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Contextual functions of antigen-presenting cells in the gastrointestinal tract

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Summary

The immune system of the gastrointestinal tract must be tightly regulated to limit pathologic responses towards innocuous antigens while simultaneously allowing for rapid development of effector responses against invading pathogens. Highly specialized antigen-presenting cell (APC) subsets present in the gut play a dominant role in balancing these seemingly disparate functions. In this review, we discuss new findings associated with the function of gut APCs and particularly the contextual role of these cells in both establishing tolerance to orally acquired antigens in the steady state and regulating acute inflammation during infection.

Keywords

dendritic cell; macrophage; monocyte; antigen-presenting cell; Treg; mucosal; commensal; pathogen; immune regulation

Introduction

The mammalian intestine is a complex environment that is under constant antigenic exposure from the high density of commensal flora and food antigens. To maintain homeostasis in light of these constitutive challenges, the gut has evolved many interrelated strategies to prevent aberrant inflammation. One critical mechanism is the presence of a distinct barrier that is primarily composed of mucus embedded with antimicrobial peptides and secreted immunoglobulin A (IgA) antibodies, which physically separates the intestinal microflora from the epithelium and underlying immune cells, while allowing food diffusion (1, 2). Due to their capacity to sense and respond to signals from their environment, gut

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resident macrophages and dendritic cells (DCs) are central players in maintenance of gut homeostasis. These specialized antigen-presenting cells (APCs), in addition to monocytes recruited from the blood during inflammation, form the mononuclear phagocyte system of the gastrointestinal tract. Recent work from our group and others has highlighted the complexity and versatility of the intestinal APC network and more particularly how the function and development of these cells are conditioned by local cues. In this review, we discuss the contextual role of gut APCs in maintaining tolerance in steady state, inducing effector responses, and limiting overt inflammation.

The antigen-presenting cell network of the gastrointestinal tract

The mucosal APC compartment of the gut is comprised of an elaborate network of DCs and macrophages within the *lamina propria* and throughout the gut-associated lymphoid tissue (GALT) (3). There are two major intestinal DC subsets, both of which express the marker CD103 but which can be distinguished by their expression of CD11b into CD103⁺CD11b⁺ and CD103⁺CD11b⁻ populations (Fig. 1). Each of these DC subsets develop from the conventional DC precursor (pre-cDC) in a manner dependent on Flt3 signaling (4, 5), but their identity as two distinct lineages is evident by their dependence on differing transcriptional programs and secreted factors for development (4). Indeed, CD103⁺CD11b⁺ DCs require the hematopoietic growth factor colony-stimulating factor-2 (CSF-2) for their survival as well as Notch2 for their differentiation, while CD103⁺CD11b⁻ DCs are dependent on interferon regulatory factor 8 (IRF8), inhibitor of DNA binding 2 (Id2), and basic leucine zipper transcription factor, ATF-like 3 (Batf3) for their differentiation (5–9). These two DC subsets also differ in terms of their location and function. The CD103⁺CD11b⁺ DC population are mucosal non-lymphoid tissue DC highly represented in the small intestine *lamina propria* that have the capacity to rapidly migrate to the mesenteric lymph nodes (mesLNs) upon antigen uptake (10–12). By contrast, the CD103⁺CD11b⁻ population is a minor subset of DCs enriched in Peyer's patches and isolated lymphoid follicles and are believed to be equivalent to lymphoid tissue-resident CD8α⁺ DCs (10, 12, 13). Whether these cells are also found in the *lamina propria* remains a matter of controversy.

A third population of intestinal APCs lacking CD103 but expressing the chemokine receptor CX3CR1 is generally accepted to be comprised of macrophages resident in the *lamina propria* (Fig. 1). Supporting this classification are the findings that these CD103⁻CD11b⁺CX3CR1⁺ cells do not migrate to the draining lymph nodes under steady state conditions (10, 14) and that their transcriptional profile resembles that of macrophages found in other tissues (15). Intestinal macrophages, defined as CD103⁻CD11b⁺CX3CR1⁺, are derived from Ly6C^{hi} blood monocytes and depend on the hematopoietic factor CSF-1 for their differentiation, proliferation, and survival (10, 12). Circulating monocytes give rise to *lamina propria* macrophages in steady state conditions. In contrast, during gut inflammation, monocytes are rapidly recruited but acquire different effector functions from those of resident macrophages and comprise a distinct APC population (16–21). Thus, intestinal APCs vary in differentiation, localization, and function and work in concert to regulate gut homeostasis.

Induction and expansion of the mucosal Foxp3⁺ Treg network

In the steady state, the mucosal immune system must be tightly regulated to prevent inflammatory responses occurring towards antigens derived from the food or commensal microbiota. Developing immune reactivity against such non-pathogenic elements constitutively present in the gut can result in severe tissue damage, as in the context of inflammatory bowel diseases (IBD). One component of the gastrointestinal immune system crucial to the establishment of a tolerogenic environment is the Forkhead box protein 3 (Foxp3)⁺ regulatory T-cell (Treg) network. This population of cells is essential for the maintenance of intestinal homeostasis, and disruption of the induction or homeostasis of these cells results in loss of oral tolerance and development of aberrant effector responses in the gut (22–24).

Although Foxp3⁺ Treg can arise as differentiated cells in the thymus, under appropriate conditions and in the proper microenvironment these cells can also develop extrathymically (25–27). Indeed, we and other groups have noted the remarkable capacity of the GALT as a site for Foxp3⁺ Treg induction (22, 28–31). The importance of this regulatory mechanism is highlighted by recent findings that a proportion of these induced Tregs are specific for antigens derived from the commensal microbiota (32). In recent years, a rapidly growing body of literature has described the superior capacity of the highly specialized APCs in the gut to support Foxp3⁺ Treg induction and expansion.

Studies by our laboratory and others demonstrated a tissue specific specialization for GALT APCs in induction of Foxp3⁺ Treg specific for oral antigens (29–31). Notably, CD103⁺ DCs isolated from the small intestinal *lamina propria* or mesLNs have a greater capacity than other DC populations to induce the expression of Foxp3 in naive T cells (29–31) (Fig. 1). This process is associated at least in part with the capability of mucosal DCs to generate bioactive transforming growth factor- β (TGF- β) from its latent form. Activation of latent TGF- β is most likely mediated by the integrin pair $\alpha\text{v}\beta 8$, which is expressed on gastrointestinal CD103⁺ DCs (29, 33). Supporting this proposed mechanism of TGF- β activation, mice lacking $\alpha\text{v}\beta 8$ expression on myeloid cells or DCs have reduced colonic Foxp3⁺ T cells and develop spontaneous colitis (34, 35).

In concert with their ability to locally generate active TGF- β , CD103⁺ DCs can also induce Foxp3⁺ Treg as a result of their ability to generate the vitamin A metabolite retinoic acid (RA). Generation of RA from dietary or stored retinol is dependent upon the expression of a number of specific enzymes (36). DCs isolated from the GALT express high levels of retinaldehyde dehydrogenases (RALDH), *Aldh1a1* and *Aldh1a2*, critical enzymes in this pathway not observed in other DC subsets (29, 37, 38). We found that RA derived from CD103⁺ DCs acts on CD4⁺ T cells specifically through RAR α to augment Foxp3 induction (30, 39).

The capacity of CD103⁺ DCs to produce RA not only plays an important role in the generation of Foxp3⁺ Tregs but also determines lymphocyte homing potential. Indeed, via their capacity to release RA, DCs isolated from the small intestine *lamina propria*, Peyer's patches, and mesLNs, are able to support the expression of the gut homing receptors integrin

$\alpha 4\beta 7$ and CCR9 on both Treg and effector T (Teff) cells (40–44). The integrin $\alpha 4\beta 7$ -integrin binds mucosal vascular addressin cell-adhesion molecule 1 (MADCAM 1), expressed by the intestinal endothelium, while CCR9 induces chemotaxis in response to CCL25, which is produced by intestinal epithelial cells.

Alongside CD103⁺ DCs, intestinal CX3CR1^{hi} macrophages also display specialized functions to support the Treg network (Fig. 1). Although a number of studies have suggested that these cells can also induce Tregs (45), their failure to migrate to the mesLN at steady state implies that this may not be their dominant mechanism of action. Rather, mounting evidence supports a role for CX3CR1^{hi} macrophages in expanding the Foxp3⁺ Treg compartment in the tissue as a result of their capacity to produce the anti-inflammatory cytokine IL-10 (28, 46). This cytokine may not only act by expanding/maintaining the Foxp3⁺ Treg network but also feed back directly on APCs to limit their capacity to produce inflammatory cytokines (47, 48).

Given the critical roles of RA synthesis and TGF- β activation by intestinal DCs in promoting Treg induction and establishment of oral tolerance in the GI tract, there is great interest in identifying the signals that drive the acquisition of these unique properties for therapeutic purposes. APCs are exquisite sensors of their environment, and in the intestine local cues such as RA can direct the differentiation and function of their precursors. Indeed, we have reported that dietary vitamin A promotes the differentiation and accumulation of CD103⁺CD11b⁺ intestinal DCs (49). RA, therefore, may play a critical role in shaping the homeostasis and function of these cells by stimulating its own synthesis.

Signals conditioning APCs can derive from the microbiota themselves. Indeed, we found that specific DNA motifs from commensal microbes can sustain the capacity of gut APCs to promote intestinal Treg generation even in the context of inflammation (50). A recent study also implicates the intestinal mucus as a factor, contributing to the functional specification of mucosal DCs. Sampling of MUC2, a mucus glycoprotein produced by intestinal goblet cells, by *lamina propria* DCs promotes their capacity to produce TGF- β and RALDH (51). This effect was mediated via β -catenin signaling in DCs, which has been shown to drive regulatory function in these cells and support their capacity to induce Tregs (52).

In light of these observations, we propose a model in which intestinal DCs reinforce their own differentiation and regulatory function under steady state conditions. In such a scenario, circulating pre-cDCs are recruited to the *lamina propria* of the GI tract where gut-tropic signals such as RA, TGF- β , defined microbial ligands, and MUC2 condition the differentiation of these cells to promote the production of RA and active TGF- β . These signals then function in an autocrine and paracrine manner to reinforce the establishment of a CD103⁺ DC subset specialized in Treg induction by a feed-forward mechanism.

Pathogen manipulation of gastrointestinal antigen-presenting cell function

For a microbe to persist for the long-term in a host, it must balance sustaining its life cycle with simultaneously preventing or delaying host mortality. Microbes have therefore developed multiple strategies targeting the innate and adaptive arms of the immune system to achieve this balance. This is well known to be the case for ‘commensal’ organisms that

make their homes in the gastrointestinal tract or skin, but it is also true for ‘pathogenic’ organisms that establish chronic infections. The immune system is a component of their ecological niche, and they have co-evolved with their host to manipulate its function to support an immune response that is tailored to ensure their continued survival. Given their highly plastic nature, it is not surprising to learn that APCs are frequently a target of this manipulation, with the aim of supporting initial microbial invasion or the long-term establishment of an immunologically hyporesponsive state towards the invader. As discussed in this review, the cell types responsible for balancing regulatory and effector responses over the course of an infection are in constant flux, and therefore, a suitable balance between these responses is dependent upon temporally targeting different layers of the regulatory network. Indeed, microbes have been shown to hijack the regulatory capacity of multiple cell types to support their own survival or dissemination (44, 53).

Foxp3⁺ Treg induction is thought to be particularly important to allow for the development of an immunologically hyporesponsive state following the re-establishment of homeostasis during helminth infection. In this context, an immune competent host can be infected for many decades with little development of protective immunity. It is apparent from both human and murine studies that augmentation of the Foxp3⁺ Treg compartment is a common feature in a number of different helminth infections (54). Regulatory T cells are critical for favoring persistence (55) and limiting overt immunopathology (56) during helminth infection. Additionally, helminth infection can support the induction of Foxp3⁺ Treg towards oral antigens (57). Manipulating Foxp3⁺ Treg conversion, therefore, is likely a major mechanism to support helminth survival and transmission.

The release of products that modulate the host’s immune response is thought to be a tactic used by which helminths to manipulate the balance between regulatory and effector responses (58). The action of complex mixtures of these products, termed excretory/secretory products (ES), as well as specific molecules isolated from them has been reported to modulate DC function to support Foxp3⁺ Treg induction. Most notably, treatment of bone marrow-derived DCs with ES from two gut-resident helminths, *Heligmosomoides polygyrus bakeri* and *Nippostrongylus brasiliensis*, has profound effects on DC responses to microbial products (59, 60). DCs treated with helminth products take on a modified state of responsiveness that is characterized by a failure to upregulate costimulatory molecules and produce pro-inflammatory cytokines including IL-6 and IL-12 (60). Manipulation of intestinal DC function may be a powerful mechanism to bias towards Foxp3⁺ Treg induction in the context of an ongoing immune response. This has been suggested by studies in *Schistosoma mansoni* – a liver dwelling helminth where exposure to ES was shown to favor Treg induction by DC due to an enhanced capacity to make TGF-β (61).

Some helminths may have found ways to overcome the necessity to manipulate DC function by directly producing factors that are capable of enhancing the Foxp3⁺ Treg induction process, such as TGF-β. Homologs of TGF-β have been identified in many helminth genomes (62, 63) and can trigger the activation of TGF-β receptors on mammalian cells (62). Most importantly, ES from the helminth *H. polygyrus* has been demonstrated to induce Foxp3 expression in naive T cells *in vitro* via TGF-β signaling (57). A number of TGF-β homologs have been cloned from the *H. polygyrus* genome (63), but as yet it has not been

possible to attribute this effect to a specific molecule. In addition to driving Foxp3 induction in T cells, TGF- β signaling stimulated by helminth ES may act directly on gut APC to limit their activation. The exact mechanisms by which secreted TGF- β homologs modulate intestinal immune responses will require further study.

Regulation of T-effector cell responses by mucosal antigen-presenting cells

Overcoming the tolerogenic milieu of the gut is a pre-requisite to the generation of a Teff cell response. In the case of highly virulent pathogens, protective responses must occur rapidly to contain and control the infection. The intestinal APC compartment is uniquely adapted to balance the induction of regulatory and inflammatory responses. Intestinal APCs express a wide range of pattern recognition receptors (PRRs) that allow them to constantly survey the intestinal milieu and dynamically control this balance (64). Paradoxically, despite their importance in establishing immunological tolerance, the very same gut APCs are also crucial to support Teff cell differentiation (Fig. 2). Such pro-effector function is evident at steady state, where Teff cells play an important role in maintaining détente with the commensal flora as well as in response to infection (65).

Although RA production is crucial for the induction of Foxp3⁺ Tregs (29, 31) in the healthy gut, in the context of inflammation RA is similarly crucial for Teff cell generation (66) (Fig. 2). This context-dependent role for RA has long been evident in human studies where vitamin A deficiency is associated with enhanced childhood mortality from gastrointestinal infections (36, 67). Indeed, in a recent study we have shown that RA is required for the differentiation of both T-helper 1 (Th1) and Th17 cells (66). Given that CD103⁺ DCs are major producers of RA due to their high expression of RALDH, we propose that during infection these DCs become potent drivers of the Teff cell response.

One effector population of particular interest for mucosal immunity is Th17 cells. Th17 cells are a distinct subset of CD4⁺ T cells originally identified in the context of autoimmune disease in humans and mice (68–71). Mucosal surfaces including the gastrointestinal tract are constitutively enriched for Th17 cells, and they play a critical role in protection against bacterial and fungal pathogens (72–74). The induction of Th17 cells is dependent on signals from the microbiota, and these cells are preferentially induced in the presence of defined commensals, such as segmented filamentous bacteria (75, 76). Supporting a role for *lamina propria* Th17 cells in controlling intestinal flora, genomic deletion or inhibition of IL-17 signaling has been shown to aggravate pathology in innate cell-mediated (77, 78) and T-cell mediated (79) mouse models of colitis. Furthermore, Th17 cells have recently been shown to drive the production of IgA and anti-microbial peptides (77). The protective function of Th17 in the GI tract has also been extended to humans and non-human primates. Loss of Th17 and other IL-17-producing cells is associated with increased mucosal damage in Simian immunodeficiency virus (SIV)-infected rhesus macaques and enhanced bacterial translocation (80). Moreover, a recent clinical trial examining the efficacy of administration of anti-IL-17A antibody to Crohn's patients was aborted after the treatment resulted in worse clinical outcomes and increased adverse events (81). One mechanism by which function of APCs can be altered is by sensing of conserved microbial ligands, many of

which shared by both commensals and pathogens. For instance, we (82) showed that sensing of bacterial DNA through Toll-like receptor 9 (TLR9) is one mechanism by which gut DCs can be deviated from their regulatory function toward the induction of Th17 cells.

Recent studies have aimed to identify the specific intestinal APC populations responsible for Th17 responses. Increased understanding of the transcriptional control of mucosal APC development has facilitated this line of research. Mice deficient in Notch2 signaling or the transcription factor IRF4 demonstrate a specific loss of the CD103⁺CD11b⁺ intestinal DC subset (as well as the related CD8α⁺, CD11b⁺ population resident in lymphoid tissue), and a resulting inability to mount Th17 responses required to control intestinal infection (9, 83–85). Supporting their central role in Th17 induction, CD103⁺CD11b⁺ DCs have been found to be potent producers of IL-6 and IL-23 (72, 82, 83, 85), signals that are critical for the differentiation and maintenance of Th17 cells. Additionally, CD103⁺CD11b⁺ DCs have been shown to be the primary cells responsible for transport of bacteria from the lumen of the intestinal tract to the mesLNs in the steady state and upon infection (10, 86). The human equivalent of the CD103⁺CD11b⁺ DC subset (CD1c⁺ DCs) has recently been identified, and these cells are likewise potent inducers of Th17 responses *in vitro* (87). Recent insights into the central role of CD103⁺CD11b⁺ DCs in Th17 differentiation in the gastrointestinal tract support earlier studies reporting the importance of TLR5 in mounting intestinal Th17 responses. CD103⁺CD11b⁺ DCs express high levels of TLR5 and were recently shown to respond to intravenous bacterial flagellin (a TLR5 agonist) injection by robustly inducing Th17 via their production of IL-23 (72).

These findings also suggest an indirect role of gut DCs in inducing barrier protection via IL-23-dependent production of IL-22 by GALT-resident lymphocytes. IL-22, a member of the IL-10 family of cytokines (88, 89) is critical for regulation of intestinal epithelial cell (IEC) function. The receptor for IL-22 is expressed in the gastrointestinal tract exclusively by IECs (90), and signaling promotes production of anti-microbial peptides such as the β-defensins and RegIIIγ (91–93). Recent studies have identified innate lymphoid cells (ILCs) as the primary source of IL-22 in steady state conditions in mice and humans (92), although Th17 cells may also contribute to production of this cytokine (92, 94). The release of IL-22 in both of these populations is induced by IL-23 (72, 89, 91, 92, 94–96), implicating CD103⁺CD11b⁺ intestinal DCs as critical regulators of this pathway.

Negative control of Treg function in the intestine

Although intestinal DCs are potent inducers of regulatory T cells at steady state, these cells can also be dominant sources of pro-inflammatory cytokines in response to pathogen-derived signals (97). As a result, we have found that, following acute mucosal infection, Foxp3⁺ Treg induction towards oral antigens fails to occur and that CD103⁺ DCs can no longer support the induction of these cells (97). Thus, in the context of acute mucosal inflammation, DCs involved in the maintenance of regulatory responses can become potent inducers of the effector response.

Our studies suggest that the capacity of gut DCs to produce IL-12, in the context of intestinal infection with the protozoan parasite *Toxoplasma gondii*, is a major mechanism by

which their capacity to favor tolerance could be negatively regulated (97) (Fig. 2). Although the precise DC subset responsible for IL-12 production has not been defined, we can hypothesize that it is the Batf3-dependent CD103⁺CD11b⁻ subset, as a Batf3-dependent population has been shown to be critical for IL-12 production in intraperitoneal *T. gondii* infection (98). Production of IL-12 by these DCs may not only result in a breakdown of Treg induction but also in conversion of Tregs into effector cells (97). Indeed, in recent years it has become increasingly apparent that T-cell lineages are not stable, rather they maintain a certain degree of plasticity, particularly in respect to their ability to produce cytokines (99, 100).

Investigation of epigenetic marks at the *Ifng* locus in *ex vivo* Tregs revealed the *Ifng* locus showed no signs of induction or repression, suggesting that this site is poised for transcriptional activation (100). We found that during *T. gondii*, Tregs began to express T-bet and that this leads to their production of IFN- γ , which is both protective and pathological in this context. Moreover, when isolated from the gut, *lamina propria* APCs were able to induce T-bet expression in Treg *in vitro* (97). Thus, gut APCs, as major producers of pro-inflammatory cytokines and RA, can support a rapid switch of the tolerogenic steady state immune system towards an inflammatory state. Such balance is not without risk as restriction of Treg conversion has been associated with enhanced gastrointestinal immunopathology (101, 102).

The impairment of Treg induction during inflammation suggests that exposure to specific antigens under these settings can prevent acquisition of tolerance towards innocuous antigens. Indeed, we found that in acute infection aberrant responses towards food and commensal antigens can occur (97, 103). Thus, excessive deviation from the tolerogenic tone of the gastrointestinal tract and sustained activation of APCs during inflammation can lead to potent effector responses towards harmless antigens, an event that can be associated with severe immunopathological outcomes.

Remodeling of the gut antigen-presenting cell network during inflammation

In addition to the capacity of mucosal DCs to adapt their function according to local cues, the APC network of the intestine is dramatically remodeled upon acute challenge to enable the establishment of an inflammatory milieu. Notably, we have previously described a collapse of *lamina propria* resident Treg population during acute intestinal infection (97). Concurrent with the disappearance of these critical regulatory cells, the mucus layer and antimicrobial peptides that in the healthy gut are crucial to maintain segregation of the commensal flora from the epithelial barrier, are lost during acute inflammation. Most likely, this results from cytokine mediated goblet cell hyperplasia and paneth cell destruction (17, 104). The loss of the intestinal mucus layer contributes to disruption of the mucosal barrier and translocation of commensal bacteria from the lumen of the intestine into the host tissue (103).

A major focus of our recent work has been to examine alterations to the composition and function of the intestinal APC network during acute infection in response to the inflammatory milieu and translocating commensal microbes. We have recently described a

striking depletion of CD103⁺CD11b⁺ DCs from the intestinal *lamina propria* during acute intestinal infection with *T. gondii* (16). These cells are the predominant DC population in the gut and are believed to be primarily responsible for induction of antigen-specific Tregs in the GALT. Indeed, their disappearance during acute mucosal infection is associated with a loss of tolerance to oral antigens (97). Gut-resident CX3CR1^{hi} macrophages also lose regulatory function during this infection, as their expression of IL-10 is dramatically diminished (16). The impairment or loss of steady state mechanisms of regulation in the gut contributes to the induction of inflammation during infection. However, new mechanisms of regulation must arise in this context to prevent immunopathology leading to tissue damage that is detrimental to the host. Monocytes expressing the surface marker Ly6C are dramatically recruited during acute gut inflammation and become the predominant APC population in the tissue (Fig. 2). The next section of this review explores mechanisms employed by these cells to limit inflammation and immunopathology in response to commensal microbes during infection.

Ly6C^{hi} monocytes as regulators of commensal-mediated immunopathology

We have described that Ly6C^{hi} effector monocytes play a key role in not only protecting against infection (105) but also preventing uncontrolled immune responses towards commensals (106). Notably, during mucosal infection, we and others have shown that there is a tremendous outgrowth of highly inflammatory commensal populations, specifically γ -proteobacteria such as *Escherichia coli* (104, 107, 108). Outgrowth of γ -proteobacteria has been reported as a common feature in a number of inflammatory settings including in human IBD (109, 110). We demonstrated that in this setting neutrophils are essential to prevent translocation of commensal bacteria into the periphery (104, 107, 108). Unexpectedly, most of the defense against the bacteria was mediated in the lumen of the gut, a site that is increasingly being recognized as major site of immune activity (111). This was associated with the generation of organized luminal structures, termed casts, which effectively contained the outgrowing γ -proteobacteria, thus preventing its interaction with the gastrointestinal epithelium (17).

In mice lacking tissue monocytes (112), gut pathology to intestinal *T. gondii* infection was dramatically enhanced independent of changes to parasite load and T-cell polarization. The increased pathology was critically dependent upon both neutrophils (106, 113) and commensal flora (106), suggesting that in the absence of inflammatory monocytes, neutrophil responses towards bacteria are uncontrolled. This finding is contrary to the previously established role of Ly6C^{hi} monocytes in infections such as *Citrobacter rodentium* (114) and *T. gondii* (105), where they are generally considered to be pro-inflammatory and protective, or their role in colitis, where they are critical to initiate inflammation (19). Frequently, monocytes and neutrophils are co-localized in the context of inflammation (16, 115) suggesting that monocyte control of neutrophil activation could be direct. Corroborating this, we found that treatment of neutrophils with supernatant from *lamina propria* monocytes limited the capacity of neutrophils to make pro-inflammatory cytokines including TNF- α and damaging reactive oxygen species (ROS) (116). Suppression occurred when neutrophils responded to TLR ligands or formyl-peptides, which were crucial for luminal cast formation (17) and are important for neutrophil trafficking at

other sites (16, 17, 116). This suppression was found to be entirely dependent upon the production of the lipid mediator prostaglandin E₂ (PGE₂) that was generated by monocytes (Fig. 2). Although, PGE₂ can, at the start of an immune response, favor inflammatory cell recruitment (117), it has also been described as a potent suppressor of human neutrophil activation (118).

Regulatory features of Ly6C^{hi} monocytes were only evident in the gastrointestinal tract and were induced in response to commensal-derived ligands. Thus during inflammation, commensals can rapidly impart tissue specific regulatory functions on mononuclear phagocyte populations. *In vitro*, PGE₂ production could be induced by lysate from a commensal form of *E. coli* or highly-purified bacterial TLR-ligands including LPS, flagellin, and CpG. However, one can speculate that *in vivo* there may be additional signals that are responsible for driving gastrointestinal specific PGE₂. In particular, stimulated epithelial cells can produce and activate IL-1 β (119) a major driver of PGE₂ from monocytes (120). Moreover, the commensal-derived dietary ligands short-chain fatty acids, such as butyrate, also augment PGE₂-production in response to commensal ligands (121). This finding also raises the question of whether altering the composition of the commensal flora could change the regulatory to inflammatory balance of Ly6C^{hi} monocytes during infection. It is interesting to hypothesize that there may be specific commensal species that favor PGE₂ production and regulatory function by monocytes. Commensal-mediated effects on APC function have been shown in the steady state where specific strains of *Clostridium butyricum* can preferentially favor IL-10 production by CX3CR1^{hi} macrophages in the colon (122).

Among the most commonly used pain medications are non-steroidal anti-inflammatory drugs (NSAIDs) that target cyclooxygenases to inhibit PGE₂ production and hence limit stimulation of pain receptors (123). However, one problem associated with NSAIDs is immunopathology specifically in the gastrointestinal tract (123). This has been dominantly attributed to the effects of NSAIDs on epithelial cells; however, these results implicate this previously undescribed Ly6C^{hi} monocyte/neutrophil regulatory loop. Thus, NSAIDs may also augment gut immunopathology as a result of their inhibition of a key regulatory pathway initiated by mononuclear phagocytes. This is particularly relevant therapeutically as there is still much controversy as to whether NSAIDs should be employed in patients with underlying gastrointestinal disorders such as Crohn's disease. Indeed, there is some suggestion that use of NSAIDs may also be associated with colitic flares(124). Thus, NSAIDs may initiate an unrestrained amplification loop in the context of gut inflammation in which epithelial damage leads to microbial translocation, causing uncontrolled neutrophil production of ROS and TNF- α . Whether monocyte derived PGE₂ can control other aspects of neutrophil activation, such as production of matrix metalloproteases (116), remains to be explored. Moreover, whether similar pathways act in other infection settings, or at other sites, where neutrophils and monocytes are recruited is unknown.

Ly6C^{hi} monocyte suppression of T cell responses during infection

Alongside their capacity to suppress neutrophil activation, Ly6C^{hi} effector monocytes are likely to play additional immunoregulatory roles during acute parasite-driven inflammation. Notably, we found that during *T. gondii* infection Ly6C^{hi} monocytes expressed other anti-

inflammatory factors. Among these is IL-10, a cytokine that can also be derived from Th1 cells during inflammation (125) and is important for immune regulation in multiple infections (126). Additionally, Ly6C^{hi} monocytes in the *lamina propria* of *T. gondii* infected mice were found to express inducible nitric oxide synthase (iNOS) (16). Although this factor, which is produced from L-arginine (127), can be pro-inflammatory and important in pathogen killing, it can also be highly immunosuppressive (127).

Metabolism of L-arginine has been implicated in suppressive activities of monocyte-derived populations in multiple infections (126). Nitric oxide (NO) is able to directly limit T-cell proliferation (127), and one well documented phenomenon is NO-mediated hyporesponsiveness of T cells at acute time points during parasite infection including *Trypanosoma cruzi* (128), *Plasmodium chabaudi* (129), and *T. gondii* (130). Recruitment of Ly6C^{hi} monocytes to the mesLN occurs early during *T. gondii* infection where their presence and production of NO could limit T-cell expansion and activation. This phenomenon has been demonstrated in *Listeria monocytogenes* infection where iNOS⁺ monocytes in the spleen suppress antigen-specific immune responses towards the pathogen (131). Moreover, NO can suppress T-cell production of IFN- γ directly (127), implying that these monocytes may also be able to limit effector T-cell function in the *lamina propria*. In the gut, it is possible that NO may not only provide a mechanism to limit over exuberant responses to the pathogen but also prevent the development of aberrant responses to food (97) or commensal antigens (103).

CD11b⁺Gr1⁺ immature myeloid cells have been described as the dominant producers of NO in a number of inflammatory settings (126), a feature reminiscent of myeloid-derived suppressor cells (MDSCs), which have been mainly described as suppressors of T-cell responses in cancerous tumors (132). While less well studied, MDSC-like cells can in fact arise during other insults including parasite and bacterial infection. Although these cells are a heterogeneous mix of myeloid cells that are in various stages of differentiation some of them display a monocytic morphology (132). It is tempting to speculate that the Ly6C^{hi} effector monocytes recruited during mucosal infection to the gut may in fact be a type of MDSC that emerges in a highly polarized Th1 setting. This is particularly likely, as MDSCs have already been described in the lung of *T. gondii* infected animals where they were found to suppress T-cell responses (133). Indeed, the acquisition of MDSC-like characteristics by Ly6C^{hi} monocytes during acute mucosal infection may help to explain their suppressive capabilities that have not been described in non-infectious settings of inflammation, such as colitis (19). Exploring whether specific signals are important to induce regulatory capacity of monocytes during infection will be an important area of future research. Whether Ly6C^{hi} monocytes can also regulate the function of additional cell types during inflammation remains to be explored.

Restoration of gastrointestinal homeostasis by mononuclear phagocyte

Following control of the invading pathogen, damage to the gastrointestinal tract must be repaired and cellular debris cleared away. In this context, APCs must once again take on a new face as regulators and effectors of the wound healing process. Subsequently, they must also restore the adaptive immune cell network to a new homeostasis. Failure to do so can

lead to ongoing inflammation as occurs in chronic gastrointestinal disorders, such as Crohn's disease or colitis. Indeed, in patients with Crohn's disease, it has been suggested that ongoing aberrant activation of recruited monocyte populations contributes to disease pathology (134).

Although understanding the restoration process is likely to yield many targets for therapeutic intervention, at this time how specific APC subsets in the gut control this response has been poorly explored. At other sites it has become very clear that it is a highly orchestrated process involving multiple APC cell-types. Perhaps the best characterized example of this is in myocardial infarction, where multiple monocyte and macrophage subsets act in tandem to orchestrate tissue repair (135). For many years it was thought that resolution was just a natural 'burning out' of the inflammatory response (136). However, in recently it has become increasingly clear that resolution of the immune response is in fact a highly active biochemical and metabolic process (136).

A key aspect during the resolution of inflammation is the production of pro-resolution mediators that prevent or reduce neutrophil infiltration as well as promote the uptake and clearance of apoptotic cells by monocytes and macrophages (136). These mediators include lipoxins, resolvins, and protectins, that can be produced by multiple cell-types, including neutrophils (137), and macrophages (138). It has been demonstrated that prostaglandins, and in particular PGE₂, favor production of lipoxins. Notably, in resolving inflammatory exudates, prostaglandin E₂ and prostaglandin D₂ stimulate the production of a functional enzyme for lipoxin production in neutrophils (139). Based on our studies, this raises the intriguing possibility that PGE₂ production in the gut by Ly6C^{hi} monocytes during acute mucosal infection may not only limit neutrophil activation but also deviate their function towards a lipoxin-producing pro-resolution phenotype. Future studies will be required to assess this possibility.

Another way in which mucosal APCs may regulate the repair of the epithelial barrier is by direct interactions with epithelial progenitors present in the crypts. Although the specific subset of monocytes/macrophages was not defined, mononuclear phagocytes were shown to mediate MyD88-dependent survival and proliferation of epithelial progenitor cells during colonic wound healing (140). This capacity was dependent upon the release of a number of mitogenic factors and extracellular matrix stabilizers. It is possible that PGE₂-production could also play a role in protection of the crypt progenitor cells as COX-2 expression by macrophages at other sites has been linked to protection of progenitor populations during inflammation (141).

Another feature of the return to homeostasis along with repair of the epithelial barrier is the restoration of the adaptive immune compartment that, as described earlier in this review, can be dramatically perturbed during an inflammatory response. In particular, it is unclear which APC subsets are critical to restore the Treg compartment following inflammation. Although at steady state intestinal DCs are responsible for Treg induction, whether they perform the same function during restoration of the compartment is unknown. Given that CD103⁺CD11b⁺ DCs are depleted from the *lamina propria* during *T. gondii* infection, other cells may have to take on this role. One possibility is that during restoration of the

compartment monocyte-derived DCs may be tuned to take on a Treg induction capacity. In this regard, it has been described that during colitis Ly6C^{hi} monocytes can differentiate to a DC-like phenotype. This is associated with upregulation of the transcription factor Zbtb46 (19), which is considered to be a defining factor of conventional DCs (142, 143). These cells upregulated CCR7, suggesting that they had a capacity to drain to the mesLNs. This implies that monocyte-derived DCs are capable of carrying luminal antigens, a pre-requisite for aiding in reestablishment of the Treg compartment following resolution of inflammation. Given that failure to restore homeostasis can lead to ongoing gastrointestinal inflammation, a better understanding of how repair is initiated in the gastrointestinal tract, and which APC subsets contribute to these responses, is an important area of continued research.

Concluding remarks

Recent studies from our laboratory and others have led to substantial advances in understanding of the specialized regulatory functions of individual mononuclear phagocyte populations in the gut. One point that is becoming abundantly clear is that the function of cells that reside in mucosal sites is highly context-specific and controlled by local cues. Most notably, CD103⁺ DCs that are crucial to induction of tolerance at steady state (31, 144) can become key negative regulators of this system in response to pathogen challenge (97). On the other hand, Ly6C^{hi} effector monocytes that are generally considered to be pro-inflammatory are essential to prevent pathologic responses towards commensals during inflammation (16).

Much therapeutic interest has been focused on using factors that favor steady state regulatory APC functions to support regulation. However, such therapies need to be used with extreme caution in settings of chronic inflammation, where regulatory signals might be dramatically altered. Of particular note is the use of vitamin A/RA supplementation that may also augment inflammatory responses depending on the context (36). Moreover, a number of studies have promoted the concept of Ly6C^{hi} monocyte depletion as a therapeutic tool to limit inflammation (145). Given their importance in generating CX3CR1^{hi} gut macrophages and also the potential regulatory roles of Ly6C^{hi} effector monocytes during acute inflammation, this may not be advisable in gut inflammation. Mononuclear phagocytes do, however, represent an important target for therapeutic manipulation due to their rapid turnover and functional versatility. By continuing to better define the regulatory and repair potential of gut antigen-presenting cells in inflammatory settings, it may be increasingly possible to specifically and rationally target these populations for therapeutic purposes.

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Fig. 1. Induction and maintenance of the steady state intestinal T-cell compartment

In the steady state, highly specialized antigen-presenting cells (APCs) enriched in the gut-associated lymphoid tissue (GALT) are crucial to establish the tolerogenic Forkhead box protein 3 (Foxp3)⁺ regulatory T-cell (Treg) network as well as effector T cells (Teff) that help maintain the mucosal barrier. CD103⁺ dendritic cells (DCs) carry dietary derived and commensal antigens to the mesenteric lymph node (mesLN), where their unique capacity to produce both active TGF-β and the vitamin A metabolite retinoic acid (RA) favors induction of Foxp3⁺ Treg from naive T cells. Additionally, RA supports the acquisition of gut-homing markers by these cells that potentiates their capacity to traffic back to the small intestine and colon *lamina propria*. Upon arrival in the gut, newly converted Foxp3⁺ Treg are maintained/expanded by IL-10-producing CX3CR1^{hi} macrophages. These induced Foxp3⁺ Tregs along with their thymus-derived counterparts act to limit local immune activation stimulated by food and commensal antigens. CD103 DCs in the mesLN also secrete IL-6, which synergizes with TGF-β signaling to induce Th17 effector cells. *Lamina propria*-resident Th17 cells modulate epithelial cell function and anti-microbial peptide secretion. Although poorly understood, a similar network of regulatory and effector T cells may be re-established in the context of chronic pathogen infection to limit unwanted ongoing immune responses towards the long-lived invader.

Fig. 2. Regulation of effector T-cell responses and recruited inflammatory cells

As well as favoring tolerance to dietary and commensal antigens, gut antigen-presenting cells (APCs) also regulate development of effector T-cell (Teff) responses towards commensals and pathogens and limit uncontrolled inflammatory cell activation during infection. Gut APCs are exquisitely sensitive to conserved commensal and pathogen-associated molecular patterns (PAMPs), due to their expression of a broad array of pathogen recognition receptors (PRRs). Notably, in response to microbial ligands, previously tolerogenic APCs become potent inducers of Th17 and Th1 responses as a result of their capacity to make IL-6 and IL-12, respectively. In this setting, the capacity of CD103⁺ DCs to produce RA supports T-cell activation and homing back to the intestine. Additionally, at the peak of the inflammatory response towards a pathogen, recruited Ly6C^{hi} effector monocytes can acquire regulatory features to prevent lethal neutrophil-mediated immunopathology. This is mediated by their ability to make prostaglandin E₂ (PGE₂), a potent suppressor of neutrophil activation, in response to commensal ligands.