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Linking genetic variation in human Toll-like receptor 5 genes to the gut microbiome's potential to cause inflammation

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ABSTRACT

Immunodeficiencies can lead to alterations of the gut microbiome that render it pathogenic and capable of transmitting disease to naïve hosts. Here, we review the role of Toll-like receptor (TLR) 5, the innate receptor for bacterial flagellin, in immune responses to the normal gut microbiota with a focus its role on adaptive immunity. Loss of TLR5 has profound effects on the microbiota that include greater temporal instability of major lineages and upregulation of flagellar motility genes that may be linked to the reduced levels of anti-flagellin antibodies in the TLR5$^{-/-}$ host. A variety of human TLR5 gene alleles exist that also associated with inflammatory conditions and may do so via effects on the gut microbiome and altered host–microbial crosstalk.

1. Introduction

The gastrointestinal tract serves as one of the most dynamic interfaces between the host immune system and commensal, as well as pathogenic, microorganisms. Due to the vast diversity of the microbiota in the gut and of the potential presence of pathogens, the intestinal immune response must be capable of (i) maintaining a basal, appropriate level of antimicrobial activity without pronounced inflammation and, (ii) mounting a pro-inflammatory response to invasive organisms when physical barriers are breached [1,2]. From recent studies we now know that genetic deletion of microbial sensors can lead to changes in cross-talk between the host and the microbiota [3–5]. Experiments in which gut microbiota of immunodeficient animals are transferred into germfree hosts have shown that alterations of gut microbial communities can be sufficient to cause disease in a new host [6–8]. Since one of the defining characteristics of inflammatory diseases including Crohn's disease and Ulcerative colitis is alteration of the microbiome [9–15], targeting gut microbiotas is an attractive strategy to ameliorate such microbially-mediated disease phenotypes [16]. Understanding how immunity shapes the composition and behavior of gut microbes remains an important task if treatments targeting the microbiota are to be developed.

Innate immune detection of microbes occurs rapidly through specific receptors that recognize conserved molecular structures found on or within the microbe [17]. These pattern recognition receptors include two key families the gut: transmembrane Toll-like receptors (TLRs), and cytosolic nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs). TLR expression has been primarily associated with innate immune cells; however, non-immune cells such as intestinal epithelial cells express TLRs and are equipped to respond to TLR engagement through the production of antimicrobial peptides and cytokines [18,19]. Disruptions in NLR and TLR expression have been associated with alterations in the microbiota composition and intestinal dysbiosis [5,6,20,21]. These studies suggest a critical and highly responsive interplay between the host and the microbiota.

This mini-review focuses on TLR5, the innate immune receptor for flagellin, the principal protein component of bacterial flagella. TLR5 response to flagellin appears to promote both innate and adaptive immune functions and to interact with bacteria in ways that may be fundamental to gut homeostasis and health. TLR5-deficient mice can exhibit either chronic intestinal inflammation, or an obese, metabolic syndrome profile, although there is some variability in penetrance of these phenotypes between colonies [6,20,22,23]. The phenotypic variability may relate to differences in mouse microbiomes between facilities, and may result from

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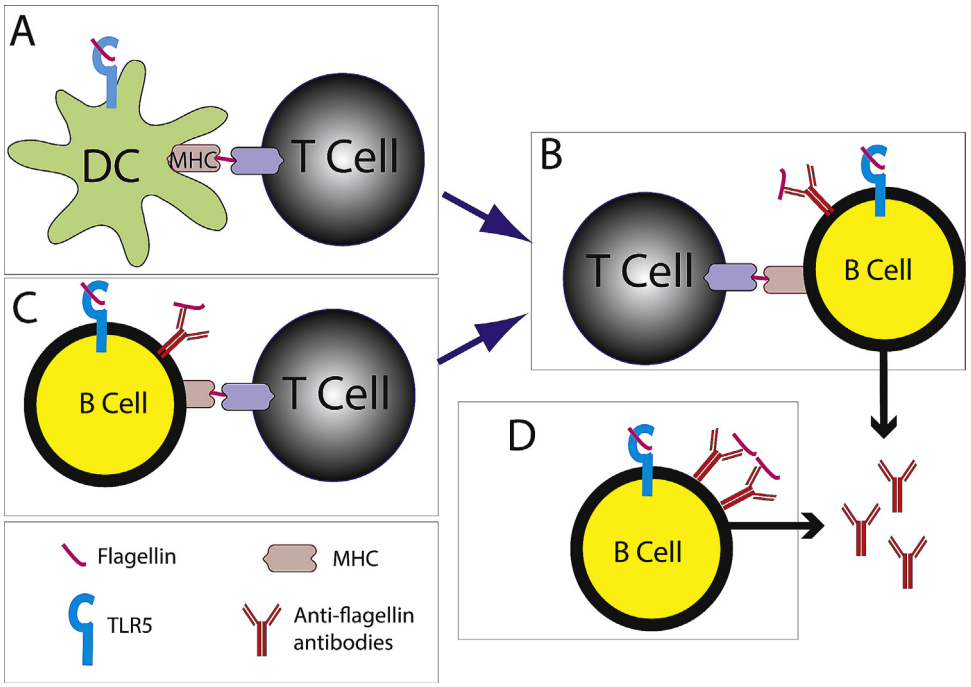

Fig. 1. Possible mechanisms for the production of anti-flagellin antibodies. (A) Dendritic cells (DCs) present flagellin to T cells via MHCII. DCs in the lamina propria express TLR5, but whether this is necessary for antigen presentation is uncertain. (B) Flagellin-specific B cells endocytose flagellin and present it to flagellin-specific T cells that have received activation signals from a DC (A). The T cell provides the necessary cytokine and costimulation for B cells to produce flagellin-specific antibody. B cells express TLR5 in the lamina propria, but whether stimulation of TLR5 by flagellin is necessary for a normal anti-flagellin antibody response is uncertain. (C) B cells can directly present flagellin in MHCII to flagellin-specific T cells after endocytosing flagellin and receiving stimulation through TLR5. In this scenario, there is no requirement for DCs since the T cells can directly provide costimulation and cytokines to the B cells. In (D), flagellin-specific B cells can engage repeating units of flagellin through the B cell receptor and TLR5, which together provide all the stimulation that is needed for the B cell to produce anti-flagellin antibodies. This would occur independently of T cells. Whether this T-independent mechanism can result in production of anti-flagellin antibody is not known.

dysregulated host–microbial interactions. When a phenotype is apparent, mice deficient in TLR5 are prone to developing two forms of inflammation, namely colitis and metabolic syndrome [6,20]. Inflammation in the TLR5-deficeint host is associated with alterations in gut microbial community composition in conventionally raised animals, yet is completely eliminated in the germfree state [3]. Transplantation of the gut microbiome of a TLR5-deficient host to wildtype (WT) germfree host is sufficient to transfer metabolic syndrome to the new host [6].

These observations have raised several questions: How is immune homeostasis impacted by loss of TLR5 signaling? What aspects of the TLR5-deficient microbiome are altered and which are required to transfer the phenotype? How does allelic variation in TLR5 genes in humans relate to microbially mediated host phenotypes? Here, we review recent research on the interactions between TLR5, adaptive immunity and the microbiota, and we discuss how variation in the TLR5 gene may alter these interactions to impact host inflammatory phenotypes in humans.

2. Location and function of TLR5

TLR5 is a receptor for bacterial flagellin [24], and is structurally composed of leucine rich repeats in the ectodomain, a transmembrane domain, and a Toll/IL-1 receptor-like domain in the cytoplasmic tail that transmits a signal to the cell [25,26]. Part of the TLR5 ectodomain (amino acids 386–407) directly associates with recombinant flagellin [27]. The region of the flagellin molecule that is recognized by TLR5 is a 13 amino acid residue that likely participates in flagellin multimerization, and thus is not accessible for TLR5 recognition in the polymerized form [28]. However, flagellated bacteria, such as *Salmonella typhimurium*, shed monomeric flagellin upon infection of epithelial cells providing a potential source of monomeric TLR5 ligand in the intestine [29]. Similar to other TLRs [30–33], restricted localization of TLR5 may be a mechanism to regulate cellular response through TLR5 and thus prevent continuous inflammatory response to bacterial flagellin in the gut [19]. Parenteral injection of flagellin in vivo induces a variety of immune outcomes including proinflammatory, Th2, IL-17/IL-22, and Treg cell activation [34–36]. In addition to this complex innate immune-activating capacity, flagellin is also a protein that elicits a flagellin-specific adaptive immune response.

3. The role of TLR5 in anti-flagellin antibody production

Since flagellin has both antigen and adjuvant activity, it has the capacity to drive anti-flagellin responses (Fig. 1). Dendritic cells receive an innate immune signal from flagellin through TLR5, and present flagellin peptides in MHC II to activate naïve flagellin-specific T cells [37] (Fig. 1A). The activated T cells will proliferate and assist B cells in producing antibodies (Fig. 1B). These B cells are likely stimulated in Peyer's patches, in a T-dependent manner, prior to trafficking to the lamina propria where they are fully differentiated, class-switched, plasma cells [38]. In this dendritic cell- and T cell-dependent scenario, B cells must also endocytose and present flagellin to receive T cell help, but there is no requirement for the B cell to express and respond via TLR5 since the dendritic cell received flagellin and activated T cells. However, B cells could also endocytose and present flagellin in MHC II directly to T cells, and receive innate signaling from flagellin through TLR5 licensing them to activate naïve T cells (Fig. 1C). The T cells will then in turn provide help to the B cells to make antibodies. However, other TLRs could provide the innate signal to B cells, and again, there is no