epithelium is associated to sub-epithelial positioning of DCs producing CCR6, the CCL20-specific receptor and to induction of mucosal adaptive immune responses [43, 44]. Recruitment of DCs by epithelial TLR signalling may therefore represent the mechanism coupling innate to adaptive immunity in mucosa.

The distribution of TLR5 along the intestinal epithelium sheds light on the physiological function of PRR in innate immunity. In the mouse intestine, TLR5 is expressed in small intestinal epithelium (our unpublished results) [30, 45, 46], but maximally in the caecum and colon epithelium [29, 31]. TLR5 is both apical and basolateral in the ileum whereas in colon expression is restricted to the basolateral membrane. Consequently, epithelial detection of flagellated bacteria in colon relies on bacteria able to deliver flagellin to the basolateral surface (transepithelial transport), i.e. invasive bacteria or following epithelial breaches. The polarized expression of TLR5 in epithelial cells could reflect different functional features related to the bacterial load in the gut segments and the control of pro-inflammatory response. In lower airways where bacteria are almost not present, epithelial cells express TLR5 at the apical surface (see below).

4. TLR-dependent discrimination mechanisms of commensal versus pathogenic motile bacteria

For many bacterial species, flagellin expression is believed to take place in the intestinal environment although not experimentally demonstrated. Interestingly bacteria like *Brucella* or entero-invasive *E. coli* considered for years as non motile have recently, been shown to synthesise a flagellum *in vitro* [47] [48].

TLR5-dependent immune response may act as a driving force for co-evolutive selection of bacterial variants. Thus, mechanisms may allow bacteria to escape TLR5 detection by the down-regulation or the abrogation of flagellin expression *in vivo* or mutations of flagellin capable to form flagellum but unable to trigger TLR5 activation (Fig. 2B). Firmicutes but not *Bacteroides*, both principal indigenous bacteria of the gut flora are capable to produce pro-inflammatory flagellin *in vitro*. These bacteria may have

been selected for the absence or the low *in vivo* expression of flagellin. Moreover, *Helicobacter pylori* or *Campylobacter jejuni*, motile bacteria that persistently colonize the gastric and intestinal environment produce flagellin mutated in the region equivalent to residues 89-96 of *S. typhimurium* molecule, thereby impairing TLR5 activation [17, 49, 50]. Molecular changes within LPS have also been described, resulting in TLR ignorance or hyporesponsiveness [14, 51]. It remains to be established whether such mutations have been selected by commensal bacteria to escape host innate defences. Alternatively, commensals have developed strategies to modulate the mucosal immunity. Thus, pro-inflammatory signalling can be turned off by avirulent *Salmonella* that produce factors that interfere with the intracellular ubiquitination machinery and, thereby the activation of NF- κ B transcription factor [52]. AvrA, an effector protein secreted by the type III secretory apparatus of *S. typhimurium* has been shown to inhibit this activation [53]. Otherwise, *Bacteroides* potentiates the nucleo-cytoplasmic export of NF- κ B in a peroxisome proliferator-activated receptor- γ -dependent manner, thus turning off the NF- κ B-mediated pro-inflammatory response (Fig. 2B) [54]. These findings illustrate the complex crosstalk between host and microflora and how commensals can modulate the host innate immune response.

TLRs were initially defined as receptors that detect pathogens. The commensal flora, however, via TLR signalling may also modulate gut homeostasis and play a role in intestinal renewal and repair [2, 4]. When TLR signalling is abolished, either by genetic manipulation using MyD88-deficient mice or by lowering the levels of molecular patterns from commensal flora using germ-free mice or antibiotic-treated animals, the differentiation/proliferation of intestinal epithelium is perturbed and expression of proteins that normally act on tissue healing is impaired. Germ-free or antibiotic-treated mice are more susceptible to injuries like dextran sodium sulfate (DSS)-induced colitis and the lesions are reverted by oral administration of TLR ligands such as LPS. Interestingly, some TLR ligands induce non-immune cells to proliferate or prevent them to exit the cell cycle [55]. TLR signalling can also activate anti-apoptotic pathways and hence control of cell proliferation [56]. Moreover, TLR – MyD88 signalling in macrophages close to epithelial stem cells contributes to the healing process (Fig. 2A) [4].

How do mucosal TLRs discriminate molecular patterns produced by commensals versus pathogens? Recent findings from Svanborg and colleagues shed light on a novel mechanism [57]. They studied the pro-inflammatory responses of the bladder mucosa to uropathogenic bacteria expressing various epithelial-attaching fimbriae. Depending on the fimbriae co-expressed with bacterial LPS, TLR4 signalling activates various adaptor molecules (MyD88, TRIF - TRAM) modulating the signalling cascade and the outcome of the immune response. The differential engagement of adaptor molecules may rely on distinct fimbriae receptors, i.e. surface glycosphingolipids and mannosylated glycoproteins (Fig. 2B). Pro-inflammatory responses did not occur in the absence of virulence factor. Modulation of immunity by accessory receptors has also been shown for yeast beta-glucans detected by TLR2 and Dectin-1, a phagocytic receptor at the surface of macrophages [58]. These observations suggest that innate responses require both a molecular pattern-TLR signal and a pathogenic signal. TLR signalling, that is often reduced to the initial TLR detection, involves many other co-factors as soluble or membrane molecules. For instance, in the systemic compartment, effective TLR4 signalling is triggered with the help of LPS-binding protein, MD-2 and CD14 that may provide additional signals for TLR activation. However, bladder and intestinal epithelial cells do not produce several TLR4 co-receptors such as CD14 and MD-2 and are therefore unresponsive to free LPS [57, 59, 60].

One can hypothesize that epithelial cells express TLRs and particular co-receptors that discriminate pathogen from commensal by interacting with pathogen-specific determinants, i.e. virulence factors (Fig. 2B). When a commensal interacts with epithelial cells, only TLRs are activated, resulting in the engagement of a specific set of adaptor molecules that turn on homeostatic functions. In response to pathogens, TLRs act synergistically with co-receptors for virulence factors thus recruiting additional adaptor molecules resulting in a pro-inflammatory response. The TLR adaptor molecule Tollip strongly expressed by intestinal epithelial cells is believed to participate in the specific modulation of TLR signalling [61]. Interestingly, flagellin binds also to gangliosides including asialo-GM1 and this interaction is important for TLR5 signalling [62, 63]. Therefore, flagellin behaves both as a TLR-specific molecular pattern and a

virulence determinant for epithelial cells. The contribution of co-receptors and adaptor molecules in TLR signalling will have to be addressed in future studies.

5. Airways response to flagellated bacteria

TLR5-dependent pro-inflammatory activation in response to flagellin is also observed within the epithelial cells lining the trachea, the bronchi and the alveoli of the respiratory tract [64-68]. The immune response to *Pseudomonas aeruginosa*, *Burkholderia cenocepacia*, *Bordetella bronchiseptica*, or *Legionella pneumophila* is partly or fully recapitulated by flagellin stimulation as reflected by the comparison of the gene expression profiles of intestinal and airway epithelial cells [26, 69]. TLR5 signalling after intranasal or intratracheal administration of flagellin is characterized by the secretion of cytokines/chemokines and the massive recruitment of neutrophils into the lung parenchyma and the alveolar compartments [70]. This process is similar to that observed within TLR-stimulated bladder mucosa [57]. The aim is to prevent any intrusion and overgrowth of microorganisms by neutrophil-mediated phagocytosis, thereby keeping the mucosa sterile. The contribution of TLR-induced production of anti-microbial molecules such as defensins needs still to be addressed.

Recent studies have shown that mucosal administration of flagellin is associated to strong adaptive immune responses, especially secretory and serum antibodies [71-73]. These mucosal adjuvant properties correlate with the capacity of flagellin to activate TLR5-positive DCs and/or epithelial cells [23, 71, 73]. Flagellin triggers a Th2- response [23, 71, 72, 74] with the production of mucosal IgAs [23, 71, 72, 74]. This is in part due to the failure of mouse and human DCs to produce IL-12 p70 in response to flagellin (Fig. 1C) [23, 36]. Epithelial cells that constitute the main flagellin-reactive cells in the airways respond to apical TLR5 stimulation by the transient production of CCL20 that attract immature DCs (Fig. 1C) [75, 76]. The respective contribution of resident versus recruited DCs in the development of flagellin-mediated mucosal response remains to be determined.

Finally, host molecules with repetitive sequences like hyaluronan-derived products are able to interact with TLRs and induce responses that control airway

epithelial homeostasis, especially during lung repair and remodelling following inflammation [5].

6. Immunopathologies associated to TLR5

The importance of flagellin-mediated TLR5 signalling in mucosal immune responses has been highlighted by recent epidemiological and genetic studies. Hawn *et al.* described a genetic polymorphism of human TLR5 coding for a deleted molecule: TLR5_{392-stop} with an allele frequency of 10%. This variant functions as a dominant negative molecule on wild type TLR5 [21]. Individuals carrying this allele are more susceptible to *L. pneumophila* infection. Interestingly, mice expressing low levels of TLR5 are more susceptible to systemic *S. typhimurium* infection. Whether these mice are more susceptible to mucosal infection has not been assessed [77]. In contrast, there is no enhanced prevalence of infection with *S. typhi*, the causative agent of typhoid fever, in individuals that are heterozygote or homozygote for TLR5_{392-stop}. Whether flagellin is not essential or poorly expressed during some crucial invasion phases remains to be established [78].

The role of TLR5 in controlling mucosal colonization is also supported by some recent findings in Crohn's disease. This multi-factorial pathology is a chronic bowel inflammation characterized by an increased permeability of the mucosa to environmental materials and an uncontrolled innate and adaptive immune response against the gut flora. Mutations in the gene encoding NOD2, a cytosolic detector of microbial peptidoglycans, are found in a subpopulation of patients with Crohn's disease [79]. Various studies showed a strong serum antibody response specific for flagellin of commensal bacteria in subjects suffering Crohn's disease but not ulcerative colitis [80-83]. In the DSS-induced colitis model, intra-colonic administration of flagellin aggravates the prognosis [31]. In addition, a negative association between TLR5_{392-stop} allele and Crohn's disease was described among Ashkenazi Jews [83]. Finally, it has been noticed that TLR5 expression is unchanged or down-regulated in the mucosa of subjects suffering colitis whereas TLR4 expression is strongly enhanced [84]. Noticeably, an association between susceptibility to colitis and differences in innate signalling of TLRs and Nod2 ligands was shown to depend on NF- κ B p50 subunit [85].

HAL author manuscript inserm-00071278, version 1

Altogether, these data suggest that early TLR5-mediated innate and adaptive responses may cause the onset of inflammation. How TLRs and Nod2 cooperate and affect the bacterial recognition and the outcome of inflammatory bowel diseases remains to be established.

TLR5 signalling may also influence the systemic response. In fact, TLR5_{392-stop} is associated with resistance to systemic erythematosus lupus (SLE), a chronic autoimmune disease characterized in part by anti-DNA antibodies [86]. Even if the role of TLR5 in SLE is not clear, it raises the possibility that flagellin-mediated stimulation in susceptible individuals may contribute to the induction of pathogenic immune responses.

7. Concluding remarks

Contribution of PRR in mucosal tissues has only recently been studied. Among PPRs, TLRs play a crucial role in detecting danger signals and triggering innate immune response that prevent pathogens from invading the host and spreading systemically. Other host molecules expressed at the mucosal surfaces are likely to participate in such defence mechanisms as shown for uropathogenic *E. coli* infection of the bladder. PPRs are also involved in mediating epithelial homeostatic functions facilitating mucosal tissue repair and remodelling following inflammation. Among PRRs, TLR5 has a unique function since it links innate and adaptive responses by recruiting DCs the only antigen-presenting cells able to activate naïve T cells. Whether TLR5 participates in bacterial clearance and/or immunopathological processes will require studies in knockout and transgenic animals.

How pathogens and commensals stimulate distinct mucosal responses although expressing similar molecular patterns starts to be understood. Recently it has been shown that commensals are able to modulate pro-inflammatory responses by interfering with TLR signalling cascades. Pathogens use additional virulence factors that participate in the onset of pro-inflammatory responses. Thus, the wide variety of responses to the myriad of microorganisms may be explained by the diversity of co-receptors that can be engaged to induce functional TLR signalling. The commensal

mediated specific TLR/MyD88 pathway allows the lasting “entente cordiale” between the microflora and the host, while pathogen-specific virulence factors are required to trigger transient pro-inflammatory responses via the usage of additional TLR co-receptor and adaptor molecules.

Finally, we have to keep in mind that TLR activation in mucosa usually does not occur as a global mucosal signalling but remains focalized to limited areas where infection takes place. When the entire mucosal tissues get activated through TLR signalling, the outcome is reminiscent to what is seen in septic shock with a systemic activation of the innate immune system. Since this situation is the most common in experimental settings, novel assays have to be developed to shed light on the function of physiological TLR signalling.

Acknowledgements

JCS and CN are supported by the INSERM (Avenir R02344ES), the Institut Pasteur de Lille, the Région Nord Pas de Calais and FEDER (ARCir émergence), the Fondation pour la Recherche Médicale, and the European Community (STREP grant VaccTIP LSHP-CT-2005-012161). MR is supported by Argentinean National Research Council (CONICET) and PICT14302 from ANPCyT. MR and JCS also thank the Franco-Argentinean ECOS-SETCIP program (A04B03). JPK is supported by the Swiss National Science Foundation grant 3100-067926.02.

Figure Legends

Figure 1. Molecular mechanisms of TLR5-induced immunity. (A) Organization of flagellum and flagellin. Motile bacteria produce flagella composed mainly of polymerized flagellin. *Salmonella typhimurium* flagellin consists of 3 domains: the terminal α -helixes (purple), the central α -helixes (blue), and the hypervariable β -sheets (yellow). The α -helixes regions are required for filament architecture and motility functions. (B) Detection of flagellin and TLR5 signalling. Flagellin monomer is the molecular pattern detected by TLR5, specifically the motif 89-96 (blue helix). TLR5 utilizes the universal TLR-specific adapter molecule MyD88 that activate downstream molecules NF- κ B and MAPKs (ERK1/2, p38 and JNK) that turns on transcription of genes involved in innate and adaptive immunity and in protection against apoptosis. (C) Flagellin-specific regulation of mucosal immunity. Two major sentinel cells are targeted by TLR5 – flagellin activation: epithelial cells and dendritic cells (DC). Epithelial cells promote innate responses by the production of microbicidal molecules and the recruitment of neutrophils that will eliminate microorganisms interacting with mucosa. Epithelial cells also stimulate the DC recruitment to start the adaptive response. Since DC express TLR5, resident and recruited cells may also be activated through TLR5, a process known to promote development of Th2-type cells. Altogether the epithelial – mucosal environment combined with the preference of TLR5-stimulated DCs for Th2 responses elicit mucosa-specific immune responses imprinting a mucosal homing program and an IgA isotype switch to B lymphocytes.

Figure 2: Discrimination of pathogen and commensal by TLR activation at epithelial sites. (A) Pro-inflammatory epithelial response to pathogens. Pathogenic bacteria colonize and/or invade epithelial cells and thereby bring microbe-associated molecular patterns (MAMP) in close contact to cells. TLR alone or in combination with pathogen receptor (PR) detect MAMP \pm pathogenic signal. In the latter case, MAMP + pathogenic signal (virulence factor or injury) may constitute a PAMP, the proinflammatory counterpart of MAMP (left panel). Remarkably, MAMP can carry both the MAMP and pathogenic moiety. For example, flagellin is the agonist of TLR5 but also

binds to gangliosides that may be a PR. TLR - PR complexes may recruit different adaptor molecules than TLR alone, giving rise to pro-inflammatory signalling. For invasive microorganisms, TLR activation may occur within vacuoles or on the basolateral surface of epithelial cells. In colon, TLR5 is basolateral: invasive flagellated bacteria trigger exclusively the innate immune response.

(B) TLR-mediated homeostasis and ignorance. Commensal bacteria that live peacefully within the host mucosa may use various strategies to activate TLR-dependent homeostasis or to prevent pro-inflammatory response. Detection of commensal bacteria by TLR signalling may trigger signalling or activate a gene expression program resulting in homeostasis response. This could operate in epithelial cells or indirectly via macrophages that, in turn, provide specific signal to sustain epithelium homeostasis. Microorganisms can also escape detection by TLR by modification of their MAMP expression or structure. For example, *H. pylori* produce flagellin that does not trigger *per se* epithelial TLR5 pro-inflammatory responses but preserve motility properties. Alternatively, bacteria can actively interfere with TLR-mediated response by downregulating pro-inflammatory signalling. In order to prevent nuclear activation of NF- κ B-dependent transcription of pro-inflammatory genes, avirulent *Salmonella* and *Yersinia* blocks the degradation of I κ B- α , the cytosol-sequestering molecule of NF- κ B whereas *Bacteroides thetaiotamicron* stimulates the nucleus-cytosol shuttling of NF- κ B. Some pathogenic bacteria may also use some of these strategies to escape the TLR-induced defences.

References

- [1] Sansonetti, P. J. (2004) War and peace at mucosal surfaces. *Nat. Rev. Immunol.* 4, 953-64.
- [2] Rakoff-Nahoum, S., Paglino, J., Eslami-Varzaneh, F., Edberg, S. and Medzhitov, R. (2004) Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell* 118, 229-41.
- [3] Hornef, M. W. and Bogdan, C. (2005) The role of epithelial Toll-like receptor expression in host defense and microbial tolerance. *J. Endotoxin Res.* 11, 124-8.
- [4] Pull, S. L., Doherty, J. M., Mills, J. C., Gordon, J. I. and Stappenbeck, T. S. (2005) Activated macrophages are an adaptive element of the colonic epithelial progenitor niche necessary for regenerative responses to injury. *Proc Natl Acad Sci U S A* 102, 99-104.
- [5] Jiang, D., Liang, J., Fan, J., Yu, S., Chen, S., Luo, Y., Prestwich, G. D., Mascarenhas, M. M., Garg, H. G., Quinn, D. A., Homer, R. J., Goldstein, D. R., Bucala, R., Lee, P. J., Medzhitov, R. and Noble, P. W. (2005) Regulation of lung injury and repair by Toll-like receptors and hyaluronan. *Nat. Med.* 11, 1173-9.
- [6] Matzinger, P. (2002) The danger model: a renewed sense of self. *Science* 296, 301-5.
- [7] Butler, S. M. and Camilli, A. (2005) Going against the grain: chemotaxis and infection in *Vibrio cholerae*. *Nat. Rev. Microbiol.* 3, 611-20.
- [8] Ramos, H. C., Rumbo, M. and Sirard, J. C. (2004) Bacterial flagellins: mediators of pathogenicity and host immune responses in mucosa. *Trends Microbiol.* 12, 509-17.
- [9] Beatson, S. A., Minamino, T. and Pallen, M. J. (2006) Variation in bacterial flagellins: from sequence to structure. *Trends Microbiol.*
- [10] Janeway, C. A., Jr. and Medzhitov, R. (2002) Innate immune recognition. *Annu Rev Immunol* 20, 197-216.
- [11] Roach, J. C., Glusman, G., Rowen, L., Kaur, A., Purcell, M. K., Smith, K. D., Hood, L. E. and Aderem, A. (2005) The evolution of vertebrate Toll-like receptors. *Proc Natl Acad Sci U S A* 102, 9577-82.
- [12] Hayashi, F., Smith, K. D., Ozinsky, A., Hawn, T. R., Yi, E. C., Goodlett, D. R., Eng, J. K., Akira, S., Underhill, D. M. and Aderem, A. (2001) The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* 410, 1099-103.
- [13] Gewirtz, A. T., Navas, T. A., Lyons, S., Godowski, P. J. and Madara, J. L. (2001) Bacterial flagellin activates basolaterally expressed TLR5 to induce epithelial proinflammatory gene expression. *J. Immunol.* 167, 1882-5.
- [14] Hajjar, A. M., Ernst, R. K., Tsai, J. H., Wilson, C. B. and Miller, S. I. (2002) Human Toll-like receptor 4 recognizes host-specific LPS modifications. *Nat. Immunol.* 3, 354-9.
- [15] Smith, K. D., Andersen-Nissen, E., Hayashi, F., Strobe, K., Bergman, M. A., Barrett, S. L., Cookson, B. T. and Aderem, A. (2003) Toll-like receptor 5 recognizes a conserved site on flagellin protofilament formation and bacterial motility. *Nat. Immunol.* 4, 1247-53.
- [16] Jacchieri, S. G., Torquato, R. and Brentani, R. R. (2003) Structural study of binding of flagellin by Toll-like receptor 5. *J. Bacteriol.* 185, 4243-7.
- [17] Andersen-Nissen, E., Smith, K. D., Strobe, K. L., Barrett, S. L., Cookson, B. T., Logan, S. M. and Aderem, A. (2005) Evasion of Toll-like receptor 5 by flagellated bacteria. *Proc Natl Acad Sci U S A* 102, 9247-52.
- [18] Choe, J., Kelker, M. S. and Wilson, I. A. (2005) Crystal structure of human toll-like receptor 3 (TLR3) ectodomain. *Science* 309, 581-5.
- [19] Bell, J. K., Mullen, G. E., Leifer, C. A., Mazzoni, A., Davies, D. R. and Segal, D. M. (2003) Leucine-rich repeats and pathogen recognition in Toll-like receptors. *Trends Immunol.* 24, 528-33.
- [20] Mizel, S. B., West, A. P. and Hantgan, R. R. (2003) Identification of a sequence in human toll-like receptor 5 required for the binding of Gram-negative flagellin. *J. Biol. Chem.* 278, 23624-9.
- [21] Hawn, T. R., Verbon, A., Lettinga, K. D., Zhao, L. P., Li, S. S., Laws, R. J., Skerrett, S. J., Beutler, B., Schroeder, L., Nachman, A., Ozinsky, A., Smith, K. D. and Aderem, A. (2003) A Common Dominant TLR5 Stop Codon Polymorphism Abolishes Flagellin Signaling and Is Associated with Susceptibility to Legionnaires' Disease. *J. Exp. Med.* 198, 1563-72.
- [22] Ren, T., Zamboni, D. S., Roy, C. R., Dietrich, W. F. and Vance, R. E. (2006) Flagellin-Deficient *Legionella* Mutants Evade Caspase-1- and Naip5-Mediated Macrophage Immunity. *PLoS Pathog.* 2, e18.

- [23] Didierlaurent, A., Ferrero, I., Otten, L. A., Dubois, B., Reinhardt, M., Carlsen, H., Blomhoff, R., Akira, S., Kraehenbuhl, J. P. and Sirard, J. C. (2004) Flagellin Promotes Myeloid Differentiation Factor 88-Dependent Development of Th2-Type Response. *J. Immunol.* 172, 6922-30.
- [24] Zhou, X., Giron, J. A., Torres, A. G., Crawford, J. A., Negrete, E., Vogel, S. N. and Kaper, J. B. (2003) Flagellin of enteropathogenic *Escherichia coli* stimulates interleukin-8 production in T84 cells. *Infect Immun* 71, 2120-9.
- [25] Berin, M. C., Darfeuille-Michaud, A., Egan, L. J., Miyamoto, Y. and Kagnoff, M. F. (2002) Role of EHEC O157:H7 virulence factors in the activation of intestinal epithelial cell NF-kappaB and MAP kinase pathways and the upregulated expression of interleukin 8. *Cell Microbiol* 4, 635-48.
- [26] Zeng, H., Carlson, A. Q., Guo, Y., Yu, Y., Collier-Hyams, L. S., Madara, J. L., Gewirtz, A. T. and Neish, A. S. (2003) Flagellin is the major proinflammatory determinant of enteropathogenic *Salmonella*. *J. Immunol.* 171, 3668-74.
- [27] Rhee, S. H., Keates, A. C., Moyer, M. P. and Pothoulakis, C. (2004) MEK is a key modulator for TLR5-induced interleukin-8 and MIP3alpha gene expression in non-transformed human colonic epithelial cells. *J. Biol. Chem.* 279, 25179-88.
- [28] Hughes, E. A. and Galan, J. E. (2002) Immune response to *Salmonella*: location, location, location? *Immunity* 16, 325-8.
- [29] Ortega-Cava, C. F., Ishihara, S., Rumi, M. A., Aziz, M. M., Kazumori, H., Yuki, T., Mishima, Y., Moriyama, I., Kadota, C., Oshima, N., Amano, Y., Kadowaki, Y., Ishimura, N. and Kinoshita, Y. (2006) Epithelial toll-like receptor 5 is constitutively localized in the mouse cecum and exhibits distinctive down-regulation during experimental colitis. *Clin Vaccine Immunol* 13, 132-8.
- [30] Bambou, J. C., Giraud, A., Menard, S., Begue, B., Rakotobe, S., Heyman, M., Taddei, F., Cerf-Bensussan, N. and Gaboriau-Routhiau, V. (2004) In vitro and ex vivo activation of the TLR5 signaling pathway in intestinal epithelial cells by a commensal *Escherichia coli* strain. *J. Biol. Chem.* 279, 42984-92.
- [31] Rhee, S. H., Im, E., Riegler, M., Kokkotou, E., O'Brien, M. and Pothoulakis, C. (2005) Pathophysiological role of Toll-like receptor 5 engagement by bacterial flagellin in colonic inflammation. *Proc Natl Acad Sci U S A* 102, 13610-5.
- [32] Zipfel, C. and Felix, G. (2005) Plants and animals: a different taste for microbes? *Curr. Opin. Plant Biol.* 8, 353-60.
- [33] Backhed, F., Ley, R. E., Sonnenburg, J. L., Peterson, D. A. and Gordon, J. I. (2005) Host-bacterial mutualism in the human intestine. *Science* 307, 1915-20.
- [34] Niedergang, F. and Kweon, M. N. (2005) New trends in antigen uptake in the gut mucosa. *Trends Microbiol.* 13, 485-90.
- [35] Ciacci-Woolwine, F., Blomfield, I. C., Richardson, S. H. and Mizel, S. B. (1998) *Salmonella* flagellin induces tumor necrosis factor alpha in a human promonocytic cell line. *Infect Immun* 66, 1127-34.
- [36] Means, T. K., Hayashi, F., Smith, K. D., Aderem, A. and Luster, A. D. (2003) The Toll-like receptor 5 stimulus bacterial flagellin induces maturation and chemokine production in human dendritic cells. *J. Immunol.* 170, 5165-75.
- [37] Steiner, T. S., Nataro, J. P., Poteet-Smith, C. E., Smith, J. A. and Guerrant, R. L. (2000) Enteropathogenic *Escherichia coli* expresses a novel flagellin that causes IL-8 release from intestinal epithelial cells. *Journal of Clinical Investigation* 105, 1769-77.
- [38] Gewirtz, A. T., Simon, P. O., Schmitt, C. K., Taylor, L. J., Hagedorn, C. H., O'Brien, A. D., Neish, A. S. and Madara, J. L. (2001) *Salmonella typhimurium* translocates flagellin across intestinal epithelia, inducing a proinflammatory response. *Journal of Clinical Investigation* 107, 99-109.
- [39] Anderle, P., Rumbo, M., Sierro, F., Mansourian, R., Michetti, P., Roberts, M. A. and Kraehenbuhl, J. P. (2005) Novel markers of the human follicle-associated epithelium identified by genomic profiling and microdissection. *Gastroenterol.* 129, 321-7.
- [40] Jenner, R. G. and Young, R. A. (2005) Insights into host responses against pathogens from transcriptional profiling. *Nat. Rev. Microbiol.* 3, 281-94.
- [41] Niedergang, F., Didierlaurent, A., Kraehenbuhl, J.-P. and Sirard, J.-C. (2004) Dendritic cells: the host Achilles' heel for mucosal pathogens? *Trends Microbiol.* 12, 79-88.
- [42] Sierro, F., Dubois, B., Coste, A., Kaiserlian, D., Kraehenbuhl, J. P. and Sirard, J. C. (2001) Flagellin stimulation of intestinal epithelial cells triggers CCL20-mediated migration of dendritic cells. *Proc. Natl. Acad. Sci. USA* 98, 13722-7.

- [43] Iwasaki, A. and Kelsall, B. L. (2000) Localization of distinct Peyer's patch dendritic cell subsets and their recruitment by chemokines macrophage inflammatory protein (MIP)-3alpha, MIP-3beta, and secondary lymphoid organ chemokine. *J. Exp. Med.* 191, 1381-94.
- [44] Cook, D. N., Prosser, D. M., Forster, R., Zhang, J., Kuklin, N. A., Abbondanzo, S. J., Niu, X. D., Chen, S. C., Manfra, D. J., Wiekowski, M. T., Sullivan, L. M., Smith, S. R., Greenberg, H. B., Narula, S. K., Lipp, M. and Lira, S. A. (2000) CCR6 mediates dendritic cell localization, lymphocyte homeostasis, and immune responses in mucosal tissue. *Immunity* 12, 495-503.
- [45] Rumbo, M., Courjault-Gautier, F., Sierro, F., Sirard, J. C. and Felley-Bosco, E. (2005) Polarized distribution of inducible nitric oxide synthase regulates activity in intestinal epithelial cells. *FEBS J* 272, 444-53.
- [46] Claud, E. C., Lu, L., Anton, P. M., Savidge, T., Walker, W. A. and Cherayil, B. J. (2004) Developmentally regulated IkappaB expression in intestinal epithelium and susceptibility to flagellin-induced inflammation. *Proc Natl Acad Sci U S A* 101, 7404-8.
- [47] Fretin, D., Fauconnier, A., Kohler, S., Halling, S., Leonard, S., Nijskens, C., Ferooz, J., Lestrade, P., Delrue, R. M., Danese, I., Vandenhoute, J., Tibor, A., DeBolle, X. and Letesson, J. J. (2005) The sheathed flagellum of *Brucella melitensis* is involved in persistence in a murine model of infection. *Cell Microbiol* 7, 687-98.
- [48] Andrade, A., Giron, J. A., Amhaz, J. M., Trabulsi, L. R. and Martinez, M. B. (2002) Expression and characterization of flagella in nonmotile enteroinvasive *Escherichia coli* isolated from diarrhea cases. *Infect Immun* 70, 5882-6.
- [49] Gewirtz, A. T., Yu, Y., Krishna, U. S., Israel, D. A., Lyons, S. L. and Peek, R. M., Jr. (2004) *Helicobacter pylori* flagellin evades toll-like receptor 5-mediated innate immunity. *J. Infect. Dis.* 189, 1914-20.
- [50] Lee, S. K., Stack, A., Katzowitsch, E., Aizawa, S. I., Suerbaum, S. and Josenhans, C. (2003) *Helicobacter pylori* flagellins have very low intrinsic activity to stimulate human gastric epithelial cells via TLR5. *Microbes Infect.* 5, 1345-56.
- [51] Guo, L., Lim, K. B., Gunn, J. S., Bainbridge, B., Darveau, R. P., Hackett, M. and Miller, S. I. (1997) Regulation of lipid A modifications by *Salmonella typhimurium* virulence genes *phoP-phoQ*. *Science* 276, 250-3.
- [52] Neish, A. S., Gewirtz, A. T., Zeng, H., Young, A. N., Hobert, M. E., Karmali, V., Rao, A. S. and Madara, J. L. (2000) Prokaryotic regulation of epithelial responses by inhibition of I kappa B-alpha ubiquitination. *Science* 289, 1560-3.
- [53] Collier-Hyams, L. S., Zeng, H., Sun, J., Tomlinson, A. D., Bao, Z. Q., Chen, H., Madara, J. L., Orth, K. and Neish, A. S. (2002) *Salmonella* AvrA effector inhibits the key proinflammatory, anti-apoptotic NF-kappa B pathway. *J. Immunol.* 169, 2846-50.
- [54] Kelly, D., Campbell, J. I., King, T. P., Grant, G., Jansson, E. A., Coutts, A. G., Pettersson, S. and Conway, S. (2004) Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. *Nat. Immunol.* 5, 104-12.
- [55] Hasan, U. A., Trinchieri, G. and Vlach, J. (2005) Toll-like receptor signaling stimulates cell cycle entry and progression in fibroblasts. *J. Biol. Chem.* 280, 20620-7.
- [56] Collier-Hyams, L. S. and Neish, A. S. (2005) Innate immune relationship between commensal flora and the mammalian intestinal epithelium. *Cell Mol Life Sci* 62, 1339-48.
- [57] Fischer, H., Yamamoto, M., Akira, S., Beutler, B. and Svanborg, C. (2006) Mechanism of pathogen-specific TLR4 activation in the mucosa: Fimbriae, recognition receptors and adaptor protein selection. *Eur J Immunol* 36, 267-77.
- [58] Brown, G. D. (2006) Dectin-1: a signalling non-TLR pattern-recognition receptor. *Nat. Rev. Immunol.* 6, 33-43.
- [59] Abreu, M. T., Vora, P., Faure, E., Thomas, L. S., Arnold, E. T. and Arditi, M. (2001) Decreased expression of Toll-like receptor-4 and MD-2 correlates with intestinal epithelial cell protection against dysregulated proinflammatory gene expression in response to bacterial lipopolysaccharide. *J. Immunol.* 167, 1609-16.
- [60] Ortega-Cava, C. F., Ishihara, S., Rumi, M. A., Kawashima, K., Ishimura, N., Kazumori, H., Udagawa, J., Kadowaki, Y. and Kinoshita, Y. (2003) Strategic compartmentalization of Toll-like receptor 4 in the mouse gut. *J. Immunol.* 170, 3977-85.
- [61] Melmed, G., Thomas, L. S., Lee, N., Tesfay, S. Y., Lukasek, K., Michelsen, K. S., Zhou, Y., Hu, B., Arditi, M. and Abreu, M. T. (2003) Human intestinal epithelial cells are broadly unresponsive to toll-like

receptor 2-dependent bacterial ligands: implications for host-microbial interactions in the gut. *J. Immunol.* 170, 1406-15.

[62] Ogushi, K. I., Wada, A., Niidome, T., Okuda, T., Llanes, R., Nakayama, M., Nishi, Y., Kurazono, H., Smith, K. D., Aderem, A., Moss, J. and Hirayama, T. (2004) Gangliosides act as co-receptors for *Salmonella enteritidis* FliC and promote FliC-induction of human beta-defensin-2 expression in Caco-2 cells. *J. Biol. Chem.* 279, 12213-9.

[63] West, A. P., Dancho, B. A. and Mizel, S. B. (2005) Gangliosides inhibit flagellin signaling in the absence of an effect on flagellin binding to toll-like receptor 5. *J. Biol. Chem.* 280, 9482-8.

[64] Tseng, J., Do, J., Widdicombe, J. H. and Machen, T. E. (2005) Innate Immune Responses of Human Tracheal Epithelium to *P. aeruginosa* Flagellin, TNF{alpha} and IL1{beta}. *Am J Physiol Cell Physiol*

[65] Zhang, Z., Louboutin, J. P., Weiner, D. J., Goldberg, J. B. and Wilson, J. M. (2005) Human airway epithelial cells sense *Pseudomonas aeruginosa* infection via recognition of flagellin by Toll-like receptor 5. *Infect Immun* 73, 7151-60.

[66] Liaudet, L., Szabo, C., Evgenov, O. V., Murthy, K. G., Pacher, P., Virag, L., Mabley, J. G., Marton, A., Soriano, F. G., Kirov, M. Y., Bjertnaes, L. J. and Salzman, A. L. (2003) Flagellin from Gram-negative bacteria is a potent mediator of acute pulmonary inflammation in sepsis. *Shock* 19, 131-7.

[67] Lopez-Boado, Y. S., Wilson, C. L. and Parks, W. C. (2001) Regulation of matrilysin expression in airway epithelial cells by *Pseudomonas aeruginosa* flagellin. *J. Biol. Chem.* 276, 41417-23.

[68] Muir, A., Soong, G., Sokol, S., Reddy, B., Gomez, M., Van Heeckeren, A. and Prince, A. (2004) Toll-like receptors in normal and cystic fibrosis airway epithelial cells. *Am J Respir Cell Mol Biol* 30, 777-83.

[69] Cobb, L. M., Mychaleckyj, J. C., Wozniak, D. J. and Lopez-Boado, Y. S. (2004) *Pseudomonas aeruginosa* flagellin and alginate elicit very distinct gene expression patterns in airway epithelial cells: implications for cystic fibrosis disease. *J. Immunol.* 173, 5659-70.

[70] Honko, A. N. and Mizel, S. B. (2004) Mucosal administration of flagellin induces innate immunity in the mouse lung. *Infect Immun* 72, 6676-9.

[71] Lee, S. E., Kim, S. Y., Jeong, B. C., Kim, Y. R., Bae, S. J., Ahn, O. S., Lee, J. J., Song, H. C., Kim, J. M., Choy, H. E., Chung, S. S., Kweon, M. N. and Rhee, J. H. (2006) A Bacterial Flagellin, *Vibrio vulnificus* FlaB, Has a Strong Mucosal Adjuvant Activity To Induce Protective Immunity. *Infect Immun* 74, 694-702.

[72] Honko, A. N., Sriranganathan, N., Lees, C. J. and Mizel, S. B. (2006) Flagellin is an effective adjuvant for immunization against lethal respiratory challenge with *Yersinia pestis*. *Infect Immun* 74, 1113-20.

[73] Pino, O., Martin, M. and Michalek, S. M. (2005) Cellular mechanisms of the adjuvant activity of the flagellin component FljB of *Salmonella enterica* Serovar Typhimurium to potentiate mucosal and systemic responses. *Infect Immun* 73, 6763-70.

[74] Cunningham, A. F., Khan, M., Ball, J., Toellner, K. M., Serre, K., Mohr, E. and MacLennan, I. C. (2004) Responses to the soluble flagellar protein FliC are Th2, while those to FliC on *Salmonella* are Th1. *Eur J Immunol* 34, 2986-95.

[75] Sha, Q., Truong-Tran, A. Q., Plitt, J. R., Beck, L. A. and Schleimer, R. P. (2004) Activation of airway epithelial cells by toll-like receptor agonists. *Am J Respir Cell Mol Biol* 31, 358-64.

[76] Thorley, A. J., Goldstraw, P., Young, A. and Tetley, T. D. (2005) Primary human alveolar type II epithelial cell CCL20 (macrophage inflammatory protein-3alpha)-induced dendritic cell migration. *Am J Respir Cell Mol Biol* 32, 262-7.

[77] Sebastiani, G., Leveque, G., Lariviere, L., Laroche, L., Skamene, E., Gros, P. and Malo, D. (2000) Cloning and characterization of the murine toll-like receptor 5 (Tlr5) gene: sequence and mRNA expression studies in *Salmonella*-susceptible MOLF/Ei mice. *Genomics* 64, 230-40.

[78] Dunstan, S. J., Hawn, T. R., Hue, N. T., Parry, C. P., Ho, V. A., Vinh, H., Diep, T. S., House, D., Wain, J., Aderem, A., Hien, T. T. and Farrar, J. J. (2005) Host susceptibility and clinical outcomes in toll-like receptor 5-deficient patients with typhoid fever in Vietnam. *J. Infect. Dis.* 191, 1068-71.

[79] Strober, W., Murray, P. J., Kitani, A. and Watanabe, T. (2006) Signalling pathways and molecular interactions of NOD1 and NOD2. *Nat. Rev. Immunol.* 6, 9-20.

[80] Lodes, M. J., Cong, Y., Elson, C. O., Mohamath, R., Landers, C. J., Targan, S. R., Fort, M. and Hershberg, R. M. (2004) Bacterial flagellin is a dominant antigen in Crohn disease. *J. Clin. Invest.* 113, 1296-306.

- [81] Targan, S. R., Landers, C. J., Yang, H., Lodes, M. J., Cong, Y., Papadakis, K. A., Vasilias, E., Elson, C. O. and Hershenberg, R. M. (2005) Antibodies to CBir1 flagellin define a unique response that is associated independently with complicated Crohn's disease. *Gastroenterol.* 128, 2020-8.
- [82] Sitaraman, S. V., Klapproth, J. M., Moore, D. A., 3rd, Landers, C., Targan, S., Williams, I. R. and Gewirtz, A. T. (2005) Elevated flagellin-specific immunoglobulins in Crohn's disease. *Am J Physiol Gastrointest Liver Physiol* 288, G403-6.
- [83] Gewirtz, A. T., Vijay-Kumar, M., Brant, S. R., Duerr, R. H., Nicolae, D. L. and Cho, J. H. (2006) Dominant-Negative TLR5 Polymorphism Reduces Adaptive Immune Response to Flagellin and negatively associates with Crohn's Disease. *Am J Physiol Gastrointest Liver Physiol*
- [84] Cario, E. and Podolsky, D. K. (2000) Differential alteration in intestinal epithelial cell expression of Toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. *Infect. Immun.* 68, 7010-7.
- [85] Beckwith, J., Cong, Y., Sundberg, J. P., Elson, C. O. and Leiter, E. H. (2005) Cdc1, a major colitogenic locus in mice, regulates innate and adaptive immune response to enteric bacterial antigens. *Gastroenterol.* 129, 1473-84.
- [86] Hawn, T. R., Wu, H., Grossman, J. M., Hahn, B. H., Tsao, B. P. and Aderem, A. (2005) A stop codon polymorphism of Toll-like receptor 5 is associated with resistance to systemic lupus erythematosus. *Proc Natl Acad Sci U S A* 102, 10593-7.

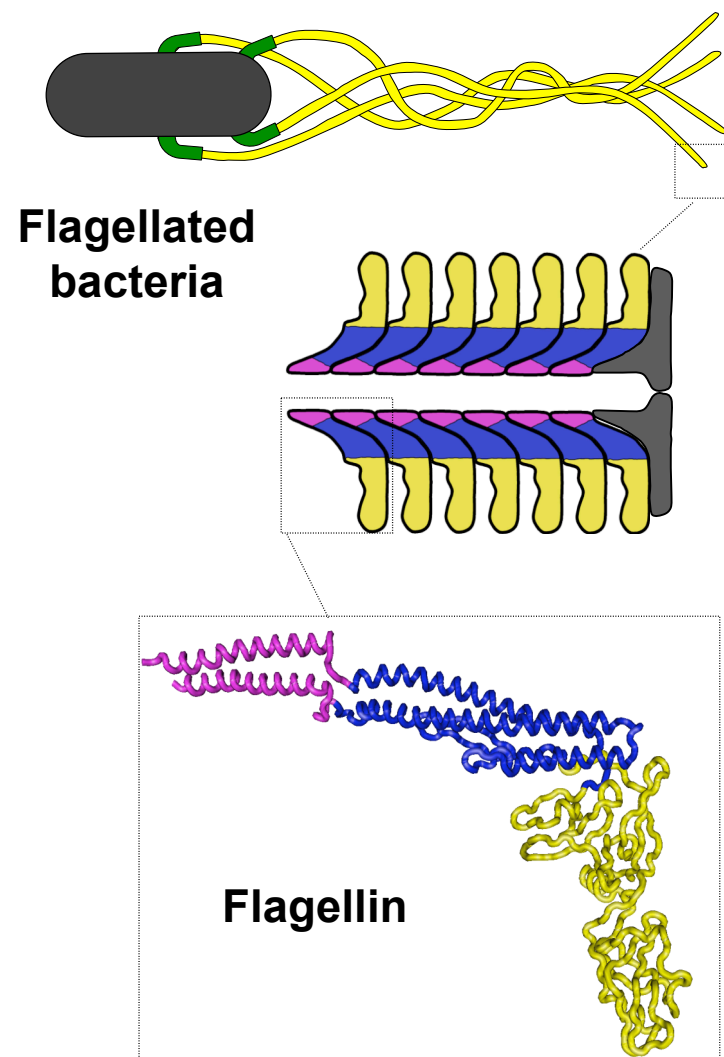


Figure 1A Rumbo *et al.*

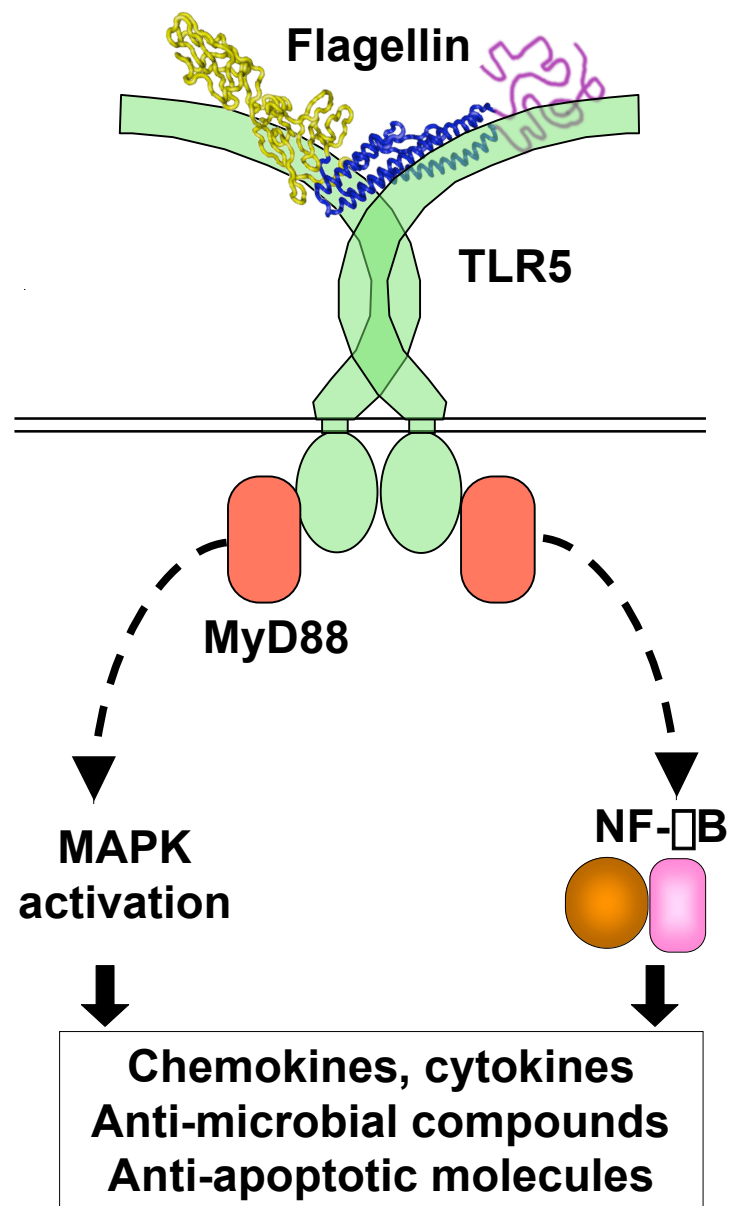
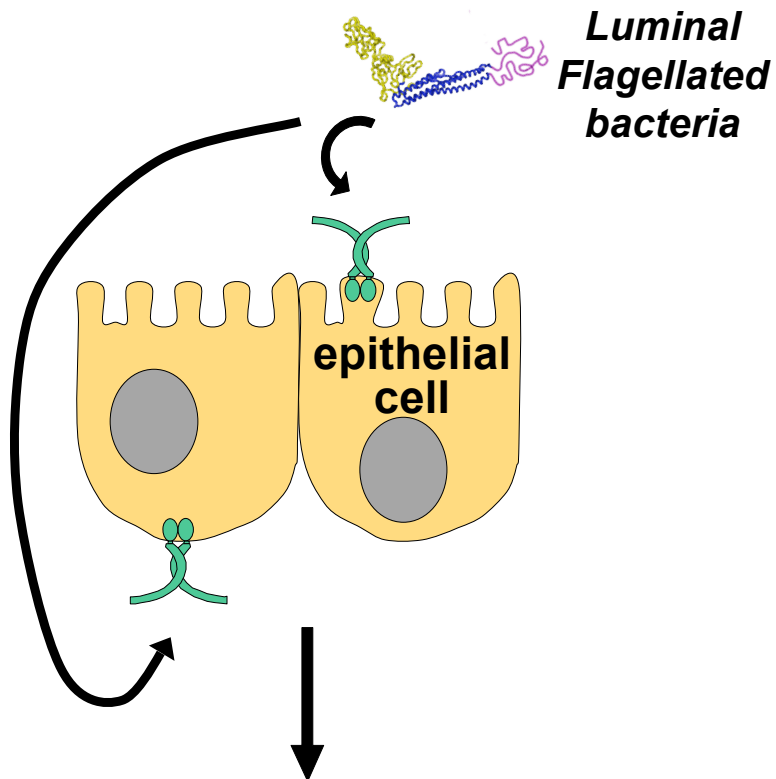


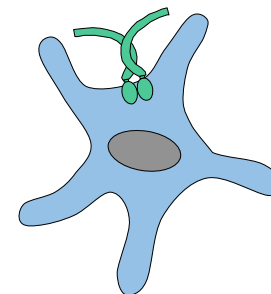
Figure 1B Rumbo *et al.*

- Invasive flagellated bacteria
- Alteration of permeability
to luminal bacteria



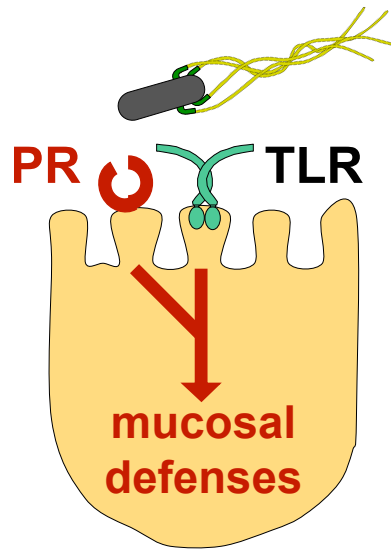
Polarized innate responses:
Chemokines specific for neutrophils and DC
Anti-microbial factors (defensins)
Th2-promoting environment?

Resident or recruited
dendritic cell



Mucosa-specific
adaptive responses: Th2 and IgA

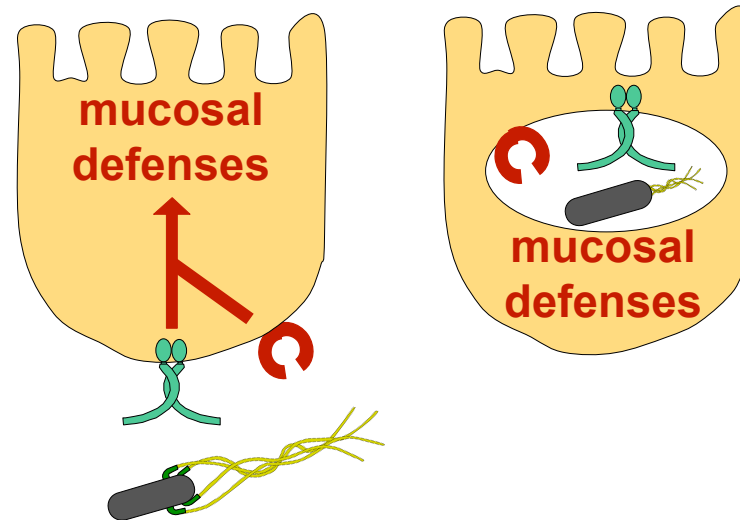
Figure 1C Rumbo *et al.*



PR = Pathogen receptor

TLR = MAMP detector

PR + TLR = PAMP detector



Invasive pathogenic bacteria

Figure 2A Rumbo *et al.*

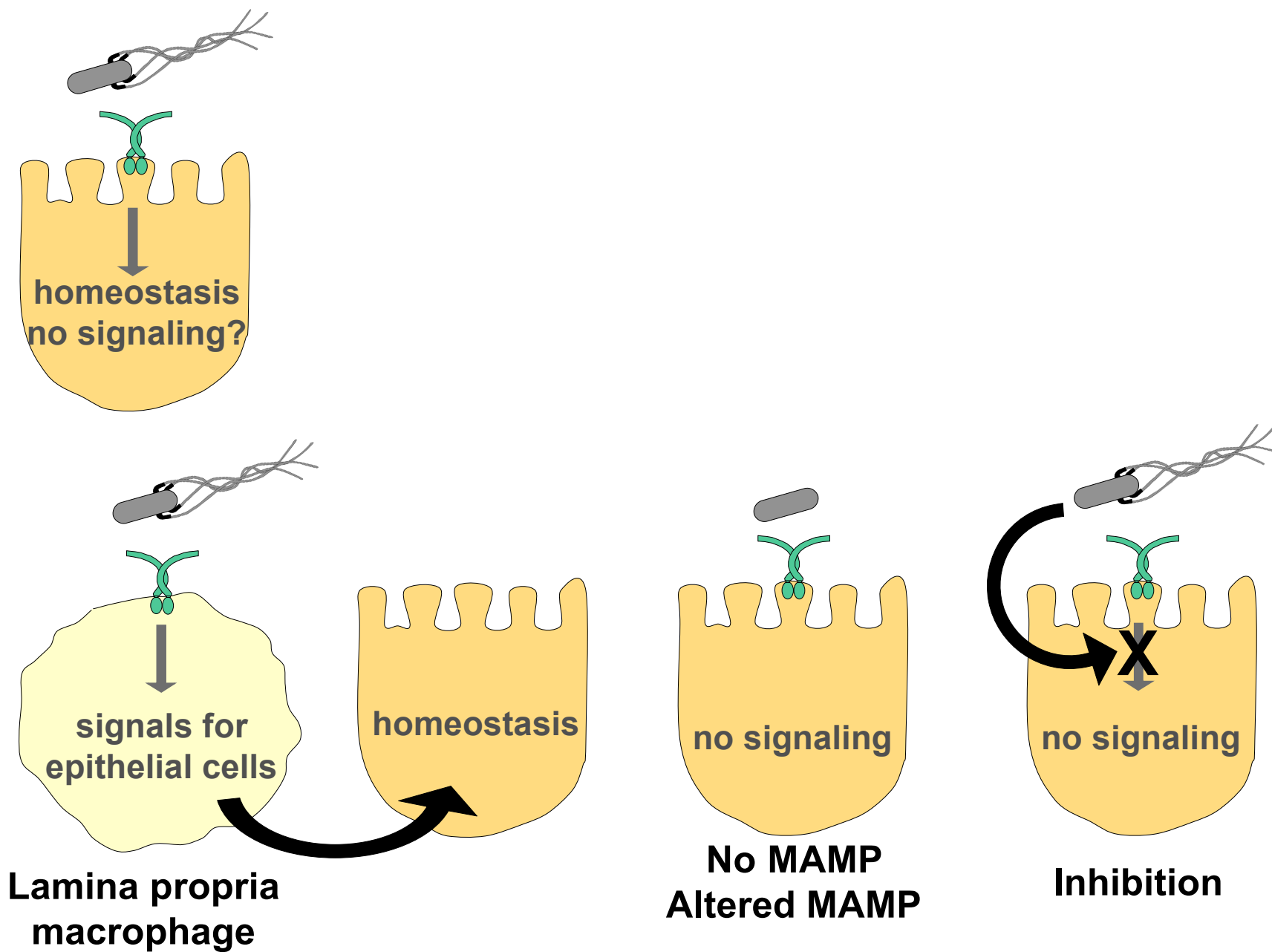


Figure 2B Rumbo *et al.*