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EDITORIAL

Intestinal dendritic cells in the pathogenesis of inflammatory bowel disease

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Abstract

The gastrointestinal tract harbors a large number and diverse array of commensal bacteria and is an important entry site for pathogens. For these reasons, the intestinal immune system is uniquely dedicated to protect against infections, while avoiding the development of destructive inflammatory responses to the microbiota. Several models have been proposed to explain how the immune system discriminates between, and appropriately responds to, commensal and pathogenic microorganisms. Dendritic cells (DCs) and regulatory T cells (Treg) are instrumental in maintaining immune homeostasis and tolerance in the gut. DCs are virtually omnipresent and are remarkably plastic, having the ability to adapt to the influences of the microenvironment. Different DC populations with partially overlapping phenotypic and functional properties have been described in different anatomical locations. DCs in the draining mesenteric lymph nodes, in the intestinal lamina propria and in Peyer's patches partake both in the control of intestinal inflammation and in the maintenance of gut tolerance. In this respect, gut-resident DCs and macrophages exert tolerogenic functions as they regularly encounter and sense commensal bacteria. In contrast, migrating DC subsets that are recruited to the gut as a result of pathogenic insults initiate immune responses. Importantly, tolerogenic DCs act by promoting the differentiation and expansion of Treg cells that efficiently modulate gut inflammation, as shown both in pre-clinical models of colitis and in patients with inflammatory bowel disease (IBD). This article reviews the phenotypic and functional features of gut DC subsets and discusses the current evidence underpinning the DC contribution to the pathogenesis of the major clinical subtypes of human IBD. It also addresses the potential clinical benefit derived from DC targeting either *in vivo* or *in vitro*.

Key words: Dendritic cell; Tolerance; Gut; Inflammatory bowel disease; Cytokine; Regulatory T cells

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INTRODUCTION

The digestive tract has a surface area nearly 200 times greater than that of the skin. Being an important port of entry for microorganisms, the gut must be protected by effective immune responses. However, immune reactivity must be prevented from damaging gut tissues in response

to benign foreign material to which the gut is continuously exposed. T cell immunity relies on the recognition of antigenic peptides processed and presented to T cells by dendritic cells (DCs), which act as initiators, stimulators and regulators of antigen-specific T cell responses, but also play a pivotal role in the maintenance of tolerance towards the commensal microflora[1,2].

DCs are specialized accessory cells distinguishable from other mononuclear phagocytes (MPs) such as monocytes and macrophages by their unique morphology and ability to capture and process antigens for presentation to effector T cells. Upon encounter with pathogens and activation, DCs undergo rapid maturation characterized by the upregulation of major histocompatibility complex (MHC) and costimulatory molecules and migrate to the draining lymph nodes. The remarkable flexibility of DC functions likely results from their ability to sense the local environment and to shape the ensuing immune response[3,4]. Intestinal MPs are distributed in organized lymphoid organs, such as Peyer's patches (PP) and mesenteric lymph nodes (MLN), and are highly abundant in the loose connective tissue underlying the epithelium, the lamina propria (LP)[5].

It is now established that DCs play a crucial role in both immunity and tolerance[1,6]. In a tolerogenic setting, DC can induce anergy in antigen-specific T cells or generate protective FoxP3$^+$ regulatory T cells (Treg) in the lymph nodes. Under steady-state conditions, DCs continuously migrate from peripheral organs *via* the lymph to secondary lymphoid organs, where they present self-antigens or innocuous environmental antigens to maintain peripheral tolerance. The chemokine receptor, CCR7, is a key regulator of the homeostatic and inflammation-induced trafficking of DCs from skin, lung and gut to their respective draining lymph nodes[7].

Human inflammatory bowel disease (IBD) consists of 2 dominant disease subtypes, Crohn's disease (CD), largely arising from a Th1 response, and ulcerative colitis (UC), largely mediated by interleukin (IL)-5- and IL-13-producing T cells or natural killer T cells[8]. The immunopathology of human IBD relates to an inappropriate and exaggerated immune response to constituents of the gut flora in a genetically predisposed individual. Amongst other cell types[9], DCs play a role in IBD pathogenesis, as suggested by mouse models of colitis and by observations in humans. The local microenvironment regulates the function of mucosal DCs through the presence of immune cells, non-immune cells and luminal bacteria[10]. In principle, DC dysfunction may promote the development of gut inflammation by priming T-cell responses against bacteria, by sustaining T cell reactivity within the inflamed mucosa and by functioning as effector cells releasing pro-inflammatory cytokines[11].

DC LINEAGE AND SUBSETS IN MICE AND HUMANS

DC origin and precursor-progeny relationships have remained a matter of controversy and debate for decades[12]. Recent landmark studies have led to a better definition of DC ontogeny in mice (Figure 1), unraveling that a macrophage and DC precursor (MDP) serves as a common bone marrow progenitor for classical or conventional DCs (cDCs), plasmacytoid DCs (pDCs) and monocytes (Table 1). Specifically, Fogg *et al*[13] have identified a clonogenic MDP with a CD117$^+$CX$_3$CR1$^+$CD11b$^-$lineage$^-$ phenotype, representing ~0.5% of total bone marrow cells and giving rise to monocytes, to several macrophage subsets and, ultimately, to steady-state CD11c$^+$CD8α^+ and CD11c$^+$CD8α^- DC. Lymphoid organ DCs in the steady state originate from a bone marrow precursor with a Lin$^-$CD115$^+$Flt3$^+$CD117^{lo} phenotype, termed common DC progenitor (CDP)[14]. Migratory DC precursors (pre-DCs) also exist in the peripheral blood and are in equilibrium with DCs in lymphoid organs and in non-lymphoid tissues, such as skin, lung, kidney and intestine. DCs actively divide *in vivo* and their lifespan varies from 5 to 7 d in the spleen, lymph node, liver and kidney and can be as long as 25 d in the lung[15].

DCs lack a unique surface marker, but rather express a distinct set of cell surface antigens. The number of DC subsets that have been phenotypically characterized and functionally designated is increasing steadily. In addition to the classical integrin marker CD11c distinguishing DCs, the integrin αE (CD103) recently gained attention and has been used to sub-classify DC subsets based on specific functional activities and anatomic location (see below for a thorough discussion)[16]. CD103 mediates T cell adhesion to epithelial cells through its binding to E-cadherin, which is expressed on the basolateral side of epithelial cells but not on endothelial cells. Mice with a targeted disruption of *cd103* show a mild reduction in T cell numbers in the intraepithelial and LP compartments, coupled with the inability to reject islet allografts[17].

MECHANISMS UNDERLYING DC-MEDIATED TOLERANCE IN THE GUT

One of the major functions of tolerogenic DCs may be the differentiation of Treg cells from naïve T cells. Two major subtypes of Treg cells have been described to date, namely, naturally occurring CD4$^+$CD25$^+$FoxP3$^+$ Treg cells (nTreg) and inducible type 1 Treg cells (Tr1).

DCs as inducers of nTreg cells

Naturally occurring Treg cells, a functionally specialized subset of CD4$^+$ T cells, have been involved in preventing T cell-mediated and innate immune pathology in a number of disease models[18]. The transcription factor FoxP3 is expressed by CD4$^+$CD25$^+$ Treg cells and is fundamental for Treg development and function. nTreg cells mainly suppress effector T cells through a cell contact-dependent and largely contact-independent mechanism. Membrane-bound transforming growth factor (TGF)-β has been implicated in nTreg-mediated inhibition of T cell responses. Moreover, TGF-β1 acts as a co-stimulatory factor for FoxP3 expression, leading to Treg differen-

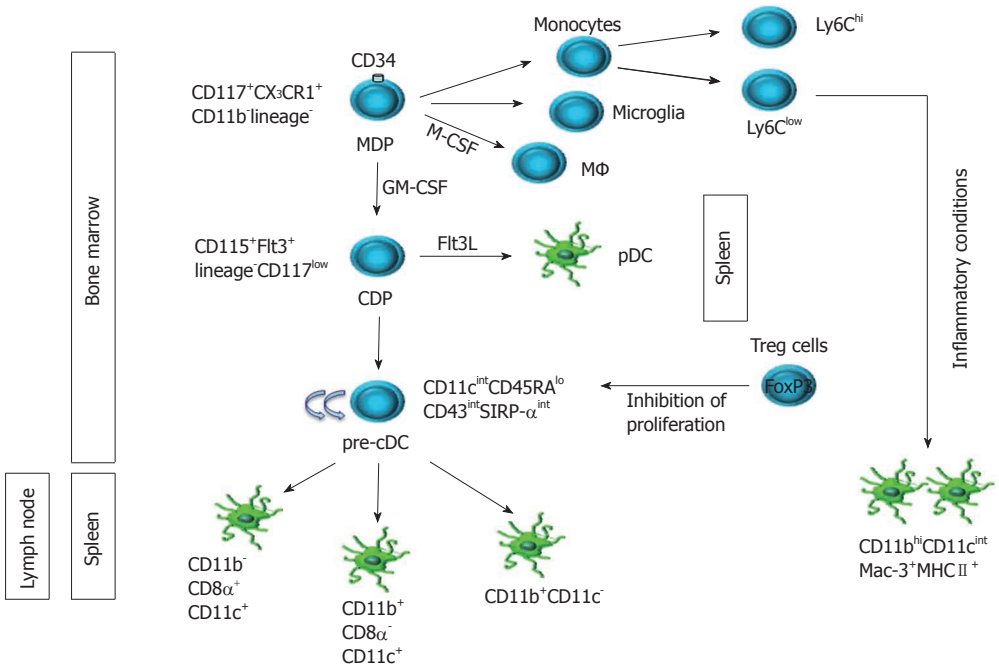

Figure 1 Ontogeny of dendritic cell subsets in mice. The most recent evidence elucidating dendritic cell (DC) ontogeny and unraveling the complexity of the DC compartment in mice is summarized. The curved arrows in cyan denote proliferation potential. Regulatory T (Treg) cells may contribute to DC development and homeostasis in mice, as suggested by studies where Treg depletion has been associated with a 2- and 12-fold increase in precursor conventional DC (pre-cDC) and cDC in spleen and lymph node, respectively[14]. MDP: Macrophage and dendritic cell progenitor; CDP: Common dendritic cell progenitor; pDC: Plasmacytoid DC.

tiation from $CD4^+CD25^-$ T cells. Interestingly, TGF-β1 production by Treg cells is not required for inhibition of colitis, suggesting that Treg cells may induce TGF-β release by other hematopoietic or stromal cells[19]. Support for this hypothesis is provided by the observation that suppression of colitis by TGF-$β1^{-/-}$ Treg cells was inhibited by anti-TGF-β antibodies, indicating that TGF-β is central to the function of Treg cells even when they do not synthesize it themselves[19]. In this respect, DCs remain a key and intriguing candidate for TGF-β production *in vivo*. It is conceivable that Treg cells be required to express TGF-β1 on the cell surface and to present it to pathogenic T cells, as previously shown[19].

Treg cells are believed to play a crucial role in inhibiting intestinal inflammation and IBD. Notably, Treg cells may contribute differentially to the modulation of experimental autoimmune gastritis and colitis. Protection from colitis, but not from gastric inflammation, has been reported to depend on IL-10 expression by $CD4^+CD25^+$ nTreg cells[20]. The T cell transfer model of colitis allows an understanding of Treg-mediated mechanisms controlling intestinal inflammation. During cure of experimental colitis, Treg cells proliferate and accumulate in MLN and colonic LP, in contact with $CD11c^+$ DCs and effector T cells[21]. Interestingly, IL-10-producing Treg cells selectively enrich within the colonic LP, whereas FoxP3-expressing Treg cells are present in similar frequencies in both the secondary lymphoid organs and LP of colitic animals[22]. Transfer of $CD4^+CD45RB^+$ T cells into $RAG^{-/-}$ mice causes colitis. Disease development requires β7-integrin-dependent intestinal localization. Importantly, β7-deficient Treg cells prevent colitis, suggesting that Treg accumulation in the intestine is dispensable for disease suppression[23]. The presence of Treg cells impacts on $CD4^+CD45RB^+$ T cell accumulation in the intestine, indicating that one major function of Treg cells may involve the inhibition of tissue localization of Th1 effector cells.

Peripheral blood $CD4^+CD25^{high}$ T cells may be decreased in active human IBD compared with inactive disease[24]. Notably, Treg cells are increased in mucosal IBD lesions, coincident with an increase in transcripts for IL-8, a hallmark of inflammation in the gut, and for FoxP3[24]. The higher degree of Treg infiltration in the gut LP of patients with diverticulitis compared with IBD suggests that an insufficient increase of Treg cells in IBD accounts for inflammation and intestinal pathology[24]. In the LP of human colon, Treg accumulation has been detected in a variety of inflammatory conditions, such as diverticulitis, pseudo-membranous colitis and cytomegalovirus-induced colitis, and may not be a specific feature of CD or UC[22]. The presence of $FoxP3^+$ T cells in the LP of patients with IBD suggests that defects in Treg numbers may not account for the pathology, and that ineffective Treg activity may rather contribute to sustained gut inflammation.

DCs as inducers of Tr1 cells

Tr1 have been described as a $CD4^+$ T-cell subset releasing high levels of IL-10, in the absence of measurable IL-2 and IL-4 production, and exerting suppressive functions in an IL-10/TGF-β-dependent but cell contact-independent manner[25]. The production and release of interferon (IFN)-γ and TGF-β by Tr1 cells are comparable with those of Th0 and Th1 clones, respectively[25].

Table 1 Intestinal dendritic cell subsets and other mononuclear phagocytes described to date

Subset	Anatomic location	Function, if known	Ref.
$CD11c^{hi}CD11b^{+}CD8\alpha^{-}$	Peyer's patches	Localized in the subepithelial dome	[57]
$CD11c^{hi}CD11b^{+}CD8\alpha^{+}$		Localized in the interfollicular regions	[57]
$CD11c^{+}CD11b^{-}CD8\alpha^{-}$		Localized in both subepithelial dome and interfollicular regions; secretion of IL-10 in response to CD40 cross-linking; induction of T-cell release of IL-4/IL-10; promotion of T-cell proliferation	[58]
$CD11c^{mid}$ plasmacytoid DC			
$CD11c^{+}CD11b^{+}CD8\alpha^{+}$ DC	Small-intestinal and colonic lamina propria	Low T-cell responses *in vitro*; $IL\text{-}12p40^{low}IL\text{-}10^{+}$; tolerance to OVA (m); secretion of IFN-α; differentiation of Tr1-like cells that secrete IL-10, IL-4 and IFN-γ	[30,55]
CX_3CR1^{+} DC		Pro-inflammatory activity dependent upon TNF-α production	[101]
$CD11c^{hi}$class $II^{hi}CD103^{+}$ DC		Generation of $CCR9^{+}\alpha_4\beta_7^{+}$ T cells with gut tropism	[51]
$CD11b^{+}CD8\alpha^{-}CD103^{+/-}$ DC		Conversion of $Foxp3^{-}$ T cells to $Foxp3^{+}$ T cells; retinoic acid and TGF-β required	[54]
$CD11c^{lo}B220^{+}CD8\alpha^{+}$	Mesenteric lymph node	Promotion of $CD4^{+}CD25^{+}$ Treg function; differentiation of Tr1-like cells ($IL\text{-}10^{+}IL\text{-}4^{+}IFN\text{-}\gamma^{+}$) from naïve T cells after repeated stimulations	
$CD11c^{hi}CD11b^{+}CD8\alpha^{+}$		See above	
$CD11c^{hi}CD11b^{-}CD8\alpha^{-}$		See above	
$CD103^{+}$ DC		~50% of $CD11c^{+}$ DC in the MLN; conversion of naïve T cells into Treg cells; retinoic acid and TGF-β required; induction of CCR9 on gut-tropic T cells	[16]
$CD103^{-}$ DC		Expression of pro-inflammatory cytokines (TNF-α, IL-6, IL-23p19) and genes such as *Tbet*, *tlr2* and *tbx21*	[16]
$CD11c^{hi}$class $II^{hi}CD103^{+}$ DC		Induction of CCR9 gut-homing receptor on $CD4^{+}$ T cells	[53]
$CD11b^{+}CD11c^{-}$ macrophages	Small and large intestine	Induction of Treg cells, secretion of IL-10 but not IL-12p40 or IL-12p70	[105,106]

DC: Dendritic cell; OVA: Ovalbumin; IFN: Interferon; MLN: Mesenteric lymph node; Tr1: Type 1 Treg cells; TGF: Transforming growth factor; IL: Interleukin; M: Mouse studies.

Colitis in the severe combined immunodeficient (SCID) mouse model involves the development of Th1 cells responding primarily to the intestinal flora. The transfer of ovalbumin (OVA)-specific Tr1 cells in SCID mice with $CD4^{+}CD45RB^{hi}$ T cell-induced colitis prevents disease manifestations, an effect that is dependent upon the *in vivo* activation of Tr1 cells by feeding mice with OVA[25]. This observation indicates that Tr1 cells can inhibit immune responses to unknown antigens by a bystander suppression mechanism. Another report has shown that $IL\text{-}10^{-/-}$ mice lack $CD4^{+}CD45RB^{lo}$ Treg cells capable of controlling intestinal inflammatory responses, pointing to IL-10 as a crucial mediator of tolerance in the gut[26]. Similarly, TGF-β is required to suppress Th1-mediated colitis induced by $CD4^{+}CD45RB^{hi}$ T cells[27], indicating that IL-10 and TGF-β play non-redundant roles in the functioning of intestinal Treg cells.

The source of IL-10 which regulates colitis remains to be unequivocally identified. Treg-derived IL-10 was recently shown to be dispensable for suppression of colitis in $Rag1^{-/-}$ mice, but host IL-10 was required to inhibit disease development[28]. Specifically, IL-10 production by myeloid $CD11b^{+}F4/80^{+}$ cells, mostly macrophages, was important for the maintenance of Foxp3 expression by Treg cells[28]. IL-10 acted directly on Treg cells, because Treg cells lacking IL-10Rβ chain failed to suppress colitis when transferred together with $CD4^{+}CD45RB^{hi}$ T cells. In addition, this study demonstrates that IL-10 is not required to maintain FoxP3 expression in non-inflammatory conditions, because Treg development and function are unaffected in $Il10rb^{-/-}$ mice[28]. It is conceivable that the differential requirement for IL-10 for FoxP3 expression and maintenance in inflammatory *vs* non-inflammatory conditions may reflect the need for an additional signal to counter inflammatory mediators such as IL-6 or tumor necrosis factor (TNF)-α[29]. It remains to be determined whether IL-10-mediated mechanisms are unique to the gut microenvironment or whether IL-10 may be required to maintain FoxP3 expression in other organs.

Other studies pointed to Treg-derived IL-10 as a major contributor to Treg-mediated suppression[25]. These discrepancies may be attributed to differences in the endogenous flora and/or in the model systems studied. Mucosal $CD8\alpha^{+}$ DCs with a $CD11c^{lo}B220^{+}$ phenotype can be isolated from mouse MLN and have been reported to promote the suppressive function of $CD4^{+}CD25^{+}$ Treg cells and to promote the conversion of naïve T cells into Tr1-like cells[30]. At variance with classical Tr1 cells, the Tr1-like cells described in this study released IL-10, IL-4 and IFN-γ and suppressed T helper proliferation[30]. The $CD8\alpha^{+}$ DC were capable of supporting Tr1-like cell differentiation also in the presence of a maturational stimulus, such as CpG, as reported for other tolerogenic, semimature DC preparations[6,31].

DC expression of indoleamine 2,3-dioxygenase 1 and gut tolerance

Indoleamine 2,3-dioxygenase 1 (IDO1) is a tryptophan-catabolizing enzyme implicated in maternal allograft acceptance and in immune tolerance to tumors[32-36]. IDO1 converts tryptophan into immune suppressive kynurenines that profoundly affect T-cell functions, promoting T-cell unresponsiveness, T-cell apoptosis and differentiation of Treg cells. IDO expression has been associated with $CD103^{+}$ DCs in the gut LP and MLN of mice[37]. Similarly, human intestinal $CD11c^{+}CD103^{+}$ DCs express higher levels of IDO mRNA compared with $CD11c^{+}CD103^{-}$ DCs. IDO inhibition of mouse $CD103^{+}$ DCs with the D

isomer of 1-methyl-tryptophan (1MT) reduced the ability of IDO$^+$ DCs to convert Treg cells and augmented the generation of IL-17-producing T cells. Mice treated with 1MT concomitant with adoptive transfer of OVA transgenic T cells and oral immunization with OVA led to a reduction in the frequency of Treg cells in the LP, PP and MLN. *Ido1*$^{-/-}$ mice displayed a decreased percentage of Foxp3$^+$ Treg cells in the LP and an almost double the proportion of IL-17$^+$CD4$^+$ and IFN-γ$^+$CD4$^+$ T cells in the intestine compared with wild-type animals. Finally, *Rag1*$^{-/-}$ mice injected with colitogenic T cells from C57BL/6 mice experienced more extensive gut inflammation and aggressive disease if treated with 1MT. Similar effects were demonstrated in mice with dextran sodium sulfate (DSS)-colitis, where 1MT administration worsened the mortality rate and colon shortening. Collectively, these experiments indicate that IDO may play a previously unappreciated and fundamental role in regulating gut inflammation through the control of Th1/Th17/Treg balance.

The expression of IDO in the murine gut may increase with age via an IFN-γ-dependent mechanism that involves commensal microorganisms[38]. IDO-deficient mice have abnormally high levels of both IgG and IgA, a phenomenon driven by the commensal flora. IDO may then physiologically restrict B-cell responses to intestinal commensal bacteria. The elevated levels of IgG and IgA in IDO-deficient mice might in principle confer resistance to enteric pathogens such as *Citrobacter rodentium*, a gram-negative bacillus similar to human enteropathogenic *Escherichia coli*. When infected orally with *Citrobacter*, IDO-deficient mice appeared well throughout the course of the experiment, at variance with wild-type animals that had decreased activity, ruffled fur and hunched posture, and had attenuated gut colonization by the pathogen[39]. IDO-deficient mice had reduced edema, inflammatory cell infiltration and epithelial damage in colonic tissue sections, associated with lower levels of TNF-α compared with wild-type mice. These observations point to IDO as a novel target to manipulate intestinal inflammation and to control diseases caused by enteric pathogens.

Crosstalk between DCs and intestinal epithelial cells

Intestinal epithelial cells (IECs) are a central component of the immune system of the gut. They express receptors for microbial-associated molecular patterns that activate signaling cascades leading to the production of antimicrobial products and chemokines[40]. IECs can also recruit leukocytes to complement their barrier function or to participate in the activation of gut adaptive immune responses, including the production of IgA and the differentiation of effector Th1, Th2 and Th17 cells.

IECs are in close contact with LP DCs and have been shown to release molecules that influence DC functions. Thymic stromal lymphopoietin (TSLP) is a cytokine secreted by IECs under steady-state conditions and imparts a Th2-polarizing phenotype to DCs[41]. IEC-derived factors also stimulate the expression of both chains of TSLP receptor on DCs, namely the common IL-7 receptor α chain and the TSLP receptor, thus conferring the ability to respond to TSLP and to drive Th2 responses. Importantly, TSLP expression by primary IECs may be deregulated in a proportion of patients with IBD. The same study also showed that mRNA signals for TSLP are readily detected in IECs from healthy controls, although the protein is consistently below the detection limit by immunoprecipitation, unless IECs are challenged with bacteria such as *S. typhimurium*. TSLP has been detected in epithelial cells of the Hassall's corpuscles and activates myeloid CD11c$^+$ DCs in the thymic medulla[42]. These apparently mature DCs promote the development of Treg cells through a mechanism that requires peptide-MHC class Ⅱ interactions, and the presence of CD80, CD86 and IL-2. Plasmacytoid DCs can be also activated by TSLP and become efficient generators of Treg cells from thymocytes through an IL-10-dependent mechanism[43]. CD4$^+$ T cells triggered through the T cell receptor, but not resting CD4$^+$ T cells, respond to TSLP with robust proliferation and acquire sensitivity to low doses of IL-2[44].

INTESTINAL DCs UNDER STEADY-STATE CONDITIONS AND IN EXPERIMENTAL COLITIS

DCs in the non-inflamed gut

Cells with antigen-presenting function within the intestine and associated lymphoid tissue include macrophages, conventional CD11c-expressing DCs and plasmacytoid DCs. Macrophages belong to a family of tissue cells that includes Kupffer cells in the liver and glial cells in the brain and have predominantly innate immune functions, such as capturing and killing of microbes, scavenging of apoptotic and dead cells, and production of regulatory cytokines[45]. Macrophages are the most abundant population of phagocytic cells in the intestine. Distinctive characteristics have also been assigned to intestinal macrophages as compared with splenic macrophages or blood monocyte-derived macrophages. Early studies identified macrophages in the small and large intestine in the mouse, based on the expression of the F4/80 glycoprotein in association with CD11b[46]. LP macrophages are detected in juxtaposition to CD4$^+$ T cells and in close contact with the epithelium[23,47]. CD11b$^+$CD11c$^-$ macrophages are scattered throughout the villus-tip axis of small and large intestine, express immune regulatory molecules such as programmed death ligand 1 (PD-L1) and PD-L2, and secrete IL-10 but not IL-12p40 or IL-12p70. They are hyporesponsive to Toll-like receptor (TLR) stimulation, suppress the differentiation of Th1 and Th17 cells, and promote the differentiation of Treg cells[23]. Local macrophages may contribute to colitis development in IL-10$^{-/-}$ mice[48]. The pharmacological depletion of macrophages in this model of colonic inflammation ameliorated colitis, suggesting that IL-10 deficiency impedes the conditioning of macrophages, leading to macrophage-mediated destructive inflammatory responses.

Under steady-state conditions, the functional properties of the DC subpopulations vary according to their anatomical location. For instance, functional differences among DCs from PP, from MLN and from small intestinal and colonic LP have been reported. Within a single anatomical site, DCs can de distinguished and further subdivided according to their surface membrane phenotype. Under inflammatory conditions, DC recruitment to the intestine occurs, although it is presently unclear whether these DC populations are separate from DCs present in the steady-state or whether DCs arriving in the inflamed intestinal microenvironment acquire the ability to foster pro-inflammatory responses as a result of their exposure to pathogens and local inflammatory mediators.

LP DCs can be isolated in the absence of overt inflammatory stimuli and perform a tolerogenic function by constitutively migrating to the draining MLN, where they present antigen to T cells. The carriage of antigens from commensal bacterial strains to the MLN might be triggered by low-level production of pro-inflammatory cytokines. In this respect, the chemokine receptor CCR9 is crucial for the positioning of plasma cells[49] and plasmacytoid DCs[50] to the small intestine, suggesting that the chemokine CCL25/TECK may regulate DC homing during inflammatory processes. After their migration to the MLN, DCs interact with T and B cells and initiate immune responses aimed at maintaining a non-inflammatory state in the intestine. Intestinal DCs have been reported to promote the peripheral induction of FoxP3-expressing Treg cells from naïve T cells. Such Treg cells with specificity for commensal bacteria and dietary antigens may prevent naïve T cells from inducing pathological responses, thus complementing the pool of thymus-derived Treg cells. In this respect, gut-associated lymphoid tissue DCs may synthesize the vitamin A metabolite retinoic acid, that selectively induces CCR9 and $\alpha_4\beta_7$ integrin on $CD8^+$ T cells with gut tropism[51]. This phenomenon occurs more efficiently after oral as compared with intraperitoneal antigen administration, indicating differential DC targeting by the 2 immunization routes[51].

CD103 is the α chain of the $\alpha E\beta_7$ integrin expressed by most mouse and human intestinal lymphocytes and mediating lymphocyte adhesion to E-cadherin-expressing intestinal epithelial cells. CD103-expressing DCs may also be required to induce gut-tropic effector T cells in the MLN[51]. Interestingly, TGF-β plays a dominant role in CD103 induction on gut-tropic $CD8^+$ T cells, as shown in a mouse model of post-transplantation graft-*versus*-host disease (GVHD) with T-cell infiltration of the intestinal epithelium[52]. T cells from 2C T cell receptor-Tg mice that express a dominant negative TGF-β type Ⅱ receptor were incapable of upregulating CD103 upon migration into the intestinal epithelium[52]. In addition, CD103 expression on host-reactive $CD8^+$ T cells was causally related to the development of GVHD pathology and mortality. Although TGF-β activity is present locally within the intestinal milieu, this study did not exclude the possibility that CD8 effectors encounter TGF-β and upregulate CD103 expression before their entry into the intestinal epithelium. $CD103^{-/-}$ T cells migrate into the host intestine but are retained much less efficiently than wild-type T cells, indicating that CD103 expression may also contribute to T-cell accumulation in the gut[52]. In a T-cell transfer model of colitis, disease-inducing $CD4^+CD45RB^{high}$ T cells were shown to promote colitis development irrespective of their expression of CD103[53]. However, anti-CD103 antibodies abrogated the suppression of colitis mediated by Treg cells. Further experiments suggested that CD103 expression by Treg cells was not essential for their function, indicating the requirement for CD103 on non-T host cells for protection from colitis[53]. Of interest, ~50% of $CD11c^+$ DCs in the MLN co-expressed CD103 at high density, at variance with ~30% of splenic DCs. Sorted $CD103^+$ DCs activated the proliferation of allogeneic $CD4^+$ T cells to a similar extent compared with the $CD103^-$ counterpart but were potent inducers of CCR9 co-expression by day 4 of culture, suggesting their ability to impart gut tropism on T cells. In addition, $CD103^+$ DCs were inefficient at inducing IL-10 and IFN-γ production by T cells. Collectively, this study suggested that DC subsets that are primed in the immunosuppressive environment of the gut may be unable to drive the release of pro-inflammatory cytokines such as IFN-γ, thus preventing the development of unwanted effector responses to ingested antigens. Another report by the same investigators has shown that $CD103^+$ DCs isolated from the MLN may both induce *de novo* expression of Foxp3 in naïve T cells and maintain pre-existing $Foxp3^+$ cells[16]. The conversion of naïve T cells into Treg cells by CD103-expressing DCs was completely inhibited by anti-TGF-β antibodies, but further enhanced by exogenous TGF-β, so that provision of 1 ng/mL TGF-β to the T-cell/DC co-cultures translated into the expression of Foxp3 by ~50% of T cells[16]. Even the provision of high concentrations of TGF-β to $CD103^-$ DCs did not allow the generation of similar percentages of Treg cells to $CD103^+$ DCs, suggesting that $CD103^-$ DCs may lack an essential cofactor. Further experiments led the authors to identify retinoic acid as the cofactor for the TGF-β-driven conversion of Treg cells from naïve T cells. Compared with the $CD103^+$ DCs, $CD103^-$ DCs released higher amounts of pro-inflammatory cytokines (TNF-α, IL-6), and expressed higher levels of IL-23p19 and Tbet[16]. Collectively, this study showed the existence of functionally distinct DC populations in the MLN of normal mice, with apparent diverging functions. A companion paper by Sun *et al*[54] has shown, both in a lymphopenic mouse transfer model and in an immunologically complete setting, that retinoic acid released by LP DCs promotes Treg conversion in the presence of TGF-β. The LP DCs expressed a $CD8\alpha^-CD11c^+$ phenotype and displayed the morphologic features of conventional DCs, consisting of a stellar shape comparable to freshly isolated splenic DCs[54].

LP DCs

The extensive phenotypic and functional characterization of mouse LP DCs so far pursued has revealed a greater complexity than previously appreciated. The majority of

LP DCs express a $CD11b^+CD8\alpha^-$ phenotype, although $CD11b^-CD8\alpha^+$ and $CD11b^-CD8\alpha^-$ DCs have also been identified[55]. Treatment of mice with Flt3 ligand increases the proportion of LP DCs without significantly altering the relative proportion of DC subsets, thus allowing the purification of a higher DC number for detailed functional analyses. Using this approach, some authors have shown that LP DCs are not fully mature *in situ* but they can be induced to differentiate in response to appropriate stimuli[55]. LP DCs were also less efficient at stimulating OVA-specific T-cell proliferation *in vitro* when compared with splenic DCs, and mediated the development of tolerance when transferred to mice fed with OVA[55]. LP DCs exhibited a unique cytokine profile, consisting of low levels of IL-12p40 mRNA associated with constitutive IL-10 and type I IFN production[55]. A specialized subset of LP DCs with a $CD8\alpha^+$ phenotype has been identified in mice[30]. Gut-derived $CD8\alpha^+$ DCs secrete IFN-α and support antigen-specific suppression mediated by $CD4^+CD25^+$ Treg cells. Furthermore, $CD8\alpha^+$ DCs favor the differentiation of Tr1-like cells that release high quantities of IL-10, IL-4 and IFN-γ upon activation with plate-bound anti-CD3 antibodies[30]. The ability of $CD8\alpha^+$ DCs to induce Tr1-like cells was not affected by their exposure to maturation stimuli, as reported for other populations of maturation-resistant, tolerogenic DCs[31].

Other LP DC subsets identified in mice include $CD11b^-CD103^{hi}$ and $CD11b^+CD103^{-/low}$ DCs. LP DCs can be further subdivided into $CD11b^-CD103^{hi}CX_3CR1^-$ DCs and $CD11b^+$ DCs with different CX_3CR1 (fractalkine receptor) expression levels[56]. The $CD103^{hi}CX_3CR1^-CD11b^-$ LP DCs originate through a DC-committed non-monocytic intermediate from MDP, a differentiation pathway that is driven by Flt3L. Conversely, $CD103^-CX_3CR1^+CD11b^+$ LP DCs derive from $Ly6C^{hi}$ monocytes and their derivation involves an extensive, granulocyte-macrophage colony-stimulating factor (GM-CSF)-driven local expansion in the mucosa. Importantly, mice that were persistently or transiently depleted of LP DCs neither developed spontaneous intestinal inflammation nor were susceptible to colitis development. In contrast, mice that harbored predominantly $CD103^-CX_3CR1^+CD11b^+$ LP DCs developed severe colitis in response to a DSS challenge, as evaluated by colonoscopy and histological examination. This pro-inflammatory activity was dependent on TNF-α secretion with ensuing epithelial damage, and might also be regulated through IL-10/TGF-β production by the $CD103^{hi}CX_3CR1^-CD11b^-$ LP DC subset[23]. This study highlighted the importance of a critical balance between LP DC subsets for tissue repair and gut homeostasis.

PP DCs

PP are the primary sites for the induction of immune responses in the intestinal mucosa and are representative of lymphoid follicles present in diffuse mucosal tissues. DCs from PP possess a unique capacity to induce T-cell responses that regulate systemic immunity through the release of IL-4 and IL-10 and that provide help for IgA B-cell differentiation. It has been shown that PP DCs reside in different anatomical sites, with $CD11b^+CD8\alpha^-$ DCs being localized in the subepithelial dome, $CD11b^-CD8\alpha^+$ in the interfollicular regions and $CD11b^-CD8\alpha^-$ [double-negative (DN) DCs] in both compartments[57]. The DN DCs constitute approximately 30% of PP DCs, are interspersed within the follicle-associated epithelium, with processes extending to the luminal surface, and occasionally associated with M cells within the M-cell pocket[58]. DN DCs express intracellular MHC molecules, indicating their immaturity, and secrete IL-12p70, suggesting functional similarity to the lymphoid DC subset[58]. Collectively, these studies indicate that DN DCs should be able to induce Th1 differentiation, at variance with myeloid DC subsets that have been implicated in IL-10 release and in skewing the immune response towards a Th2 profile. It has been proposed that orally delivered antigens may initially encounter the myeloid and DN DCs located underneath or within the follicle-associated epithelium. As a result of feeding with low-dose antigen, antigen uptake by the DCs would not result in DC activation and migration but rather in the differentiation of Th2 or Th3 cells with regulatory properties. The T cells interacting with antigen would then secrete IFN-γ in the absence of activation signals by the DN DCs, thus becoming anergized. If soluble protein antigen is given at high dose, T-cell activation would occur in the PP and LP as a result of DC stimulation and migration to the interfollicular regions or the MLN. In this scenario, IL-10 produced by the myeloid DCs may serve to control detrimental inflammation induced by microbial antigens, whereas DN DCs and lymphoid DCs may be acting as the primary source of IL-12 for the induction of Th1 responses[58].

DCs in experimental colitis

The availability of mice expressing the diphtheria toxin receptor under the control of the *Cd11c* promoter has allowed the selective depletion of DCs and the study of DC role in the development of intestinal inflammation. DC ablation has been correlated with the amelioration of DSS-induced colitis[59]. DSS-stimulated bone marrow-derived DCs release high quantities of proinflammatory cytokines and chemoattractants *in vitro*. Furthermore, DC adoptive transfer exacerbated disease manifestations, whereas DC ablation attenuated disease severity as shown by histological examination of tissue sections. However, DC activation with TLR9 ligands before colitis induction with DSS exacerbated disease manifestations. Since DSS injures the colonic epithelium, it is conceivable that, at least in this model, DCs exerted protective effects through stimulating repair of colonic epithelial cell layers rather than modulating the immune response.

There is evidence that DCs may play both protective and detrimental roles in intestinal pathology. In DSS colitis, an experimental model resembling acute colitis, DC ablation during DSS administration ameliorated disease manifestations[60]. Conversely, colitis was exacerbated if DCs were ablated before DSS treatment, suggesting that DCs are protective in initial phases of colitis but play a pathogenic role during the disease course[60].

In a T-cell transfer model of colitis induced by CD45RB^{hi}CD4$^+$ T cells, transplanted T cells formed aggregates with sub-epithelial CD11c$^+$ DCs in the MLN[61]. Blocking OX40-OX40L interactions prevented the development of colitis. DC activation *via* CD40 has been reported to cause colitis in the absence of T and B cells and through a cytokine-dependent mechanism[62].

Intestinal inflammation is correlated with significant changes in the cellular composition of the colonic LP. Gut inflammation in mice is accompanied by a marked infiltration of CD11c$^+$ DCs within the LP[63]. From a phenotypic standpoint, these DCs express high levels of CD80 and resemble mature activated DCs, while secreting low levels of IL-10 and IFN-α. Of interest, CD103$^+$ DCs were dramatically reduced in the LP of colitic mice but were detectable in the spleen, suggesting that intestinal CD103$^+$ DCs may migrate to lymphoid organs during inflammation.

The observation that IL-10-deficient and TGF-β-deficient mice develop spontaneous colitis point to IL-10 and TGF-β as important determinants of DC function in the gut. In a T-cell transfer model of colitis, Treg production of IL-10 was dispensable for disease suppression but IL-10 secreted by LP CD11b$^+$ macrophages was crucial to maintain FoxP3 expression in Treg cells[28]. It is conceivable that intestinal bacteria are a fundamental trigger of IL-10 production by LP macrophages through the activation of TLR signaling. It is presently unknown whether IL-10 signals are also required to maintain FoxP3 expression in Treg cells from other organs during inflammation[29]. Serum IL-10 is reportedly normal in patients with IBD[64]. However, LP mononuclear cells are impaired in the ability to release and respond to IL-10[64], suggesting that IL-10 provision might be beneficial in human IBD through effects on the DC compartment[65].

DCs IN HUMAN IBD

DCs accumulate at sites of inflammation in patients with IBD, whereas both myeloid DC and pDC populations are depleted in the peripheral blood of patients with active disease. DC recruitment to the gut may be the result of an increased expression of chemokines such as CCL20 or of addressins, such as mucosal vascular addressin cell adhesion molecule-1 (MAdCAM-1). CCL20 is a nuclear factor-κB-regulated molecule that mediates the CCR6-dependent recruitment of DCs and T cells to mucosal surfaces. CCL20 has been detected at increased levels in the mucosal epithelium covering lymphoid follicles in patients with both types of IBD, in proximity to CCR6-expressing cell types, such as langerin$^+$ DC, B cells and memory T cells[66]. Phenotypically heterogeneous populations of DCs have been identified in colonic tissues and MLN from patients with IBD[67]. One population consists of immature DCs expressing DC-specific intercellular adhesion molecule-grabbing non-integrin (SIGN) and mainly located at antigen-capturing sites in the mucosa and medullary cords. A second population expresses CD141 and has a similar localization as that of DC-SIGN$^+$ DCs. The third DC subset consists of mature DCs expressing S-100 and CD83 and is located in the T-cell areas in both the colonic lymphoid follicles and the MLN. In contrast, pDCs were hardly detected in the colon and MLN. The fraction of circulating DC precursors has been found to correlate with established IBD activity indices[68]. Specifically, higher percentages of pDC and myeloid DCs were measured during disease remission compared with acute flares, suggesting DC migration to secondary lymphoid organs. In line with this hypothesis, DC precursors from patients with IBD expressed $\alpha_4\beta_7$, a gut-homing integrin marker and receptor for MAdCAM-1 also detected on LP T cells[69]. Importantly, immature DCs are significantly reduced in active IBD, indicating that potentially tolerogenic DC subsets may be defective during disease reactivation.

M-DC8$^+$ DCs have been detected in the subepithelial dome of ileal PP from 3 patients with untreated CD[70]. In one of these patients, an ileal biopsy performed 6 mo after glucocorticoid-induced clinical remission documented the complete disappearance of M-DC8$^+$ DCs from the ileal mucosa. The observation that M-DC8$^+$ DCs secrete large amounts of TNF-α but not IL-10 upon stimulation with lipopolysaccharide (LPS) suggests that these cell types might contribute to the pathogenesis of IBD[70].

Colonic CD11c$^+$ DCs from patients with either CD or UC express higher levels of TLR2 (interacting with peptidoglycan and bacterial lipoproteins), TLR4 (a receptor for LPS) and CD40 compared with non-inflamed CD tissues and tissues from healthy controls[71]. This may lead to enhanced recognition of bacterial products and an increased response to them. Importantly, treatment with TNF-α blocking antibodies translated into the downregulation of CD40 expression on DCs, irrespective of resolution of inflammation at the tissue level. Also, production of IL-6 and IL-12 at the single-cell level was increased in DCs from patients with CD but not with UC compared with healthy controls.

Although the DC abnormalities documented in UC generally resemble those evidenced in CD, differences may exist when comparing these 2 major forms of IBD. Epstein-Barr virus-induced gene 3 (EBI3) encodes a secreted protein that shares 27% amino acid sequence identity with IL-12p40. EBI3 can substitute for p40 to form a heterodimer with IL-12p35, and is an IL-27 subunit[72]. EBI3 expression is upregulated by macrophage/DC-like cells within the LP of patients with active UC but not CD[73]. These data are consistent with a scenario in which EBI3 opposes the IL-12p40/p35 heterodimer and downregulates the cytotoxicity promoted by IL-12. In addition, this study reinforced the view that macrophages and DCs serve more than one role in the pathogenesis of IBD, being either protective or detrimental.

DCs AS TOOLS AND TARGETS FOR THERAPY IN IBD

Different approaches have been proposed to restore and/or enhance the tolerogenic properties of DCs, including *in vitro* treatment with growth factors and use of drugs that target DC number and/or function (Figure 2)[74,75].

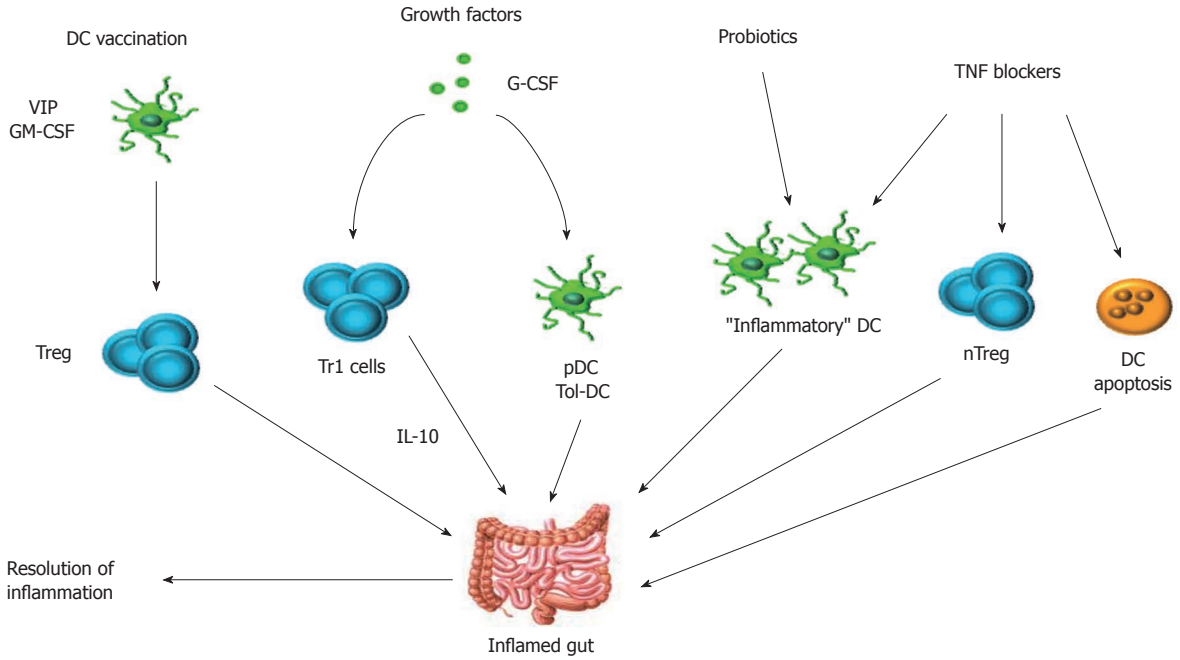

Figure 2 Potential strategies to modulate dendritic cell functionality in human inflammatory bowel disease. *In vitro* differentiated tolerogenic dendritic cell (DC) have been administered to mice with inflammatory/autoimmune disorders. Vasoactive intestinal peptide (VIP) has a unique ability to skew DC function towards a tolerogenic profile and has been used to vaccinate animals with colitis, rheumatoid arthritis and post-transplantation graft-*versus*-host disease[76,77]. Selected growth factors have shown to modulate immune reactivity *in vivo*. For instance, granulocyte colony-stimulating factor (G-CSF) has been successfully given to patients with Crohn's disease, leading to accumulation of pDC in the lamina propria and increase in IL-10 production, with favorable repercussions on disease manifestations[89]. GM-CSF: Granulocyte-macrophage colony-stimulating factor; IL: Interleukin; TNF: Tumor necrosis factor.

Adoptive transfer of cytokine-modulated DCs

DCs have been targeted in animal models of intestinal inflammation. Regulatory DCs differentiated with vasoactive intestinal peptide (VIP) and GM-CSF (DC-VIP) have been transferred to BALB/c mice with colitis induced by trinitrobenzene sulfonic acid (TNBS), a Th1-mediated disease requiring T-cell activation with subsequent macrophage recruitment and activation[76]. Mice received the DC preparations either 8 h after TNBS instillation or 6 d after colitis induction in order to assess their therapeutic effects both on colitis induction and on established disease. DC infusion ameliorated disease severity and histopathology, being associated with inhibited Th1 responses and with the *in vivo* differentiation of IL-10-producing Treg cells. DC-VIP decreased the production of proinflammatory cytokines both systemically and locally, and deactivated spleen macrophages, blunting the *in vitro* production of TNF-α and IL-12 in response to LPS challenge. Importantly, DC-VIP augmented the number of TGF-β/IL-10-secreting $CD4^+$ T cells within LP mononuclear cells cultured in the presence of colonic proteins extracted from colitic mice. Also, $CD4^+$ T cells obtained by the MLN of DC-treated mice suppressed proliferation and IL-2 production by autoreactive $CD4^+$ T cells in response to colonic proteins. These $CD4^+$ T cells were also capable of reversing the body weight loss which is characteristic of TNBS-induced colitis when transferred to colitic mice, suggesting the acquisition of a potent regulatory activity after their *in vivo* encounter with DC-VIP. Finally, the therapeutic effect of $CD4^+$ T cells was dependent on TGF-β and IL-10 production, being reversed by *in vivo* blocking of these cytokines. This elegant study suggests that *in vitro* conditioning of DC preparations with VIP and self-antigens might be pursued as therapeutic strategy in colitis and possibly other inflammatory disorders[77,78], also to minimize patients' dependence on non-specific immune suppressive drugs currently in use for human IBD.

TGF-β1 gene-modified immature DCs with enhanced tolerogenicity undergo efficient transport to inflamed colonic tissues and delay the progression of murine IBD induced by DSS[79]. DC injections in mice with established colitis alleviated weight loss and reduced intestinal bleeding, translating into a lower disease activity index compared with control DC or untreated mice. DC vaccination was associated with increased Treg numbers in the MLN and with increased TGF-β1 levels in mouse colon tissues[79].

Probiotic bacteria

Probiotics, mainly belonging to the lactic acid bacteria (LAB) family, exert beneficial effects in human or animal health and are presently considered as peace-keepers in the gut[40,80]. The regular intake of probiotic bacteria may contribute to immune homeostasis by altering microbial balance or by interacting with intestinal immune cells. Dysbiosis, namely, an imbalance between pro-inflammatory and anti-inflammatory bacteria in favor of the former, may have a causative role in patients with IBD. Probiotics have been tested both in animal models of colitis and in patients with IBD. The potential mechanisms of action of probiotic bacteria include their interaction with

TLR and DCs in the gut. The demonstration of anti-inflammatory effects after systemic administration of probiotics suggests that regulatory cell populations may be induced distant from the site of inflammation[81].

Importantly, probiotics may present strain-specific *in vitro* immune modulating actions, that are strictly correlated with their *in vivo* anti-inflammatory effects. For instance, *L. salivarius* Ls33 and *L. rhamnosus* Lr32 possess high immunoregulatory capacities and efficiently protect from murine TNBS-induced colitis, at variance with other strains such as *L. acidophilus* NCFM and *L. lactis* MG1363 that exhibit an opposite immunological profile[82]. The protective effect of probiotic-treated DCs was attributed to a downregulation of proinflammatory mediators such as IL-12 and IL-17, paralleled with an acute overexpression of IFN-γ and IDO[82]. Of interest, pre-formed naturally occurring Treg cells were required for the protective effect of probiotic-treated DCs, as shown by experiments with an anti-CD25 rat monoclonal antibody.

The probiotic mixture designated IRT5 contains 5 different probiotic strains. IRT5 has been shown to induce T-cell and B-cell hyporesponsiveness when administered for 20 d to mice by the oral route[83]. Even more intriguingly, IRT5 increased FoxP3 expression in MLN as a result of the enhanced conversion of naïve T cells into Treg cells and the augmentation of the suppression function of pre-existing natural Treg cells. These effects were mediated through the promotion of DC tolerogenic activity, with high expression of IL-10, TGF-β, IDO and COX-2 mRNA. IRT5 retarded the progression of TNBS-induced colitis and was also efficacious in other immune-mediated disorders, such as atopic dermatitis and collagen-induced arthritis. Both the atopic ear and the inflamed colon of IRT5-treated mice were enriched with FoxP3-expressing Treg cells, likely as a result of increased tissue levels of CCL1 and CCL22, chemokines involved in Treg attraction.

The probiotic mixture VSL#3, which contains 8 different bacterial strains and is clinically beneficial in human IBD and pouchitis, has been reported to downregulate IL-12 and upregulate IL-10 production by human blood and colonic LP DCs in a dose-dependent fashion[84]. This change in DC functional polarization translated into the inhibition of *in vitro* generation of Th1 cells from allogeneic $CD4^+$ T cells.

A comprehensive analysis of previously published studies detailing the activity of different probiotics in animal models of colitis suggests that the colitis model used may affect the results[85]. An interesting study has evaluated the ability of 3 *Lactobacilli* strains (*plantarum*, *LGG* and *paracasei* B21060) to activate DCs either directly or indirectly through epithelial cells. While inducing similar degrees of DC phenotypic maturation, the different strains elicited differential cytokine release, with *L. paracasei* inducing lower levels of IL-12p70, TNF-α and IL-10. The lactobacilli also affected epithelial cell function, and supernatants of *L. paracasei*-treated epithelial cells drastically reduced the ability of DCs to activate T cells and drive their polarization towards a Th1 phenotype. Finally, the *in vitro* activity of probiotics was predictive of their *in vivo* efficacy in an acute model of colitis. Taken together, these studies indicate that probiotics interact both with immune cells and with non-immune cells and that the clinical use of individual bacterial strains should be proposed and recommended only after taking into account *in vitro* immunostimulatory or immunoregulatory activity.

Immune modulating drugs

Granulocyte-CSF (G-CSF) has remarkable immune modulating activities[86]. Indeed, G-CSF mobilizes DC2, differentiates tolerogenic DCs *in vitro* through IL-10 and IFN-α, and polarizes naïve T cells to a Tr1-like functional profile[31,87,88]. G-CSF has been administered to patients with CD in order to modulate immune reactivity and induce potential clinical benefit[89]. Nine patients with active CD received subcutaneous G-CSF for 28 d at 5 μg/kg of body weight. Six patients reported improvement in the CD activity index (CDAI) and achieved either a clinical response (4 patients) or remission (2 patients). The 3 non-responding patients had a longer duration of disease, had had bowel resections and one was the only CD patient with active fistulae. In responder patients, IL-10 production by isolated memory $CD4^+$ T cells was significantly higher at the end of G-CSF treatment compared with non-responders. Conversely, IFN-γ production in post-G-CSF peripheral blood samples was significantly higher in non-responders. G-CSF also affected the relative proportion of circulating myeloid DCs and pDCs, inducing a decrease in the myeloid DC-to-pDC ratio in responding patients. Notably, 4 patients in the responder group showed an increase in LP $CD123^+$ DCs[89]. In sharp contrast, accumulation of $CD123^+$ pDCs could not be evidenced in the LP of non-responders. Finally, the percentage of FoxP3-expressing cells within LP $CD25^+$ cells decreased significantly in non-responders at the end of treatment. In line with this, the fraction of $CD25^{hi}FoxP3^+$ cells increased in the LP of responding patients at the end of treatment, although these differences failed to achieve statistical significance. Collectively, this study provided proof-of-principle in favor of IL-10-mediated immune regulation by G-CSF in patients with IBD and suggested that treatment with this cytokine may translate into disease control. G-CSF at 3 μg/kg of body weight was also highly effective at controlling an UC-like syndrome in a 23-year-old patient with glycogen storage disease Ib[90]. G-CSF therapy was maintained for 16 years, with good control of gastrointestinal symptoms and dramatic improvement of colon histology.

There is evidence that TNF-α antagonism translates into changes in DC function. Although this has been primarily shown in patients with rheumatoid arthritis, it is likely that modifications of DC functions by TNF blockers may also impact on the clinical manifestations of IBD. Both etanercept and adalimumab were shown to downregulate CD83, CD80 and CD86 expression on monocyte-derived DCs and to reduce their T-cell stimula-

tory capacity[91]. Anti-TNF-treated DCs polarize T-cell responses *in vitro* and favor T-cell release of IL-10, IL-4 and IL-17. Although no correlation was found between the clinical response to TNF blockade and the functional modulation of DCs *in vitro*, DCs derived from patients with rheumatoid arthritis given TNF blocking agents enhanced T-cell production of IL-10, while decreasing the release of IL-4, IL-17 and IFN-γ. Infliximab may also suppress the antigen-presenting capacity of DCs derived from patients with psoriasis by reducing the expression of CD1a and costimulatory molecules, an effect that is not reversed by LPS[92].

Mesenchymal stromal cells

Mesenchymal stromal cells (MSC) are cells endowed with multi-lineage differentiation capacity and have been isolated from bone marrow, adipose tissue, amniotic fluid, placenta and umbilical cord blood. MSC affect both innate and adaptive immune responses and have reduced immunogenicity, thus being a promising therapeutic tool for inflammatory, autoimmune and degenerative diseases[93]. Adipose tissue-derived MSC have been shown to ameliorate experimental colitis through the promotion of IL-10 release with subsequent inhibition of activated macrophages and differentiation of Treg cells[94]. Importantly, the intrafistular injection of *in vitro*-expanded MSC (median number: 64×10^6 for each patient) has resulted into sustained complete closure of fistula tracks, reduction of perianal disease activity index, and with rectal mucosal healing in 10 patients with CD[95]. Intriguingly, the percentage of mucosal and circulating Treg cells significantly increased during treatment and remained stable until completion of the 12-mo follow-up period. T cells isolated from the inflamed mucosal areas released higher amounts of IL-10 when co-cultured with MSC *in vitro*. Based on previously published data on the ability of IL-10 to skew DC differentiation towards a tolerogenic profile[96,97], it is tempting to speculate that MSC therapy may target pro-inflammatory DCs *in vivo*, through the promotion of IL-10 release by colitogenic T cells.

Based on the experience reported in patients with GVHD[98], MSC have also been infused intravenously, at a dose of $1\text{-}2 \times 10^6$ cells/kg of body weight, in 10 patients with chronic active CD, refractory to all currently available medical therapeutic options[99]. Although MSC-based therapy did not induce clinical remissions as defined by a CDAI < 150, reductions of 70 points in CDAI were recorded in 3 patients. The biological effects of MSC intravenous infusion included a trend towards higher percentages of $CD4^+CD127^+$ *bona fide* Treg cells. *In vitro*, patient-derived MSC inhibited the proliferation of autologous peripheral blood mononuclear cells and decreased their production of TNF-α. Collectively, the studies published so far demonstrate that MSC from patients with CD can be expanded *in vitro* and may induce favorable therapeutic effects *in vivo*, including differentiation of Treg cells, and possibly functional inhibition of DCs within the inflamed gut.

CONCLUSION

It is now clear that DC activation is a contributing factor in generation of IBD, as indicated both by mouse models of gut inflammation and by human disease. The recent advances in the phenotypic and functional characterization of DC populations in humans have unveiled a remarkable and previously unappreciated heterogeneity within the DC compartment but have also led to the identification of potential targets for therapeutic manipulation. A thorough understanding and knowledge of DC subsets and functionality in humans is a prerequisite for delivering interventions aimed at correcting DC malfunctioning. Several other cell types with APC function cooperate to ensure appropriate immune responses in the gut. They include IEC, basophils, MSC and other non-immune cells[10,41]. Theoretical strategies to interfere with DC activity include vaccination with gene-modified DCs or cytokine-treated DCs to restore tolerance, growth factor administration, therapy with DC-modulating drugs, and use of probiotics. The impact of TNF blocking antibodies on DC functions needs to be further investigated. Some of these approaches have been successfully applied to animal models of gut inflammation and other autoimmune/inflammatory disorders such as multiple sclerosis and arthritis. In this respect, "tolerogenic vaccination" with cytokine-modulated DCs may hold promise for the treatment of intestinal inflammation. However, there is a theoretical concern that tolerogenic DCs suppress beneficial anti-infective and anti-tumor responses, in addition to unwanted immune reactivity. These issues must be carefully addressed before this approach is translated into the clinic. Studies in murine GVHD are somehow reassuring, having clearly indicated that the injection of cytokine-treated DCs preserves $CD8^+$-mediated cytotoxic responses against leukemia while blunting GVH reactivity[100,101]. Finally, the patient categories that may benefit from DC-based therapeutic approaches need to be identified. It should be emphasized that other cell-based interventions such as the intravenous infusion of MSC have not induced any clinical remission in severe refractory CD[99]. Conceivably, patients with IBD should be offered DC-centered treatments earlier in the disease course, following patient profiling and stratification on the basis of molecular predictors for complicated disease (genetic markers such as NOD2 homozygous or compound heterozygous, and anti-microbial antibodies) as well as clinical features at diagnosis[102]. There is evidence from both pediatric and adult IBD that treatment of short-duration CD with TNF antagonists is associated with better response and remission rates[103,104]. Whether DC-based approaches have the potential to slow disease progression and alter the natural history of IBD will hopefully be determined in the near future.

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