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Review

Intestinal microbiota: Shaping local and systemic immune responses

Michael J. Molloy, Nicolas Bouladoux, Yasmine Belkaid*

Mucosal Immunology Unit, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 4 Center Drive, Room 4/243, Bethesda, MD 20892, USA

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ABSTRACT

Recent studies have highlighted the fundamental role of commensal microbes in the maintenance of host homeostasis. For instance, commensals can play a major role in the control of host defense, metabolism and tissue development. Over the past few years, abundant experimental data also support their central role in the induction and control of both innate and adaptive responses. It is now clearly established that commensals are not equal in their capacity to trigger control regulatory or effector responses, however, the molecular basis of these differences has only recently begun to be explored. This review will discuss recent findings evaluating how commensals shape both effector and regulatory responses at steady state and during infections and the consequence of this effect on local and systemic protective and inflammatory responses.

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The human intestine harbors and is in constant contact with 1000 trillion microbes, composed of an estimated 4000 strains [1,2]. Recent studies have changed our perspective of commensal microbes from benign passengers, to active participants in both the post-natal development of mucosal and systemic immunity, and in its long-term steady-state function. To maintain its interaction with commensals and sustain its function as a digestive organ, the gastrointestinal tract environment requires the constant induction and maintenance of various classes of regulatory responses. However, immune tolerance does not represent the only fate of immune responses at mucosal sites as a certain degree of constitutive effector responses and inflammation is beneficial for the host, not only to reinforce the barrier, but also to allow for the development of protective responses when required. This implies that the regulation of this environment is more complex than initially thought and relies on the maintenance of a constant balance of antagonistic signals. This review will discuss recent findings evaluating how commensals shape both effector and regulatory responses at steady state and during infections and the consequence of this regulation on protective responses against pathogens.

1. Commensals control the first line of defense

Various aspects of host protective structures and innate immunity develop extensively after birth, due in large part to the interaction with the recently acquired microbiota [3]. Studies performed in germ free (GF) animals revealed that the microbiota plays a critical role in secondary lymphoid structure development [4,5]. This also includes fortification of the intestinal barrier through epithelial cell maturation and angiogenesis of a capillary network that facilitates transport of white blood cells [6,7]. The molecular mechanism responsible for this development remains incompletely understood, but at least in part involves a variety of pattern recognition receptors (PRRs) which are capable of detecting microbe-associated molecular patterns (MAMPs) including toll-like receptors (TLRs), NOD-like receptors (NLRs) and RIG-like receptors (RLRs) [8]. Several lines of evidence indicate that microbial signals are also responsible for the induction and development of isolated lymphoid follicles (ILFs) from cryptopatches in the intestinal tract. Notably, ILFs appear within the first weeks after birth of mice and can eventually number in the hundreds [9]. The current model hypothesizes that Gram-negative bacterial derived peptidoglycans are sensed by NOD1 expressed on intestinal epithelial cells (IECs) [10]. The IECs subsequently express CCL20 and β-defensin 3 which activate LTi cells leading to the formation of ILF [11–13]. An alternative but not exclusive model proposes that the activated LTi cells would engage lymphoid tissue organizer (LTo) cells, which are mesenchymal in origin, to express CCL20 for the recruitment for B cells.

Other critical components of host defense are represented by the mucus layer and antimicrobial peptides both under the tight control of the flora [14–16]. Engagement of PRRs by commensally derived products induces expression of a variety of anti-microbial peptides, which are critical in preventing translocation of bacteria through the rest of the host tissue [17]. One of the best-characterized mucosal anti-microbial peptides is RegIIIγ, which is expressed soon after birth or following colonization of GF mice [18]. Production of this lectin is controlled by the flora

* Corresponding author.
E-mail address: ybelkaid@niaid.nih.gov (Y. Belkaid).

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in an MyD88 dependent manner and has a direct microbicidal effect on Gram-positive bacteria [18–20]. Similarly, the PRR NOD2 controls expression of a subset of α-defensins and cryptdins by Paneth cells [21]. Thus, by virtue of favoring structural development and innate immune responses at the intestinal interface, the flora plays a dominant role in controlling primary encounter with pathogens.

2. The homeostasis of the GI tract: balance of inflammatory and regulatory signals

Although mucosal surfaces have to constitutively integrate a multitude of microbial derived signals, new evidence suggest that defined bacteria or microbial products can play a dominant role in the induction of distinct class of immune responses. At steady state, the gut is home to a large number of lymphocytes, a high fraction of them with the potentiality to produce cytokines such as IL-17, IL-22, IFN-γ, and/or IL-10 [22,23]. The flora tightly controls this constitutive production of cytokines, as GF mice show extensive deficiencies in basal cytokine production [22,24]. Further, in absence of flora, the $CD4^+$ T cell population is diminished, disproportionately affecting Th1 and Th17 cells, although, Treg frequencies are maintained or increased in the small intestine [22,23]. Colonization of GF mice with complex microbiota orchestrates a broad spectrum of T helper (Th1 and Th17) and regulatory T cell responses [25]. Some of the factors that govern the induction of constitutive effector and regulatory responses in the GI tract and how such conditioning affect subsequent effector responses against pathogens will be discussed in this review.

3. Regulatory responses in the GI tract

A complex regulatory network including specialized population of antigen presenting cells, lymphocytes and innate cytokines, controls GI tract homeostasis and converge to favor the induction of regulatory responses toward antigens present at mucosal sites. Early reports suggested that commensals played a central role in maintaining such regulation. Indeed, oral tolerance [26] – the active suppression of inflammatory responses to food and other orally ingested antigens – could not be induced in the absence of gut flora or gut flora derived signals [27,28] and feeding germ free mice with LPS was sufficient to restore this process [27]. The presence of commensals has been also associated with the suppression of IgE and Th2 responses following antigen feeding [29]. Oral tolerance could also be rescued by reconstitution of germ free mice with *Bifidobacterium infantis*, a dominant commensal bacteria [29]. Some of this effect of the flora has been associated with the cross talk between commensal derived LPS and TLR4 [30,31]. Intriguingly, recent evidence demonstrate that conditioning of naïve T cells with LPS, a phenomenon that would occur in gut associated lymphoid structure, provides a tonic inhibitory role for TLR4 signaling on subsequent-dependent $CD4^+$T cell responses [32]. Such phenomenon may account for the limitation of aberrant responses to orally derived antigen. Although LPS has been associated with the acquisition of oral tolerance, the precise molecular mechanism accounting for such phenomenon and the targets of microbial derived signals remains incompletely understood.

4. Induction of Treg cells at mucosal sites

Although immunological tolerance is likely to be achieved via multiple and redundant mechanisms [26], over the past few years, several actors including TGF-β, IL-10 and in particular Foxp3 regulatory T (Treg) cell have taken central stage in our understanding of this process. Treg cells maintain both peripheral and mucosal homeostasis throughout the lifespan of the host [33]. Treg cells typically develop during thymic selection processes; however they can also develop extra-thymically in response to chronic antigen stimulation or exposure to environmental and food antigen at mucosal sites [34]. In particular, the gut-associated lymphoid tissue is a preferential site for the peripheral induction of Treg cells [35–38]. Development of inducible Treg (iTreg) cells requires transcription factor binding to the intronic enhancer element (enhancer-1) of the foxp3 locus, also known as conserved non-coding sequence 1 (CNS1) and is dependent on several soluble mediators, including: TGF-β, IL-2 and the vitamin A metabolite retinoic acid (RA) [35–37,39–42]. This process is tightly controlled at steady state by the capacity of a specialized population of gut tropic DCs expressing CD103 to produce RA [35,36,43]. In addition to supporting iTreg differentiation, RA derived traffic signals are required for a sustained expansion of iTreg cells in the gut [44]. This expansion is propagated through IL-10 mediated interactions with lamina propria resident CX3CR1 + macrophages [44]. In previous studies, similar interactions were shown to contribute to both the induction and maintenance of Treg cells [43,45]. Recent findings demonstrated that the physiological relevance of iTreg induction at mucosal sites is associated with their central role in oral tolerance [46,47]. Further, RA is also required to elicit pro-inflammatory helper T cell responses to infection and mucosal vaccination [42,48]. Antagonism of RA receptor (RAR) signaling results in a cell-autonomous CD4 T cell activation defect, which impairs intermediate signaling events, including calcium mobilization. Altogether, these findings reveal a fundamental role for the RA–RAR axis in the development of both regulatory and inflammatory arms of adaptive immunity [49]. The precise factors that govern the activation of enzymes involved in the metabolism of RA as well as how commensals or pathogens modify the metabolism of vitamin A remains poorly understood. However, interaction with microbial products and in particular via TLR2 can promote vitamin A metabolism [50]. A reciprocal regulation between the flora and vitamin A metabolism is further supported by the observation that vitamin A deficiency leads to dramatic shift in commensal populations [51]. Of note, some of this alteration may result from the role of RA in controlling IgA responses and or homing receptors on effector cells required for the proper establishment of the mucosal firewall [52,53]. An important future area of study will be to understand the complex interplay between vitamin A and commensal populations in the induction of regulatory and effector responses to pathogens. Another mean by which the flora may control oral tolerance is associated with its role in the control of antigen sampling of luminal contents by DCs from the underlying lamina propria compartment [54]. Recent reports also indicate that the gut flora can directly contribute to the expansion of lamina propria resident CX3CR1 macrophages that have been associated with local expansion of Treg cells [55]. Flora derived products can also control the status of activation of liver resident DC and elevate the threshold of activation needed for induction of immune responses [56]. Such control appears to be tissue specific as *ex vivo* analysis of DC status of activation revealed a similar pattern of activation between GF and conventionally raised mice in secondary lymphoid organs including mesenteric lymph nodes [57,58]. However, it is worth noting that the diet of GF mice contains endotoxins that can provide surrogate signals to the ones normally provided by the flora. Coupled with activation induced by tissue dissociation, this is likely to blunt any potential differences resulting from the absence of commensals. Based on the known role for gut microbiota in promoting both regulatory and effector populations, a better understanding of the role of commensals in shaping tissue resident and peripheral DC activation