Rupture, invasion and inflammatory destruction of the intestinal barrier by *Shigella*: The yin and yang of innate immunity

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PJ Sansonetti. Rupture, invasion and inflammatory destruction of the intestinal barrier by *Shigella*: The yin and yang of innate immunity. Can J Infect Dis Med Microbiol 2006;17(2):117-119.

Shigella is a Gram-negative bacterial species of the family *Enterobacteriaceae* that causes bacillary dysentery in humans. This acute colitis reflects the capacity of the microorganism to disrupt, invade and cause the inflammatory destruction of the intestinal epithelium. The pathogenesis of the *Shigella* infection can be seen as a disruption of the homeostatic balance that protects the gut against inflammation in the presence of its commensal flora. This provides the unified view that enteroinvasive pathogens allow for the identification of key signalling molecules and pathways involved in the regulation of intestinal inflammation, and more generally, in the regulation of the innate and adaptive immune response.

Key Words: *Inflammation; Intestine; Invasion;* Shigella; *Stanier Institut/Institute Stanier*

La rupture, l'invasion et la destruction inflammatoire de la barrière intestinale par le *Shigella* : Le yin et le yang de l'immunité innée

Le *Shigella* est une espèce de bactérie gram négative de la famille des *Enterobacteriaceae* qui provoque une dysenterie bacillaire chez les humains. Cette colite aiguë reflète la capacité du microorganisme à perturber, à envahir et à provoquer une destruction inflammatoire de l'épithélium intestinal. La pathogenèse de l'infection à *Shigella* peut être perçue comme une perturbation de l'équilibre homéostatique qui protège l'intestin de l'inflammation en présence de flore commensale. Elle procure un point de vue unifié, selon lequel les pathogènes entéroinvasifs permettent de repérer les molécules et le voies de signalisation clés qui participent à la régulation de l'inflammation intestinale et, plus généralement, de la réponse immunitaire innée et adaptative.

In addition to its functions in digestion, nutrient, water and electrolyte transport, and hormone production, the intestinal epithelium also plays the role of barrier between the host and the external environment. The colon is exposed to the huge bacterial load of its commensal microflora; thus, intestinal epithelial cells (IEC) are on the frontline to interact with luminal bacteria (1). This barrier function protects efficiently against invasion and systemic dissemination of the commensal flora, but can be subverted at various degrees by pathogens. The intestinal epithelium achieves three barrier functions at once (2): physical, functional and immunological. It is in permanent cross-talk with microorganisms, with IEC being a major partner due to their strategic interface position (3). Microorganisms are constantly sampled from the intestinal lumen into the inductive sites of the intestinal immune system, and the intestinal flora plays a role in gut maturation (4). The coevolution of mammals with their intestinal flora has led to a situation of tolerance, the mechanisms of which have only recently been analyzed in molecular and cellular terms (5). The homeostatic balance that prevails is a complex and fragile equilibrium because the exposed surface is large (ie, 200 m^2) and the microbial density is enormous (ie, 1×10^{11} bacteria/g to 1×10^{12} bacteria/g of stool in the colon). This fragile balance can be subverted by toxins or pathogens. Innate immunity and its regulation are central to this homeostatic balance, marked by a situation of controlled 'physiological inflammation' (Figure 1), the disruption of which, particularly by enteroinvasive bacteria, leads to inflammatory destruction of the barrier (3,6). Identification of the receptors and signalling pathways that enteroinvasive microorganisms interact and interfere with is essential because tracking bacteria in their 'proinflammatory journey' may allow for the identification of key molecules that regulate intestinal inflammation. Many pathogens have coevolved with mammals, particularly humans, for so long that under the selective pressure of the host's immune response, they have accumulated genes encoding effectors that regulate this response to allow successful colonization. Not surprisingly, recent discoveries regarding the genetic determination of chronic inflammatory bowel diseases, such as Crohn's disease, reveal that common signalling pathways are affected, central to which is the Nod family of intracellular sensors of bacterial peptidoglycan (PGN) (7,8).

SHIGELLA AS A PARADIGM OF RUPTURE, INVASION AND INFLAMMATORY DESTRUCTION OF THE EPITHELIAL BARRIER

Shigella represents an extraordinary 'tool' to address the mechanisms of rupture, invasion and inflammatory destruction of epithelial barriers (9). A series of molecular mechanisms reflect stepwise interactions mediated by bacterial effectors

Third Stanier Institute Lecture (delivered November 3, 2003, in Montreal, Quebec, by PJ Sansonetti of the Howard Hughes Medical Institute, Chevy Chase, Maryland, USA and Institut Pasteur, Paris, France)

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Received and accepted for publication January 18, 2006

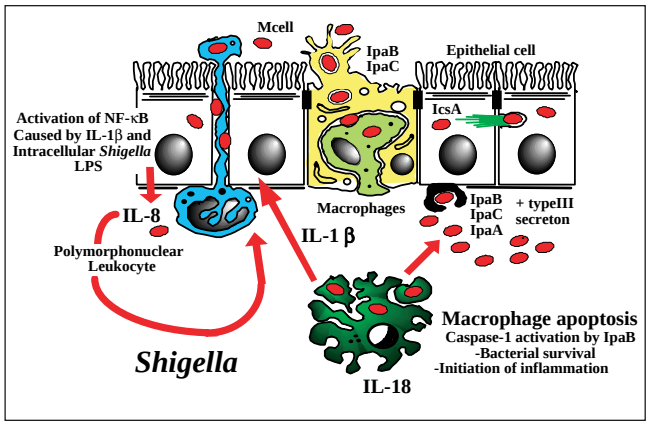

Figure 1) *Summary scheme of the mechanisms of epithelial invasion by* Shigella. *The intertwined nature of the process of epithelial cell invasion and the triggering of inflammation are emphasized here. Inflammation destabilizes the epithelial barrier by disrupting its impermeability, thus facilitating bacterial invasion (30,31). IL Interleukin; Ipa Invasion plasmid antigen; LPS Lipopolysaccharide; Mcell Microfold cell; NFkB Nuclear factor kappa B. Reproduced with permission from reference 3*

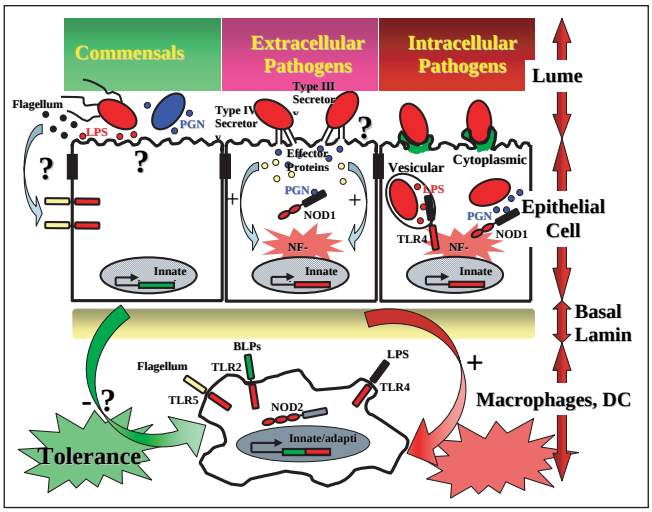

Figure 2) *The complex border between commensals and pathogens regarding the cross-talks controlling tolerance and inflammation (32). BLPs Bombesin-like peptides; DC Dendritic cell; LPS Lipopolysaccharide; NF Nuclear factor; PGN Peptidoglycan; TLR Toll-like receptor*

encoded by a virulence plasmid (10) and delivered to eukaryotic cells by a type III secretory system (TTSS) (11). The kinetics of effector delivery by the TTSS into epithelial cells have recently been unravelled in real time by our group (12). This series of cross-talks allows entry of the bacteria into epithelial cells by a macropinocytic event reflecting massive reorganization of the host cell cytoskeleton that is triggered by activation of small GTPases of the Rho family (13) and activation of a cascade involving Src and its phosphorylated substrate cortactin (14), both of which induce actin nucleation and assembly via activation of the Arp2/3 complex. Following entry and lysis of the phagocytic vacuole, bacteria escape into the cytoplasm and move intracellularly in an actin-dependent process involving a plasmid-encoded outer membrane protein, IcsA (15), the host-cell protein neural Wiskott Aldrich syndrome protein and the actin nucleating complex Arp2/3 (16). Bacteria then spread from cell to cell following engagement of adherence junction components (17). Paracrine regulation, mediated through hemi-channels by which infected cells release ATP and cause Ca^{2+} fluxes in yet uninfected cells of the epithelial lining, creates a highly permissive state of invasion that amplifies the efficiency of invasion (18).

The whole process leads to massive intracellular colonization of the intestinal epithelium, which accounts for the development of mucosal inflammation because each invaded cell gets reprogrammed by intracellular microorganisms to express proinflammatory molecules dominated by the expression of interleukin-8 (19), thus triggering massive recruitment of polymorphonuclear leukocytes (20). Acute recruitment of this mucosal infiltrate of polymorphonuclear leukocytes is essential to eradicate shigellae (21), but this occurs at the cost of massive epithelial destruction (22). The role of antibacterial peptides expressed by IEC in the control of pathogens in the course of an acute infection remains to be definitely demonstrated, but even more interestingly, poor expression of cryptdins by Paneth cells may be related to the triggering of uncontrolled inflammation in Crohn's disease (23). Intracellular sensing and proinflammatory programming is based on recognition of the *Shigella* PGN by Nod proteins (24), particularly Nod1, which is able to discriminate between PGN from Gram-negative and Gram-positive bacteria (25,26). This has set a new paradigm of intracellular sensing of bacteria leading to 'inside in' signalling that activates the nuclear factor-kappa B and c-Jun N-terminal kinase pathways (Figure 2), and thus the transcription of numerous proinflammatory genes. Nod molecules are composed of three domains: a leucine-rich C-terminal domain, which is likely to achieve recognition of the PGN fragments, a central nucleotide binding site domain, and one or caspase recruitment domains. Nod1 recognizes a muropeptide characterized by a tripeptide with a molecule of mesodiaminopimelate in position 3. Nod2 recognizes a shorter and generic muropeptide present in all PGN, the muramyl dipeptide (27). This whole process accounts for the break in tolerance induced by invasive pathogens, making Nod molecules an essential system for the perception of bacterial aggressions to the epithelium.

The number of pending questions are numerous; however, three dominate: do Nod molecules also participate in the homeostatic balance controlling the commensal flora? In other words, can Nod molecules recognize pathogen-associated molecular patterns (PAMPs) from extracellular bacteria? What level of cross-talk is achieved between the Nod and toll-like receptor molecules in the global network of cell signalling in the presence of PAMPs? How are bacterial PAMPs presented to intracellular Nod molecules?

In the case of *Shigella*, an intracellular pathogen that escapes into the cytoplasm, presentation of PAMPs to Nod-sensing molecules is direct. It was not clear, on the other hand, whether extracellular pathogens could be recognized by this system. Recent work on *Helicobacter pylori* interacting with gastric epithelial cells (28) has shown that the type IV secretory system encoded by the *cag* operon was able to introduce *H pylori* PGN fragments into target cells along with the Cag protein effectors.

Last but not least, another emerging theme is how invasive shigellae cope with the strong, innate response they elicit, and how they manage to survive and efficiently colonize the epithelial surface before proceeding to invasion. Accumulating evidence indicates that a group of effector proteins encoded by the *Shigella* virulence plasmid and secreted through the TTSS

are likely to be strong regulators of the innate and possibly adaptive immune responses. These proteins, such as outer surface protein(s) and invasion plasmid antigen(s), are expressed only during periods of activation of the TTSS, even though it is not yet clear whether the TTSS is constantly activated in vivo, or simply turned on every time bacteria meet the eukaryotic cell surface, particularly the epithelium. In any event, one of these proteins, outer surface protein G, has recently been shown to downregulate activation of the nuclear factor-kappa B signalling pathway. It does so through a very original process in which it binds to the ubiquitine-transfering enzymes ubiquitine-conjugating enzyme 5 and ubiquitine-conjugating enzyme 7, thus blocking the degradation of inhibitory kappa B (29). One may thus expect that a battery of anti-inflammatory proteins will soon be unravelled in *Shigella*. One should, however, look beyond the mere aspect of inflammation; it is expected that such effectors will not only affect the innate immune response in the broad sense, but also the adaptive immune response, as well as some basic aspects of the physiology and homeostasy of the epithelial barrier.

CONCLUSION

We may be ready for a unifying approach to intestinal inflammation by developing, in parallel, studies aimed at understanding the homeostatic mechanisms allowing tolerance of the intestinal flora, deciphering how (enteroinvasive) pathogens cause intestinal inflammation and break this tolerance process, and understanding the molecular mechanisms of inflammatory bowel diseases. These are clearly interconnected topics that provide a fascinating intrusion into the basic mechanisms of intestinal physiology and immunity.

ACKNOWLEDGEMENTS: The author thanks the members of Unité de Pathogénie Microbienne Moléculaire and Group Immunité Innée et Signalisation for their contributions, which were briefly summarized in the present paper. The author also thanks Dr Morris Goldner and the members of the Stanier Institute/Institut Stanier for the opportunity to disseminate the author's research findings to Canadian colleagues.

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