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Interactions among dendritic cells, macrophages, and epithelial cells in the gut: implications for immune tolerance

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The intestine is described as an immune privileged site where immunoregulatory mechanisms simultaneously defend against pathogens, yet preserve tissue homeostasis to avoid immune-mediated pathology in response to environmental challenges. Additionally, tolerance to ingested antigens promotes the development of systemic unresponsiveness towards the same antigens. It is increasingly clear that this tolerance is a complex process that derives from the coordinated action of both canonical immune and non-immune cells at mucosal sites, including dendritic cells, macrophages and epithelial cells. Recent evidence suggests that dysregulation in gut-induced tolerance and commensal bacterial handling affects both local and systemic compartments and contributes to autoimmune disease. Understanding how tolerance is achieved at mucosal sites may thus be exploited to re-establish tissue homeostasis.

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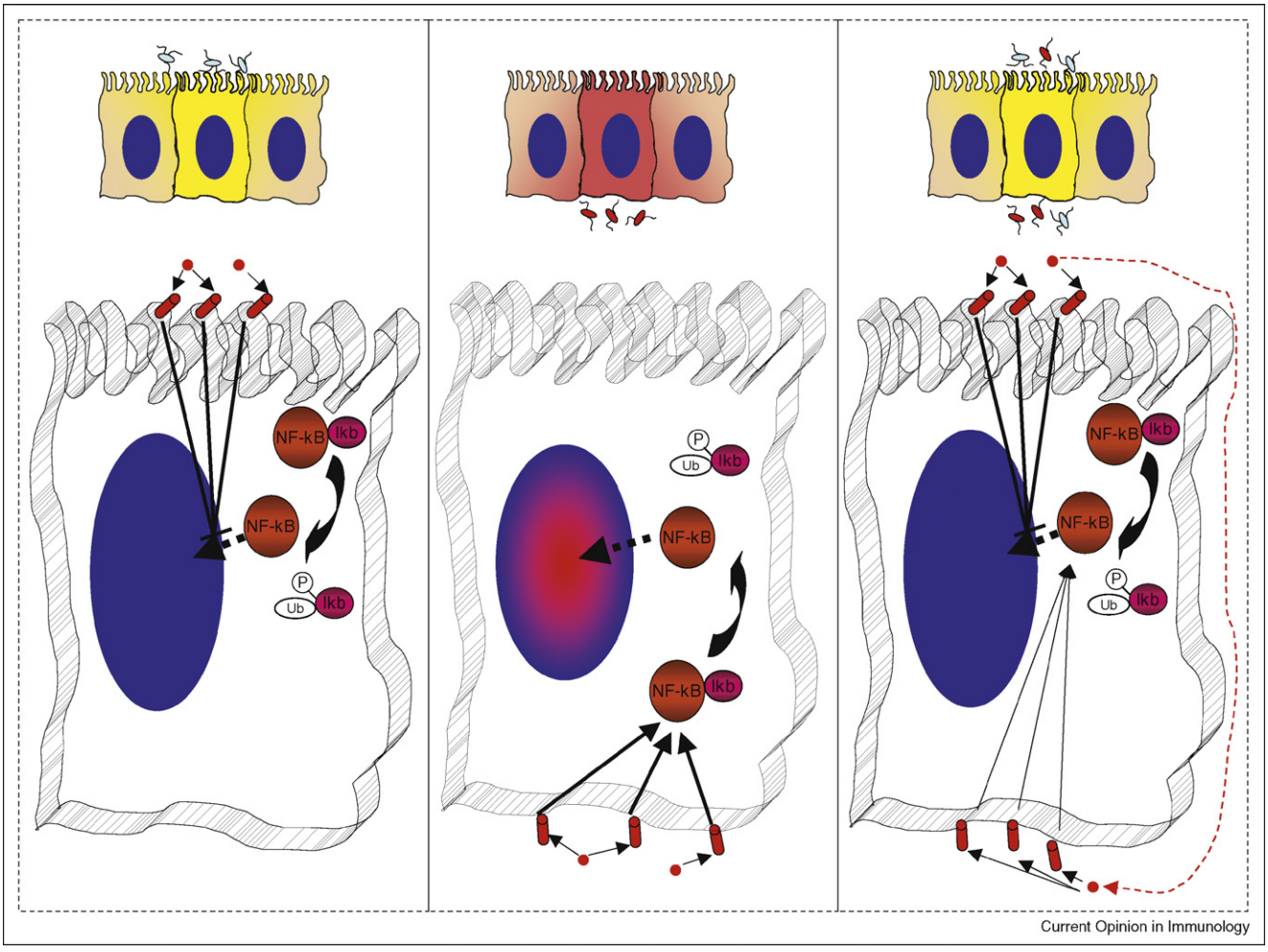
Introduction

The digestive apparatus is likely the first discrete organ to develop. Nutrients are forced into a 'lumen' to favor absorption. The luminal content is separated from the epithelial cell membrane by a mucous layer [1]. However, this mucous barrier provides imperfect protection against the diversity of bacteria residing in the same luminal content. Thus rich arrays of immune cells are scattered both between epithelial cells (ECs) and deeper in the lamina propria (LP). These cells participate in the discrimination between food and pathogenic organisms, in the induction of IgA response, and in the inhibition of inflammatory responses to the continuous challenge of intestinal bacteria. Furthermore, commensal bacteria normally considered as safe may become pathogenic and contribute malabsorption, inflammatory bowel disease, and colorectal cancer [2]. How can the intestinal immune system handle this load of information is not completely understood, but it is becoming clear that the interaction between luminal bacteria, epithelial cells, and immune cells is crucial to preserve intestinal homeostasis. In this review, we will focus on three important cell types: epithelial cells, dendritic cells, and macrophages that via a coordinated action help keeping peace at mucosal surfaces but are ready to fight, when needed.

Epithelial cells: not simply a barrier

Most pathogen recognition receptors, including Toll-like receptors (TLRs), were thought to be exclusively expressed either intracellularly or on the basolateral membrane of epithelial cells, leaving the apical membrane unable to interact with bacteria. Subsequently, several findings suggested that this may not be the case. Rather it has been shown that the binding of TLR ligands can occur both at apical and at basolateral membranes, giving different outcomes [3]. A typical example is TLR9, the receptor for unmethylated CpG-containing bacterial DNA. Unlike myeloid cells, TLR9 is expressed on the cell surface of epithelial cells, both apically and basolaterally, *in vitro* and *in vivo* [4•]. TLR9 engagement on the apical surface of epithelial cells induces partial activation of NF-κB, a master regulator of the inflammatory response, without stimulating the release of proinflammatory cytokines (Figure 1). By contrast, basolateral engagement of TLR9 leads to a robust inflammatory response, which can be inhibited by preincubation with apical CpG [4•]. This suggests that the apical engagement of TLRs is protective. In agreement, mice lacking TLRs or TLR signaling are more prone to develop experimental colitis [5–7]. These experiments also indicate that partial NF-κB activation is protective rather than inflammatory. In agreement, mice with intestinal epithelial cell deletion of different subunits of IKK, kinases required for NF-κB activation, display severe chronic intestinal inflammation [8••] or are more susceptible to infections [9••]. Finally, commensal bacteria were found to interact with epithelial cells and induce inhibitory signals in ECs [10–12]. Incubation of epithelial cells with noninvasive strains of *Salmonella enteriditis* or *Bacteroides thetaiotaomicron* leads to the reduction of NF-κB activation and translocation to the nucleus [11,13] or premature egress of RelA subunit from the nucleus [10], respectively. Interestingly, reactive oxygen species (ROS) induced by commensals are responsible for the inactivation of the catalytic cysteine residue of Ubc12

Figure 1



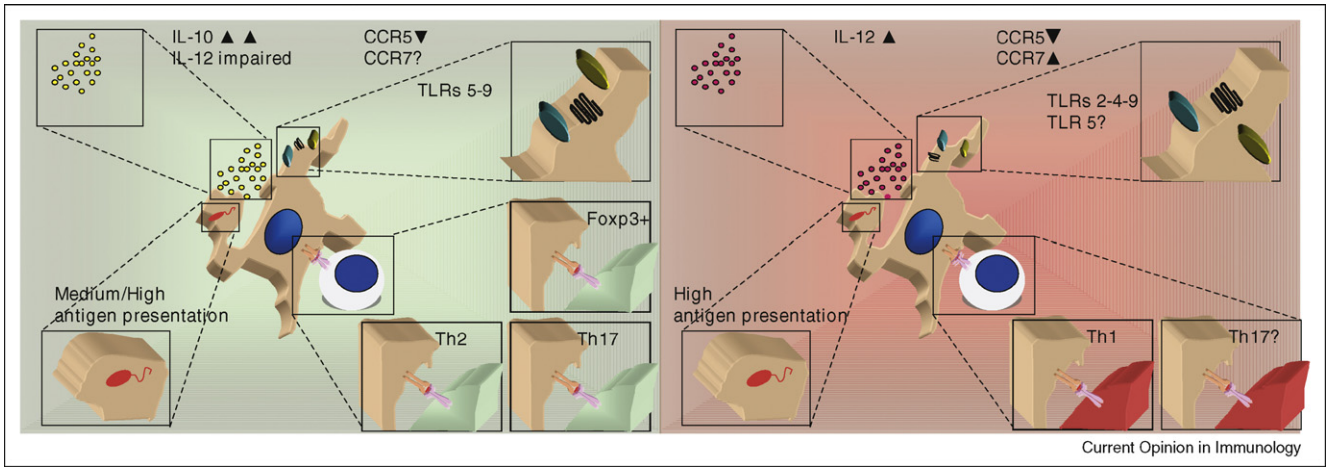
Epithelial cells are not simply a barrier. Three different conditions of epithelial cell (EC) activation are depicted. Unlike myeloid cells, ECs express TLR9 on their cell surface (red cylinders) and respond to bacterial DNA (red circles). During steady state (left panel), ECs sense the presence of commensal bacteria (light blue) and are 'set' to a noninflammatory mode. There is partial activation of NF-κB that does not result in nuclear translocation. During infection (middle panel), invasive bacteria (red) reach the basolateral membrane. Here TLR9 triggering leads to full NF-κB activation and the release of inflammatory mediators. As apical engagement of TLR9 inhibits full activation of NF-κB from basolateral TLR9 engagement, it is not clear whether ECs under steady state are inflammatory at all (right panel). We hypothesize that ECs located at the tip of the villi are more exposed to bacteria resulting in tolerization, while those located closer to the crypts are in a more 'sterile' environment because of the release of antimicrobial peptides by Paneth cells and retain their inflammatory potential. Invasive bacteria that reach the deeper cells may initiate an inflammatory cascade. Alternatively, *in vivo*, invasive bacteria could transform the tolerogenic phenotype of ECs, perhaps as a consequence of binding to basolaterally expressed TLRs. These possibilities remain to be explored.

resulting in impaired I-κB ubiquitination and NF-κB activation [14•]. Altogether, these data suggest that epithelial cells are not simply a barrier to intestinal bacteria [15]. It is unclear though if bacteria contact EC surface receptors somehow bypassing the large mucus layer covering the epithelial membrane, or if TLR ligands released during bacterial degradation deliver anti-inflammatory signals. Nonetheless, it appears that as long as the bacteria remain in the lumen they are sensed as innocuous and the result is not ignorance, but induction of a protective tolerogenic response.

Epithelial cells during infection

In general, pathogenic bacteria are invasive and reach intracellular compartments as well as the basolateral membrane to initiate a proinflammatory signaling cascade [12]. These generate 'alarm' signals to underlying immune cells and recruit circulating leukocytes. However, as mentioned above, if epithelial cells are tolerized by the presence of commensals or bacterial products at their apical surface, how do they respond to invasive bacteria? One possibility is that epithelial cells located at the tip of the villi are more exposed to bacteria resulting in tolerization, while those

Figure 2



Specialized functions of gut DCs. Resident LP-DCs (left panel) have been shown to be impaired in their ability to release inflammatory cytokines like IL-12, but produce more IL-10. DC precursors ($CD11b^{lo}CD11c^{lo}$) express TLRs but are not responsive to TLR ligation, while differentiated DCs express only TLR5 and TLR9, and respond to flagellin. Depending on the analyzed subset, DCs can either induce Th2, Foxp3+ T_{regs} or Th17 T cell development. The location and precise phenotype that distinguishes these different subsets remains to be understood. During inflammation (right panel) it is not yet clear whether resident DCs lose their noninflammatory properties, or whether fresh nonconditioned DCs are recruited from blood as differentiated cells or monocytic precursors. The immunostimulatory environment drives their full activation. DCs release IL-12 and drive the development of Th1 T cells. It is not yet clear whether activated cells are better inducers of Th17 T cells.

located closer to the crypts are in a more 'sterile' environment because of the release of antimicrobial peptides by Paneth cells and retain their inflammatory potential. Invasive bacteria that reach the deeper cells may initiate an inflammatory cascade. Alternatively, *in vivo*, invasive bacteria could transform the tolerogenic phenotype of ECs, perhaps as a consequence of binding to basolaterally expressed TLRs. These possibilities need to be explored, but it has been shown that TLR3 ligation induces severe mucosal injury, suggesting that some apically applied TLR ligands may be inflammatory [16].

Immune cells in the gut

Peyer's patches (PPs) that represent the major gut-associated lymphoid tissue, differ from other lymphoid organs. They contain a higher proportion of B cells versus T cells [17] and are rich in cytokines with IgA-inducing functions, including transforming growth factor (TGF)-β [18]. Therefore, PPs are considered a site conducive to antigen-specific IgA induction, as in response to *Salmonella* [19]. By contrast, PPs are dispensable for the generation of T-cell-independent IgA responses that seem to occur directly in the LP [20]. Dendritic cells (DCs) are professional antigen-presenting cells characterized by the ability to migrate to mesenteric lymph nodes (MLNs) both during the steady state and during infection [21,22]. In the PPs, DCs are located in the subepithelial dome, just below the follicle-associated epithelium, and migrate to T and B cell areas after stimulation.

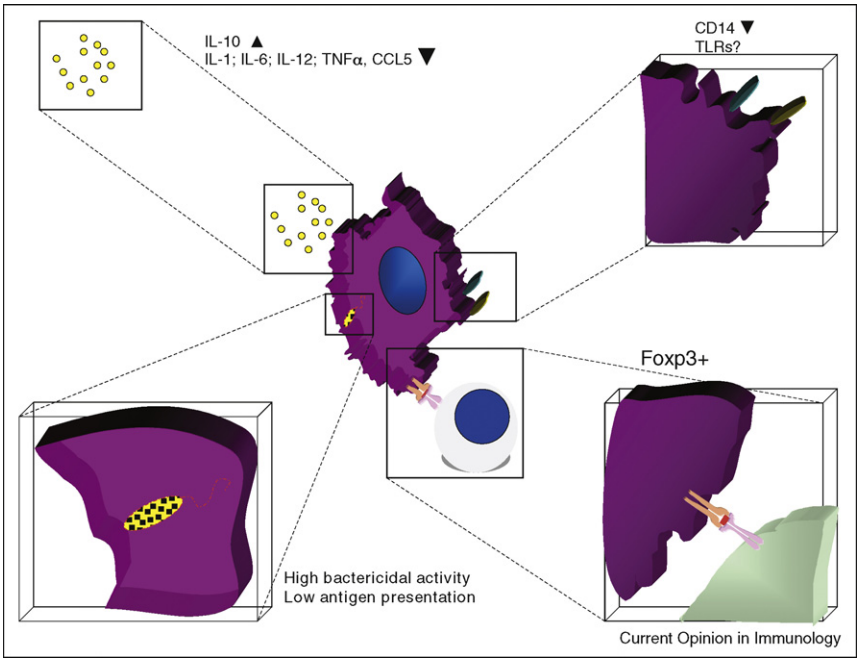
The vast majority of LP is colonized by immune cells. The LP is home to a large number of T regulatory cells, invariant T cells, natural killer cells and noncanonical CD8αα intraepithelial T cells [23,24]. All these cells seem to play a role in protecting against commensal-driven inflammation. This allows a dynamic control of the immune response depending on the external environment. DCs are not only deeply infiltrated into villi but are also in close contact with the intestinal epithelium [25,26]. Epithelial TLR engagement provokes DCs to extend processes into the intestinal lumen for direct bacterial uptake [27•].

Gut dendritic cells

It is becoming clear that there is neither systemic immunity nor tolerance to commensals [28]. Commensal-laden DCs are retained in MLNs and do not reach the spleen, thus impeding the induction of commensal-specific systemic IgG responses [29]. As anticipated, a key requirement of the intestinal immune system is to generate oral tolerance to food antigen, while preserving immunity to pathogens. A major site for this process is MLN [30]. Within the MLN, DCs are potent mediators of tolerogenic and inflammatory phenomena. A population of DCs characterized by the expression of CD103 has been described to have the ability to induce the de novo differentiation of naïve T cells into CD4+CD25+Foxp3+ T cells, via a TGF-β and retinoic acid (RA)-dependent mechanism [31••]. This population is presumably coming from the LP [32••,33].

Also DCs from other gut district display specialized functions [34,35] (Figure 2). LP-DCs express only TLR5 and TLR9 and induce IgA class switching and

Figure 3



Specialized functions of gut macrophages. Macrophages isolated from the gut are impaired in the release of proinflammatory mediators in response to bacteria, but retain their bactericidal activity. Intestinal macrophages also have reduced CD14 surface expression and selective TLR expression. Macrophages can also release TGF-β and retinoic acid and drive the development of Foxp3+ T_{regs}. Recently, a population of cells with intermediate phenotype between macrophages and DCs has also been described.

Th17 cells when challenged, however the clinical consequence of this response is unclear [36•,37]. A subpopulation of LP-DCs ($CD11b^{lo}CD11c^{lo}$) although expressing high levels of TLRs fails to produce proinflammatory cytokines when challenged [38•]. This explains why the capacity of DCs to extend dendrites across the intestinal lumen depends on TLR engagement on epithelial cells and not on DCs [27•].

Finally, PP-DCs can impart gut-homing properties to T cells, B cells, and T_{regs} via a RA-dependent mechanism [33,39,40,41•,42]. PP-DCs can also drive IgA class switching via a RA-dependent mechanism [41•,43].

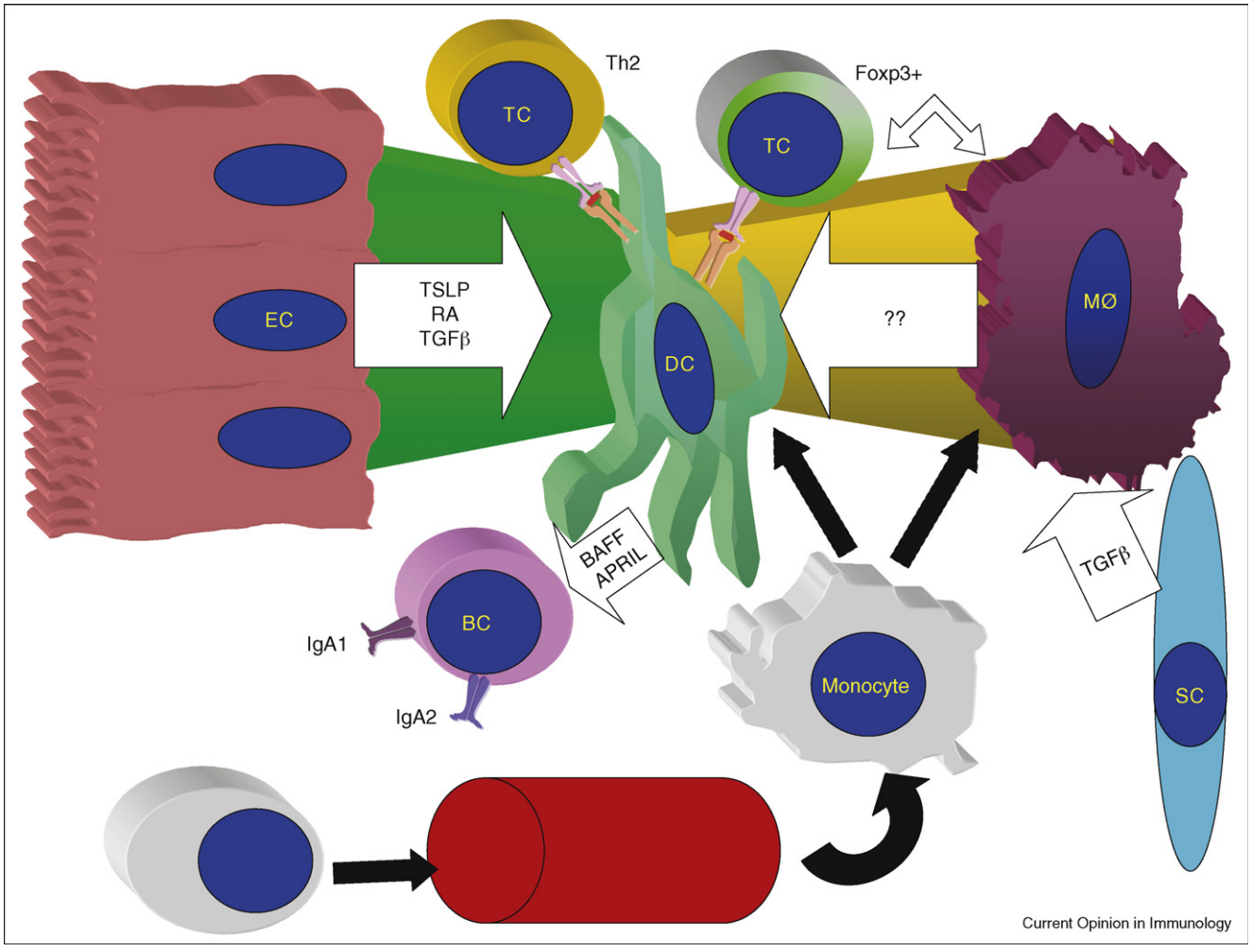
Macrophages

Macrophages display specialized functions in the gut in a fashion similar to DCs (Figure 3). Macrophages also display selective expression of TLRs, and are unresponsive to TLR ligation in terms of proinflammatory cytokine production, but retain fully competent bactericidal activity [44]. Recently, a new function for intestinal macrophages has been proposed. Like CD103+ DCs, they have been shown to support the induction of CD4+CD25+Foxp3+ T_{reg} cells [45•]. The function of tolerogenic macrophages is not clear, as it is not known whether they can migrate to MLN. However, cell migration to MLN has been shown to be required for the induction of oral tolerance [30]. One hypothesis is that macrophages support locally the maintenance of a T_{reg} phenotype in an environment continuously exposed to bacteria and bacterial products. Macrophages have also been described to tune DC function by inhibiting their potential to drive Th17 T cells [45•]. A unique CD14+ inflammatory cell with intermediate phenotype between DCs and macrophages has also been recently described in the human gut [46]. This cell type could represent a recently recruited monocyte that is undergoing a transition to either macrophages or DCs.

Epithelial cell–dendritic cell–macrophage interactions

The previous observations indicate that intestinal epithelial cells, DCs, and macrophages are profoundly non-inflammatory. This state is characterized by the inability of these cell types to initiate inflammatory responses to intestinal bacteria. However, these intestinal immune cells are not paralyzed and bacteria are not ignored. For instance, commensal reactive IgAs are found in mice reared under specific pathogen-free conditions [47]. These IgAs could be either specific or polyreactive and serve as anchors, which may either prevent internalization or promote a controlled entrance of bacteria via M cells [48]. Gut DCs also have the peculiar capacity to induce T_{reg} cell development; however, there is no systemic

Figure 4



Epithelial cell–dendritic cell-macrophage interactions. What drives the development of the specialized functions of gut DCs? Human intestinal epithelial cells release TSLP, TGF-β, and RA that drive the development of tolerogenic DCs able to induce Th2 and Foxp3+ T_{regs}. TSLP is also shown to favor the release of BAFF and APRIL by conditioned DCs and supports IgA class switching of B cells directly in the LP or the generation of protease-resistant IgA2 after sequential class switching from IgA1. Additionally, macrophages appear to tune the inflammatory potential of DCs, but the factors involved are not yet known. On the other hand, macrophages are shaped by stromal cell derived TGF-β and induce Foxp3+ T_{regs} in a fashion similar to DCs. DCs and macrophages derive from circulating monocytes that could undergo 'mucosal' conditioning during their terminal differentiation into the tissue. In agreement, T_{regs} can steer the differentiation of monocytes into regulatory macrophages. Hence, the concerted action of immune cells, stromal cells, and epithelial cells is required to keep peace at intestinal surfaces.

tolerance to commensal bacteria. One might argue that these T_{regs} are retained within the gut without spreading to systemic districts, as would be hypothesized by the expression of gut-homing receptors [42]. Alternatively, T_{regs} specific for commensal bacteria are not generated. These issues remain to be elucidated (Figure 4).

What drives the development of the specialized functions of gut DCs? We hypothesize that the local microenvironment, and in particular intestinal epithelial cells, plays a role in shaping DC function. The simple incubation of human monocyte derived DCs with epithelial cell supernatant is sufficient to induce a 'mucosal' phenotype to DCs [49]. Epithelial cell conditioned DCs are unable to release inflammatory cytokines and to drive Th1 T cells. In humans, thymic stromal lymphopoietin (TSLP) was identified as one of the conditioning factors released by epithelial cells [49]. Interestingly, TSLP is also shown to favor the release of BAFF and APRIL by conditioned DCs and supports IgA class switching of B cells directly in the LP [50], or the generation of protease-resistant IgA2 after sequential class switching from IgA1 [51••]. TSLP is not the only factor released by epithelial cells that confers mucosal DC properties. Both mouse and human epithelial cells also release RA and TGF-β. These factors are required to drive the development of CD103+ tolerogenic

DCs (Iliev *et al*., unpublished). Epithelial cells are not the only cells able to modulate DC function. As mentioned above, macrophages also appear to tune the inflammatory potential of DCs [45•]. Further, macrophages are shaped by stromal cell-derived TGF-β [44] and by T_{regs} that direct their differentiation from monocytes into anti-inflammatory cells [52]. Altogether these findings suggest that the reciprocal action of immune cells, stromal cells, and epithelial cells is required to keep peace at intestinal surfaces.

Conclusions

In conclusion, intestinal resident immune cells have been shown to display specialized functions aimed at maintaining immune homeostasis. These cells interact and control the reciprocal function of the others. Epithelial cells, in particular, both at steady state and during infection sense the external world and relay this information to underlying immune cells. These interactions can lead either to tolerogenic or to inflammatory immune responses depending on the local status of the intestine. As our knowledge of these players expands, we may understand the contribution of other components like intraepithelial lymphocytes, mast cells, stromal cells, and glial cells in the overall immune homeostasis.

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