Microbiotal shaping of antigen presenting cell signaling during intestinal immune response

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SUMMARY

Macrophages, dendritic cells (DCs) and to less extent B cells are professional antigen presenting cells (APCs). They sample antigens from the intestinal lumen, process, and present them to cells of the adaptive immune system. While DCs are capable of priming T cell responses, macrophages do polarize the responses. The intestinal lumen contains diverse range of antigens from food proteins, microbiota, and pathogenic microbes that constantly challenge the immune system. While antigens from food proteins and microbiota are not harmful, those from pathogenic microbes are detrimental to the body. The immune system detects both types of antigens and drives immune responses geared at inducing tolerance or reaction to maintain immune homeostasis. Uptake of these antigens is done by the APCs. These cells present antigens to effector cells of the adaptive immune system which generate responses corresponding to particular antigen. The microbiota influences the nature and type of responses generated by APC-activated effector cells by shaping the APC signaling to the adaptive immune system. In a steady state gut environment, the shaping is towards tolerogenic responses while a protective inflammatory reaction results from antigens sampled from harmful microbes. Understanding the interaction between microbiota and APCs in driving immune responses would pave way to improving human and animal health through promotion of maneuvers that maintain immunity.

Keywords: Microbiota, intestine, immunity, dendritic cells, macrophages

INTRODUCTION

The gastrointestinal tract is constantly exposed to an array of non-harmful antigens from food proteins and microbiota and harmful antigens from pathogenic organisms, mainly bacteria. These antigens are accommodated through the tightly regulated gut immune response that maintains immunological tolerance to non-harmful antigens or elicits protective immune responses against pathogenic organisms (Mann *et al.,* 2007; Amu *et al.,* 2010; Lee *et al.,* 2011). Antigen sampling, processing and subsequent presentation to cells of the immune system is done by professional antigen presenting cells (APCs) that include macrophages, dendritic cells (DCs) and to a lesser extent B cells (Batista and Harwood, 2009; Steinman, 2012). These cells are pivotal to initiating, maintaining, shaping and linking innate and adaptive immune systems in the gut (Fujimoto *et al.,* 2011). Specifically, APCs recognize enteric antigens through their Toll-like receptors (TLR) (Fujimoto *et al.,* 2011), drive regulatory responses such as differentiation, expansion, and maintenance of regulatory T cell (Treg) populations and induce immunoglobulin (Ig)A against the microbiota (Manicassamy and Pulendran, 2009).

The microbiota influences APC functions pertaining to intestinal immune homeostasis and responses to foster gut immunity. Imbalances of microbiota and the subsequent defective APC signaling lead to intestinal disorders. The cascade of events involves induction of TLR activity that activates innate immune response exhibited by sampling of gut antigens by APCs, APC-mediated differentiation of naïve T cells into effector T helper (Th) cells, alteration of the gut homeostasis and induction of diseases (Bermudez-Brito *et al.,* 2014; Janelsins *et al.,* 2014). On the contrary, during homeostasis, the microbiota drives a balance between pro- and anti-inflammatory mechanisms for immunity. This is through microbiotal-potentiated generation of pro-inflammatory Th17 cells or anti-inflammatory Treg cells expressing Foxp3 (Rivollier *et al.,* 2012; Arpaia *et al.,* 2013).

Besides influencing APC functions, the microbiota shapes the signaling of these cells to influence effector responses (Rodes *et al.,* 2013; Trapecar *et al.,* 2014). An intact normal microbiota profile shapes the signaling towards homeostasis by inducing tolerogenic responses towards harmless antigens or reactive responses against noxious antigens. A disturbed microbiota may shape the APCs signaling towards inflammatory responses that usually predispose to diseases (Abdelouhab *et al.,* 2012; Baker *et al.,* 2012). Restoring normal microbiota profile to such patients treats the

diseases due partly to re-shaping the distorted APC signaling during disease.

MACROPHAGES

Macrophages and gut tolerance

Gut macrophages are mostly located in the LP of the gut mucosa throughout the entire gastrointestinal tract (Hume *et al.,* 1984). A small population is also present in the smooth muscle layers of the gastrointestinal tract (Mikkelsen and Rumessen, 1992; Tajima *et al.,* 2012). They are involved in maintaining microbiota-host homeostasis, intestinal epithelial renewal and protective immunity. The contribution of macrophages to gut tolerance can be through their constitutive production of anti-inflammatory IL-10, generation and maintenance of Tregs, and suppression of effector Th1, Th2 and Th17. The cytokine IL-10 produced by $CX3CR1^{+}$ macrophages drives the expansion and differentiation of $FoxP3^{+}$ Treg in the intestinal mucosal LP (Hadis *et al.,* 2011). Tolerance can also be mediated through their F4/80 molecules (Lin *et al.,* 2005). Furthermore, Treg can directly target APCs to induce gut tolerance (Shevach 2009). It has been shown that constitutive surface expression of cytotoxic T lymphocyte antigen (CTLA)-4 enables the interaction with co-stimulatory molecules CD80 and CD86 on macrophages. This interaction leads to down-regulated expression of CD80 and CD86 resulting in tolerance (Onishi *et al.,* 2008; Shevach 2009). Individuals without CTLA-4 lack tolerance and develop multi-organ inflammation and premature death (Read *et al.,* 2006; Wing *et al.,* 2008).

Influence of microbiota on macrophage functioning during health and disease

The effect of microbiota to the macrophage functioning is depicted during development and differentiation of these cells as well as functioning for tolerance and active immune response. Trapecar and others (2014) observed that microbiota affects early development of macrophages. From the weaning time, the microbiota drives a process of replacing intestinal macrophages by chemokine receptor CCR2-dependent influx of $Ly6C^{hi}$ monocytes that differentiate locally into mature, anti-inflammatory macrophages. This microbiota driven process continues throughout adult life to maintain a normal intestinal macrophage pool and tolerance (Bain *et al.*, 2014).

At steady state, the microbiota maintains tolerance partly by inhibiting $CX3CR1^{hi}$ macrophages from transporting commensal and pathogenic bacteria from the intestinal lumen to MLNs. However, the trafficking of non-invasive bacteria to the MLNs is resumed during dysbiosis. This transport is in a CCR7-dependent manner and leads to induction of immune reaction mediated by both T cell responses and IgA production (Diehl *et al.,* 2013). The microbiota can also engender immune homeostasis by promoting a crosstalk between innate myeloid and lymphoid cells. This phenomenon depends on the ability of macrophages to sense microbial signals and produce IL-1β that triggers production of granulocyte macrophage colony-stimulating factor (GM-CSF) by retinoic acid-related orphan receptor $(ROR\gamma t)^{+}$ innate lymphoid cells (ILCs). Deficient production of GM-CSF alters mononuclear phagocyte effector functions leading to reduced number of Tregs and impaired tolerance (Mortha *et al.,* 2014). Such scenario predisposes to intestinal diseases like IBD.

The microbiotal shaping of macrophage production of cytokines has been observed in experiments involving specific microbiota species. *Lactobacillus reuteri, L. rhamnosus, L. plantarum, Bifidobacterium animalis, B. bifidum, B. longum,* and *B. longum* subsp. *infantis* have been demonstrated to suppress macrophage production of pro-inflammatory TNF-α and IL-1β but increase production of anti-inflammatory cytokines IL-4 and IL-10. Both macrophage-shaped responses protect against intestinal lipopolysaccharide (LPS)-induced colitis (Rodes *et al.,* 2013). Under disease conditions, the anti-inflammatory IL-10 produced by intestinal macrophages maintains the expression of Foxp3 on Treg to suppress colitis (Murai *et al.,* 2009). Furthermore, the microbiota negatively regulates macrophage production of pro-inflammatory cytokines via production of IL-10 to maintain intestinal immune homeostasis and prevent intestinal diseases (Ueda *et al.,* 2010) (Figure 1).

The shaping of macrophage signaling towards protective direction has been observed to occur during recovery of previously disrupted microbiota. For instance, small bowel resection (SBR) that disrupts colonic microbiota by decreasing the *Firmicutes*, increases the number of macrophages and their pro-inflammatory cytokines IL-1β, IL-6, IL-8, IL-18 and TNF-α in colonic mucosa leading to clinical short bowel syndrome (SBS) (Lapthorne *et al.,* 2013). A similar decrease in *Firmicutes* occurring in mice predisposes the animals to *Citrobacter rodentium* infection. This infection causes acute colitis characterized by increased colonic mucosal macrophages, pro-inflammatory macrophage inflammatory protein (MIP)-2α,

inducible nitric oxide synthase (iNOS), IFN-γ, IL-22, and TNF-α expression (Baker *et al.*, 2012).

Maneuvers that specifically enrich colonic *Firmicutes* and decrease γ-*Proteobacteria*, and those manipulating the microbiota in general suppress macrophage induced pro-inflammatory responses and protect against infectious and chemical colitides (Abdelouhab *et al.*, 2012; Baker *et al.*, 2012).

DENDRITIC CELLS

DCs are bone marrow derived APCs. Although they are present in small numbers in the intestine, they have a large influence on immune responses. For example, one DC can influence the function of 300 to 1000 T cells (Stagg *et al.*, 2003). Their main functions are acquisition of antigen and stimulation of lymphocytes.

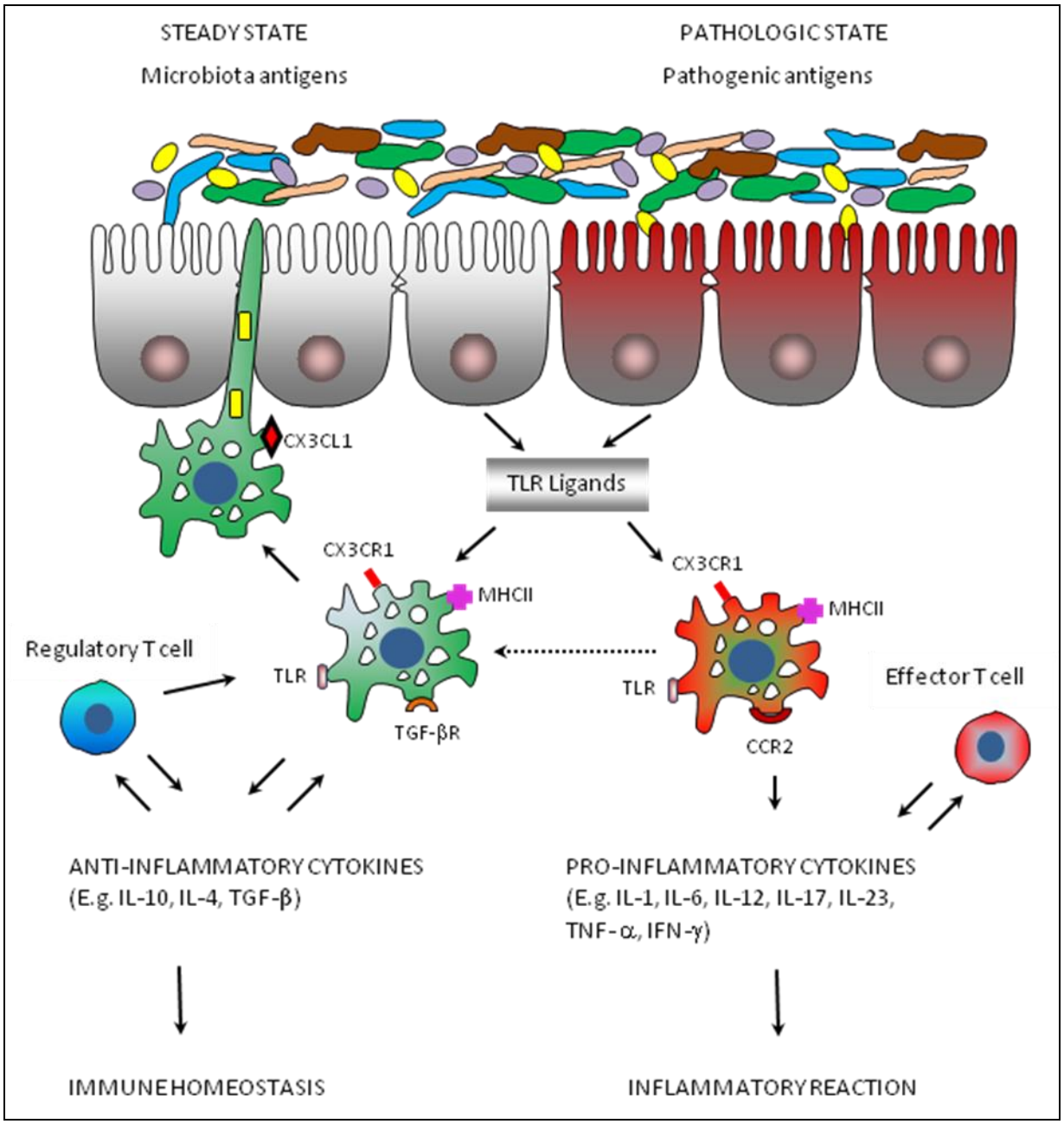

Figure 1. Microbiotal influence on macrophage functioning during health and disease. During intestinal steady state, macrophages sample microbiota antigens, produce anti-inflammatory cytokines, and polarise regulatory T cells to induce immune homeostasis. On the contrary, during infections, macrophages sample pathogenic antigens, produce pro-inflammatory cytokines and polarise effector T cells to cause inflammation. IFN, interferon; IL, interleukin; MHC, major histocompatibility class; TGF, transforming growth factor; TLR, toll-like receptor; TNF, tumour necrosis factor.

Influence of microbiota on antigen sampling and presentation by DCs

Intestinal DCs continually sample luminal antigens and present them to the immune cells in the LP. This can be via M cells of the follicle-associated epithelium of Peyer's patches (PP). The M cells take up luminal antigens and translocate them to subepithelial dome regions of PP where they deliver them to DCs. In addition, DCs found at the base of villi and those residing in lymphoid aggregates sample luminal antigens (Mowat, 2003; Iwasaki, 2007). After sampling, DCs present the antigens directly to GALT or migrate to MLNs to induce immune responses for tolerance or reaction (Niess, 2008). Microbiotal uptake by DCs limits bacterial penetration through the intestinal mucosa, neutralizes LPS, and hinders the microbiota from activating the immune system in the LP (Macpherson and Uhr, 2004). Antigen sampling and presentation by DCs can be enhanced by specific microbiota species, *Bifidobacterium* species, in IBD patients (Strisciuglio *et al.,* 2014). Similarly, food derived *Lactobacillus* species (*L. plantarum* WCFS1, *L. salivarius* UCC118, and *L. lactis* MG1363) upregulate the activity and numbers of $CD11c^+$ $MHCII^+$ DCs in the immune-sampling PP of healthy mice (Smelt *et al.,* 2013).

Influence of microbiota on DC-mediated tolerance

The microbiota, through pathogen-associated molecular patterns (PAMPs) is recognized by DC TLRs that are highly expressed by DCs. Subsequently, DCs are activated via intracellular signaling molecule TNF receptor associated factor (TRAF)6 to institute gut immune homeostasis. A loss of intestinal mucosa tolerance characterized by development of Th2 cells, eosinophilic enteritis and fibrosis occurs following DC-specific deletion of TRAF6 Han *et al.,* 2013). The microbiota can also maintain gut homeostasis by activating DCs to express low levels of MHCII that avoid damage to commensal bacterial (Bell *et al.,* 2001). The MHCII production profile changes during diseases associated with disruption of microbiota in which case DCs express high levels of MHCII (Sun *et al.,* 2013).

Influence of microbiota on DC-mediated induction of T cells and implication during disease

The influence of microbiota to DC-driven T responses has been observed in naïve T, effector Th1, Th2, and Th17 as well as Treg cells involved in gut immunity. Specific microbiota species that have been observed to activate DC to prime naïve T cells include *Lactobacillus acidophilus* that drives DCs to polarize production of Th1, *L. reuteri* and *Bifidobacterium bifidum* for generation of Th2, *L. rhamnosus* that induce production of Th17, and *Bifidobacterium breve* that facilitates production of Tr1 (Figure 2) (Jeon *et al.,* 2012; Dongarrà *et al.,* 2013). Polarization of Th1 response by DCs responding from microbiota is depicted from a combination of *L. casei* and TLR3 ligand poly(I:C) that selectively induce DCs to produce substantial amount of IL-6, IL-1β and IL-23 and enhanced levels of IL-12p70. These DCs prime T cells to generate IFN-γ-producing T-bet-positive cells (Th1 cells) without driving a Th17 response. The *L. casei* plus poly (I:C) is considered an *in vitro* model of viral intestinal infection. As such, the ability to polarise Th1 responses could have protective role in intestinal viral diseases. Besides *L. casei*, *Bifidobacterium infantis* 35624, acting through retinaldehyde dehydrogenase (RALDH) also influences DC signaling by inducing production and increase in the number of DCs which in turn, increases production of Treg $Foxp3^+$ cells but reduce that of Th1 and Th17 in the intestinal LP. The *B. infantis* 35624-induced decrease in Th1 and Th17 reduces the severity of dextran sulphate sodium (DSS)-induced colitis (Konieczna *et al.,* 2013).

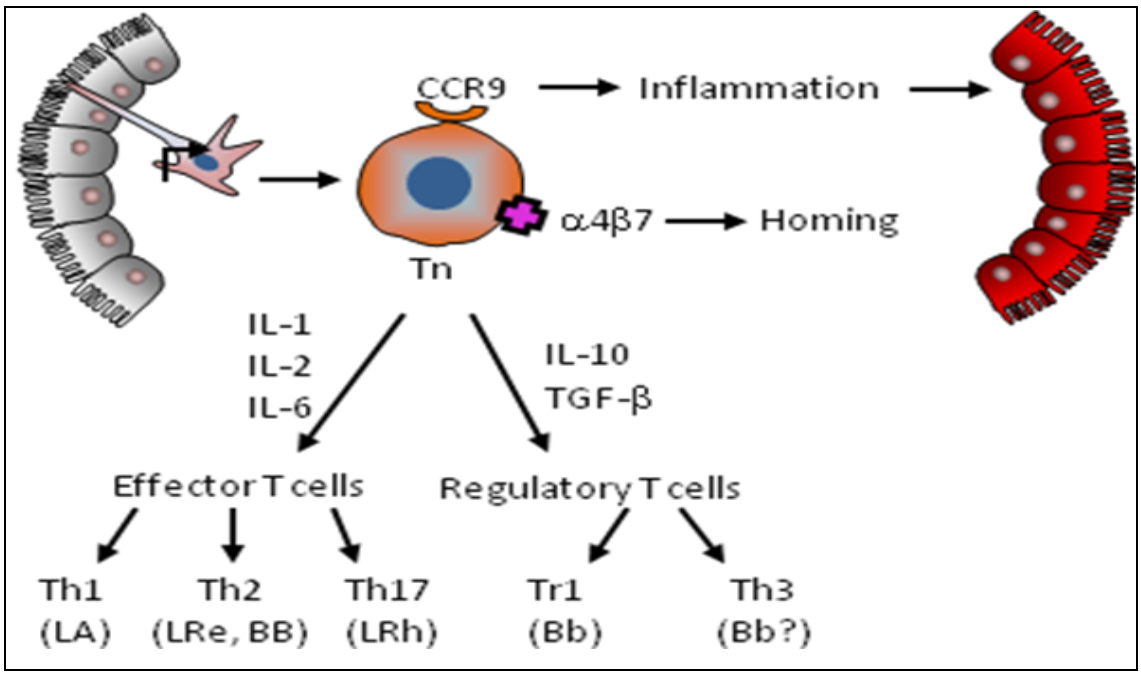

Figure 2. Dendritic cell-mediated microbiotal influence on T cells. Presentation of microbiotal antigens by dendritic cells to naïve T (Tn) cells induces expression of CCR9 and α4β7 integrin receptors on Tn cells for inflammatory and homing responses respectively. The antigen presentation also drives differentiation of effector (e.g. Th1. Th2, Th17) and regulatory (e.g. Tr1, Th3) T cells. Different microbiotal antigens may have different T cell type differentiation. Bb, *Bifidobacterium breve*; BB, *Bifidobacterium bifidum*; IL, interleukin; LA, *Lactobacillus acidophilus*; LRe, *Lactobacillus reuteri*; LRh, *Lactobacillus rhamnosus*; TGF, tumour necrosis factor.

According to Goto and others (2014), commensal segmented filamentous bacteria (SBF) sampled by DCs, primes and induces generation of Th17 cells in a MHCII dependent antigen presentation manner. The generated Th17 contributes to intestinal protection and is essential for immunity. Deficiency of MHCII promotes production of Th17 through different pathways independent from SBF while dysregulation of Th17 cells results in autoimmunity. The ability of DCs to polarize Th17 cells is affected by microbial products like bacterial LPS. DCs express acyloxyacyl hydrolase (AOAH) enzyme that inactivates microbiota LPS to polarize and generate Th17 effector cells (Janelsins *et al.*, 2014). Another way by which DCs can shape effector responses is by expressing myeloid C-type lectin receptors (CLRs) that enable them bind to glycan structures present on self or foreign antigens. Clinically, the significance of CLRs stems from their involvement in IBD pathogenesis. Both the macrophage-restricted C-type lectin (MCL) and the DC immunoreceptor (DCIR) bind to intestinal microbiota to modulate production of pro-inflammatory cytokines by these cells and impact subsequent T cell responses. MCL-/- as well as DCIR-/- mice exhibit an increased severity of colitis compared to wild-type counterparts indicating a role for MCL and DCIR in the regulation of intestinal immunity (Hütter *et al.*, 2014)

Effect on Treg

Mucosal DCs express high levels of TLR5 protein in a steady state gut. This expression is downregulated following treatment with various bacterial ligands. The DC signaling through TLR5 restrains Treg cell generation whereas mice lacking TLR signaling ($TLR5^{-/-}$) have increased $Foxp3^{+}$ Treg cells in the intestinal LP (Feng *et al.*, 2012). The microbiota also influences DC-mediated Treg cell functioning via its dietary fiber fermentation products, butyrate and niacin. These chemicals promote the anti-inflammatory activity of DCs by stimulating them to induce differentiation of Treg cells and IL-10-producing T cells through their receptor GPR109A (Singh *et al.*, 2014). Furthermore, the microbiota maintains balance between pro- and anti-inflammatory mechanisms through the fermentation products by inhibiting histone deacetylase (HDAC) (Arpaia *et al.*, 2013) to facilitate extrathymic differentiation of Treg cells in an intronic enhancer CNS1 (conserved non-coding sequence 1, essential for extrathymic Treg-cell differentiation)-dependent manner. A crosstalk between intestinal DCs and *Bifidobacterium breve*

induces development of Tr1. The crosstalk starts with *B. breve* activating intestinal $CD103^+$ DCs to produce IL-10 and IL-27. The produced cytokines drive generation of Tr1 which subsequently produce IL-10 as a potent anti-inflammatory mediator, and express cMaf, IL-21, and Ahr (aryl hydrocarbon receptor) in the large intestine. $CD103^+$ DCs from IL-$10^{-/-}$, Tlr$2^{-/-}$, and Myd$88^{-/-}$ mice exhibit defective *B. breve*-induced Tr1 cell development and may predispose to intestinal diseases. IL-10 (Jeon *et al.*, 2012).

CONCLUSION

The intestinal microbiota influences macrophage and DC development, differentiation and functioning for tolerance and active immune response geared at maintaining immune homeostasis. In the absence of microbiota or during dysbiosis, macrophages and DCs drive inflammatory responses leading to diseases that can be deleterious to the host. The microbiota-mediated macrophage and DC protective responses are manifested by the ability of the commensals to shape the effector products of these cells. As a result, the microbiota prevents intestinal diseases that would otherwise develop from activated macrophages and DCs. Insight in this understanding could pave way to establishing medicaments against intestinal diseases by employing the microbiota.

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