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Toll-Like Receptors in Gastrointestinal Diseases

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Abstract
Innate immune cells – and many other cells – express evolutionarily conserved, germline-encoded receptors that recognize seemingly pathogen-derived ligands (also termed pathogen-associated molecular patterns), thereby allowing the host to perceive infection. Although they were the first to be discovered, Toll-like receptors (TLRs) are not the only pattern recognition receptors. TLRs are unlikely to discriminate between commensals and pathogens in the gut microbiota. There is, however, increasing evidence that TLRs shape intestinal function. In addition, certain bacteria appear to drive either Th1/Th17 proinflammatory immune responses, or T regulatory responses. Furthermore, TLRs appear to trigger 'sterile' autoinflammatory responses by sensing metabolically altered host (self) components.

Introduction

In 1908, the Nobel Prize for Physiology/Medicine went to Paul Ehrlich and Ilya Ilrych Metchnikoff for 'recognition of their work in immunology'. While Ehrlich realized the specificity of adaptive immune responses (he had collaborated with E.A. von Behring in developing 'diphtheria serum' which inspired him to phrase his famous 'side-chain theory'), innate and nonspecific immune effectors such as phagocytosis of bacteria and toxins were advocated by Metchnikoff [1, 2]. This dichotomy of immunity – unspecific innate versus specific adaptive immunity – has mirrored the wisdom of textbooks throughout the 20th century.

The infusion of molecular biology and the integration of the central dogma of genetics, which holds that cellular information flows from DNA to RNA to proteins, have been of upmost importance in the development of immunology. As a consequence, knowledge on adaptive immunity has advanced by quantum leaps during the past decades, driven also by the clonal selection theory and subsequently by crucial discoveries on the role of the thymus and bone marrow in T and B cell genesis and function.

Although microbes have long been recognized as the cause of infectious diseases, and Metchnikoff's nonspecific phagocyte model has been with us since the end of the 19th century, the question as to how the immune system perceives infection has remained largely unknown. Interestingly, the late Charles Janeway speculated in 1998 [3] that pathogen-associated molecular patterns (PAMPs) cause upregulation of costimulatory molecules on antigen-presenting dendritic cells (DCs), thus allowing the latter to activate naïve T cells. Another clue came from the observation that the inbred mouse strain C3H/HeJ

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resisted otherwise lethal doses of lipopolysaccharide (endotoxin) [4]. Could it be that these mice harbored a nonfunctional (mutated) lipopolysaccharide receptor?

Using Toll gene mutants generated by others for embryonic studies in the fruit fly Drosophila, Bruno Lemaitre and Julius Hoffmann discovered in 1996 that a functional Toll gene was essential to control fungal infections in adult flies [5]. That a germline-encoded and ligand-specific receptor controlled aspects of a fly's innate immune system was a revelation to many immunologists.

Orthologues of the fly gene Toll were soon detected in the human (h) germ line – termed Toll-like receptors – and Janeway and Medzhitov reported a year later that enforced expression of a constitutive active hTLR (it happened to be TLR4) caused NF-κB-dependent cytokine production and induction of costimulatory molecules [6]. Independently, Bruce Beutler, who had previously shown together with Cerami [7] that LPS triggers in macrophages the cytokine 'tumor necrosis factor' (TNF), used C3H/HeJ mice to search for the postulated LPS receptor. He reported in 1998 that TLR4 senses LPS [8]. And in generating an ever-increasing stock of TLR pathway gene knockout mice, Kawai and Akira [9] further and profoundly advanced today's knowledge of TLR signal pathways. Thus, we owe to the pioneering work of Akira, Beutler, Hoffmann and Medzhitov (initially together with the late Charles Janeway) the answer as to how TLRs perceive infections. For their pioneering work, the 2011 Nobel Prize award went to Julius Hoffmann, Bruce Beutler and Ralph Steinmann (the work of the latter laureate is not discussed here).

Toll-Like Receptors

Humans and primates express the transmembrane glycoproteins TLR 1–10, while mice and most other mammalian additionally harbor the orphan receptors TLR 11–13. They are located on the cell surface or on endosomes, and specifically recognize the presence of microbes through PAMPs such as LPS (TLR4), flagellin (TLR5), certain lipopeptides (TLR 2/1/6), DNA (TLR9) and RNA (TLR3 or-TLR7/8). In addition, TLRs recognize damage-associated molecular patterns (modified host components). TLRs contain 16–28 leucine-rich repeats that mediate ligand binding. On ligand binding, the C termini of the extracellular domains come together (dimerize). This brings the intracellular Toll/Il1 R domains into close proximity, which in turn is required for docking of the respective adaptor molecules such as Myd88, Mal, TRIF and TRAM. As a consequence, NF-κB-dependent production of proinflammatory cytokines, such as IL-12, IL-6, TNF, etc., become induced, or via interferon regulatory factors the production of type 1 interferons [9]. Many additional pattern recognition receptors (PRRs) are found in the cytoplasm, including the 'retinoic acid gene I inducible helicases', 'nucleotide-binding domain leucine-rich repeat-containing receptors' and several other DNA sensors, including the stimulator of IFN genes (Sting). In addition, the pioneering work of the late Jürgen Tschopp highlighted the caspase-1-activating function of the 'inflammasome' formed in the cytosol after ligand-driven activation of certain nucleotide-binding domain leucine-rich repeat-containing receptors [10]. Once activated, caspase 1 controls maturation of members of the IL-1 family, and IL-1 is known to cause fever as well as inflammation.

TLR Signaling in the Intestine

The intestinal epithelium provides a physical barrier that separates the gut microbiota in the intestinal lumen from the underlying lamina propria and deeper intestinal layers. Four cell types characterize the intestinal epithelium: intestinal epithelial cells (IECs), mucosa-producing goblet cells, hormone-producing enteroendocrine cells and Paneth cells, with the latter producing antimicrobial peptides and lectins. Furthermore, specialized intraepithelial T cells and DCs localize between the structurally and functionally polarized IECs. Given the juxtaposition between gut flora and IECs, the question arises as to how TLRs expressed by IECs avoid an indiscriminate response to PAMPs from commensal bacteria? In fact, IECs seem to tolerate the presence of luminal PAMPs, but require TLR signals for their normal function. The expression of TLRs by IECs is believed to be low, but during intestinal inflammation (ulcerative colitis, Crohn's disease) TLR expression is upregulated, presumably via γ-interferon [11]. TLR signaling in the intestine is also thought to be regulated spatially; there is 'regulated' apical or basolateral TLR expression. While apical TLR signaling might be tolerogenic [12], basolateral expressed TLR signaling appears to come into play upon injury of the physical barrier of gut's IEC monolayer. Deleterious TLR activation may also be inhibited by negative regulators of TLR signaling, such as TOLLIP or SIGIRR.

While under normal conditions (no injury), proliferation of TLR signaling defective IECs equals that of wild-type mice; mice deficient in TLRs are more susceptible to dextran sulfate-induced injury. Since treatment of wild-type mice with broad-spectrum antibiotics converts mice similarly susceptible to dextran sulfate-induced injury, both the bacterial gut flora and TLRs appear to be required for optimal IEC proliferation. The mechanisms linking TLRs to IEC proliferation includes induction of ligands for epithelial growth factor family members. TLRs also regulate barrier function (tight junction). During *Citrobacter rodentium*-induced colitis, IEC expression of TLR2 protects against apoptosis and maintains zona occludens 1 protein at the apical tight junction region. In addition, the expression of antimicrobial peptides (defensins) in IECs is induced by TLR signaling, but Paneth cells remain the major source.

TLR signaling also has an impact on IgA2 production. While in humans the production of IgA1 is T cell-dependent and antigen-specific, class switching to the protease-resistant IgA2 requires TLR activation of IECs to induce the secretion of 'a proliferation-inducing ligand' (APRIL). TLR signaling of IECs also enhances the ability of DCs to emit projections into the lumen to sample commensal and pathogenic bacteria. Finally, TLR signaling affects the composition of mucus that is composed of mucin glycoprotein and trefoil factor 3 since TLR2 induces expression of trefoil factor 3 in IECs. It therefore appears that most TLR signaling in intestinal epithelium has a beneficial role in maintaining intestinal homeostasis.

The Immune System and the Gut Microbiota: 'Peacekeeping' and Polarizing Lessons

Given that under homeostatic conditions there is a persistent host association with trillions of obligate and facultative beneficial symbionts in the gut, one might speculate that the immune system evolved to accommodate colonization by symbiotic bacteria while retaining the capacity to fight pathogens. How bacterial colonization of the gut influences the development and the function of the immune system has become a major focus of interest. Colonization is initiated by maternally acquired bacteria during birth, followed mainly by two environmentally acquired phylotypes, the Firmicutes and the Bacteroidetes [13]. This led to the view that the host and his symbionts have coevolved towards mutualistic interactions – while highly flexible defense mechanisms cope with the potential bacterial threat. Accordingly, PRR-expressing IECs are a central component of the immune system of the gut, able to recruit leukocytes to complement immune defenses, and the development of gut-associated lymphoid tissue is initiated before birth.

Severe forms of autoimmune enteropathy characterize IPEX (immunodysregulation polyendocrinopathy enteropathy X-linked syndrome) patients and scurfy mice that lack the key transcription factor of regulatory T cells (Tregs), Foxp3 [14]. This underscores the importance of Tregs for the 'peacekeeping' control of the immunes system against antigen in the gut. It has been known that postthymus naïve T cells acquire in the intestine regulatory function by upregulating Foxp3. As it turns out, *Bacterioides fragilis* (a common culturable member of the microbiota) possesses an unusual capsular polysaccharide A that functions as TLR2 ligand for naïve CD4 cells, and is able to drive their differentiation in 'peacekeeping' Tregs in the presence of specialized DCs secreting TGF-β and retinoic acid. Furthermore, these induced Tregs have a T cell receptor repertoire which is specific for an individual's microflora [15, 16].

While certain members of the microbiota have adopted 'peacekeeper' activities, others are endowed with proinflammatory activities, an example being 'segmented filamentous bacteria'. These uncultivable species colonize the rodent intestine at the time of weaning and stimulate postnatal immune-maturation toward Th1 and Th17 immunity. Thus, the gut flora and the respective cytokine milieu which is induced appear to act as a driving force either for induced Treg formation or differentiation of (Th1/Th17) effector T cells. While the former protect against IBD, the latter potentially cause IBD. Most likely, dysbiosis – also driven by antibiotics – can play a causative role in gut inflammation. Bacterial colonization differs between neonates born vaginally or by caesarian delivery. Interestingly, these differences have been linked to an increased risk for atopic diseases such as asthma in children born by caesarian delivery.

Unforeseen, another paradigm shift has appeared on the horizon in the past years. There is now compelling evidence that germline-encoded PRRs not only perceive pathogen-induced inflammation, but also 'sterile' autoinflammation by sensing metabolically altered self (host) components including modified lipids and proteins. These data support Matzinger's view [17] that 'danger' as sensed by the innate immune system comes mainly 'from the inside'. To date it is unknown to what extent these mechanisms operate in IBD.

In conclusion, the interplay of 'proinflammatory' and 'peacekeeping' bacteria of an individual's microbiota

which are indiscriminately recognized by PRR-expressing IECs possibly impacts on the host peripheral immune system. As a consequence, dysbiosis may not only drive IBD but also the development of extraintestinal immune-mediated diseases, such as allergy and autoimmunity.

Disclosure Statement

The author declares no conflict of interest.

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