

Antigen Presenting Cells and T Cell Interactions in the Gastrointestinal Tract

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Introduction

Dendritic cells (DCs) are the key players in the immune response since they are the only cell type capable of presenting antigens to virgin T cells thereby initiating cell-mediated immunity and T cell-dependent antibody responses (IgG, IgA and IgE). They are called dendritic cells since in vitro, they extend long processes (dendrites) away from the cell body. Derived from the bone-marrow and relatively short lived, they are present in all tissue in low numbers, but are relatively prominent in the skin (Langerhans cells), airways (within the epithelium) and gut (in the lamina propria and organised lymphoid tissue). At these sites they are immature, expressing low levels of co-stimulatory molecules (such as CD80, CD86 and CD40) and relatively low surface expression of MHC molecules (1). However they are highly endocytic. When they encounter vaccines in adjuvants or infections, bacterial and viral products signal via toll-like receptors to increase co-stimulatory molecules, and the dendritic cell then processes and presents peptide ligands on MHC. The DC's then leave the tissue and migrate via afferent lymph to the T cell zones of draining lymph nodes where they initiate T cell responses (Figure 1). Even in nodes however they make up less than 1% of total mononuclear cells. (2)

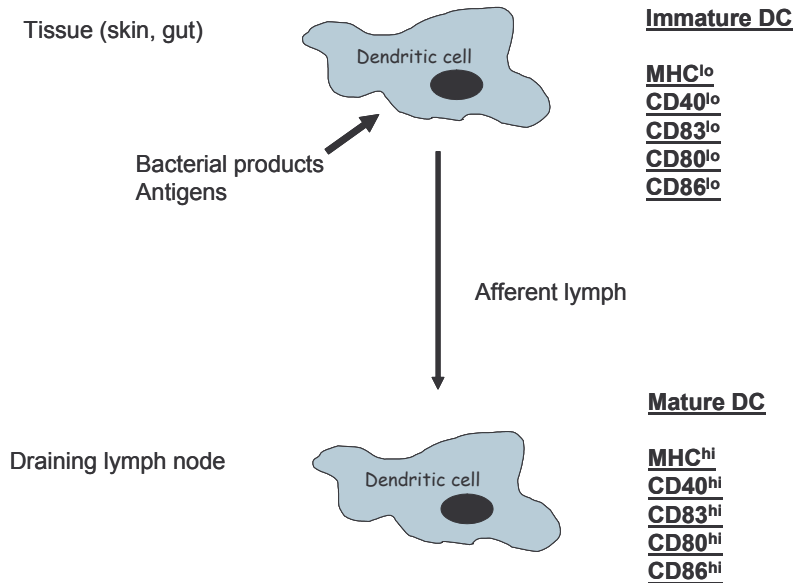


Figure 1

Dendritic cells also control T cell responses both quantitatively and qualitatively. Immature DC's presenting antigens to T cells tend to lead to T cell anergy rather than activation because of the lack of the co-stimulatory signals to the T cell. The profile of cytokines made by DC's also controls Th1/Th2 and Treg differentiation. Thus DC which make IL-12 (the major Th1-inducing cytokine in man) promote interferon- γ secreting T cells and DC which make IL-10 promote T reg cells or Th2 cells. It is probably simplistic to assume that all immature DC's tolerise T cells and all DC's in lymph nodes are mature, since there is ample evidence that immature DC's can prime T cells and that lymph node DC's are immature

Dendritic cells are also very heterogeneous. Broadly speaking in man they can be subdivided into 2 main categories (1). Plasmacytoid DC's are found in blood and the T cell zones of lymph nodes. Blood precursors, perhaps of lymphoid origin are dependent on Il-3 and CD40L for survival. They are CD11c- (CD11c is the α_x integrin which associated with CD18 forms the ligand for CD54 and fibronectin), CD123+ (the IL-3 receptor) and CD11b- (CD11b is the α_m integrin which associates with CD18 to form the Mac-1 antigen which is also a receptor for ICAM-1 and fibronectin) . Myeloid DC's are closely related to monocytes and when cultured with GM-CSF, and IL-4 blood monocytes become myeloid DC's. Skin Langerhans cells are the prototypic myeloid DC., expressing CD11c, and CD11b, but lacking CD123. Interstitial DC's

are also myeloid DC's but are found in lymph nodes and tissues. One of the major differences between plasmacytoid and myeloid DC's is that the former make abundant interferon-alpha which functions both as an anti-viral molecule but is also a potent inducer of Th1 cells in man.

Although there is a large literature on *in vitro* generated DC's, and blood DC's, much less is known about DC's in the gut. Technical difficulties make studying gut DC's problematic. First, they are present at very low numbers in both the mucosa and organised lymphoid tissue of the gut. Second, being in tissues, enzymatic digestion is needed to isolate them, but the process of digestion can affect their phenotype and function. For example, DC's are exquisitely sensitive to lipopolysaccharide, present in abundance in the gut lumen from commensal bacteria. Exposure of immature DC's to very low levels of LPS rapidly increases co-stimulatory molecules and cytokine production (3). In rats it is possible to study the DC's draining from the gut very close to their natural state by cannulating the thoracic duct of mesenteric lymphadenectomised rats, so that DC's can be collected minutes after they have left the gut wall (4), but this is not possible in man.

In this brief review we will try to focus on what is known about DC's in human gut and sample the rodent literature where there are important lessons to be learned.

What are the types of DC's in the human gut?

Two techniques have been used to look at DC's in normal human gut, immunohistochemistry and flow cytometry. The first has the advantage of looking at cells in situ, but is non-quantitative, whereas the second is quantitative but suffers from the fact that the isolation procedure might alter cell function and phenotype. In tissue sections, a few CD11c+ cells can be seen in the colonic lamina propria. Some appear to express CD83, a marker of mature DC's (5,6). There is essentially no staining for CD123 or other markers of plasmacytoid DC's in the lamina propria of the lower bowel (MacDonal and Pickard, personal observation). By flow cytometry, CD11c+ cells can be identified in cell suspension s of colonic mucosa and they express low levels of CD80, 83 and 86, so they are probably immature DC's (7). In gut inflammation, as in inflammatory bowel disease, some workers have shown higher expression of co-stimulatory molecules on DC's (6). However other workers have not confirmed these findings ((5,7) and

indeed claimed a reduction in DC number in IBD (5); but an absence of mature DC's in IBD is so counter-intuitive that these anomalous results probably reflect the difficulties of working with such a rare and poorly characterised cell type in the gut.

The organised lymphoid tissue of the gut, the Peyer's patches and solitary lymphoid follicles in small and large bowel, are the inductive site of mucosal immune responses and are probably where DC's control mucosal T cell responses (8). Because of their site, they are unique lymphoid tissues in that they have no afferent lymphatics, instead antigen crosses into the PP across the follicle associated epithelium (FAE) between the gut lumen and the dome region. M cells within FAE promiscuously transport gut antigens into the lymphoid tissue. In PP, plasmacytoid DC's are found in the T cell zones, but CD11c+ myeloid DC's are present primarily below the FAE (Figure 2 and ref 9). There is no published information on whether these cells are mature or immature in man, but they do express DC-SIGN, a C-type lectin which functions as a co-receptor for HIV (9). In rodents, the DC's in the dome of the Peyer's patch are immature and are attracted to this site by the chemokine CCL20, produced by FAE and which binds to CCR6 on the immature DC's (10).

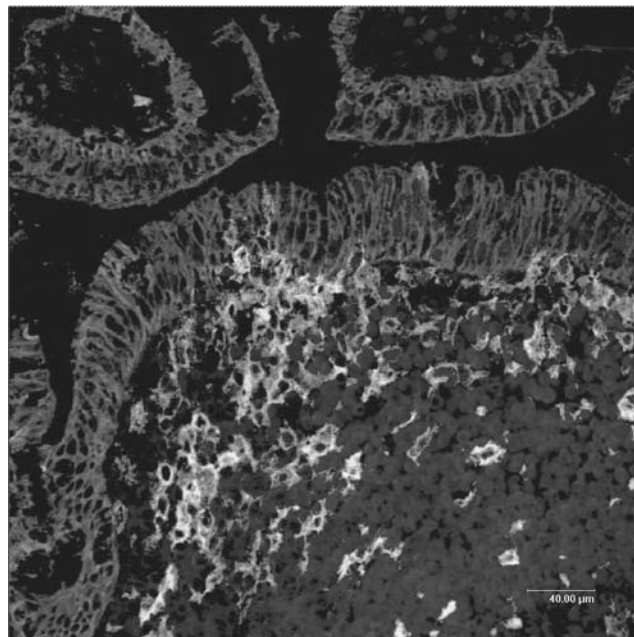


Figure 2

Animal studies which reveal novel insights into mucosal DC's

DC's play at least 2 important roles in mucosal immunity. The PP are the site of the induction of mucosal immune responses. T and B cells activated by gut antigens leave the PP and migrate via the mesenteric lymph nodes, thoracic duct and blood, back to the lamina propria. PP DC's preferentially induce expression of $\alpha 4\beta 7$ integrin on CD8T cells (11), allowing them to home to the gut since lamina propria vessels express the gut specific addressin, MAdCAM-1. Moreover PP DC's preferentially metabolise vitamin A (retinol) to retinoic acid which also induces the $\alpha 4\beta 7$ integrin on T cells (12). Thus the gut homing tropism of PP derived T cells is probably controlled by DC's.

A second novel function is the ability of DC's in the lamina propria to reach through the epithelium and sample antigens from the lumen (Figure 3). This was first observed in vitro in co-cultures of DC's and gut epithelial cell lines, then in vivo in ileal loops in mice challenged with a bacterial infection (13). In the ileum of mice over-expressing IL-23, DC's also send processes into the lumen (14). More recently mice have been produced which express the fractalkine receptor in DC's, linked to GFP, so that DC's can be visualised in real time in vivo (15). These mice demonstrate that DC's in the villus cores reach through the epithelium and sample commensal microbes. There is also evidence that DC's transport luminal commensals to the mesenteric lymph nodes (16).

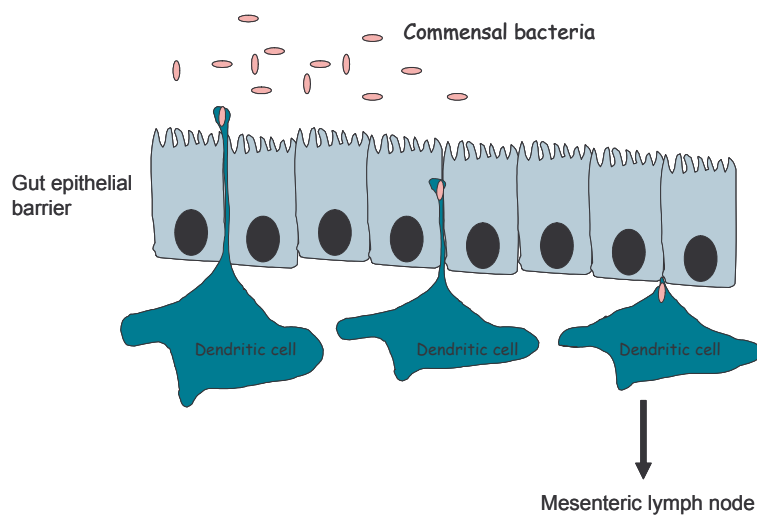


Figure 3

Dendritic cell cytokines which polarise T cell responses

Given the primacy of DC-derived cytokines in controlling T cell responses, there has been interest in cytokines made by DC's in the gut in health and disease. Most data derives from mice. In Peyer's patches, 2 populations of DC's in the subepithelial dome produce different cytokines. The CD11b⁺/CD8 α - population make IL-10 and drive Th2 cells whereas the CD11b^{low}/CD8 α population make IL-12 and drive Th1 responses (17). It is not clear how these 2 populations interact to prime particular T cell responses in PP tissue. In the normal lamina propria of the mouse, where these 2 populations are also present, the predominant cytokine produced appears to be IL-10 (18).

In man, there is very little data on DC's in PP in terms of cytokine production. T cells from normal human Peyer's patches are strongly Th1 polarised with abundant production of interferon- γ , and there is spontaneous production of IL-12 by PP cells cultured in vitro (19). However the source of this IL-12 (macrophages or dendritic cells) is not known. In the lamina propria of normal gut it has been reported that a few DC-SIGN⁺ cells contain immunoreactive IL-18, but that 70% contain IL-12 (20). There must be doubt about this observation since IL-12p40 is undetectable in normal gut by PCR (21). In IBD, the number of immunoreactive IL-18 and IL-12 containing DC's is increased (20). Using flow cytometry, very few DC's in normal colon make IL-6, IL-10 or IL-12. In Crohn's disease, both IL-6 and IL-12 containing DC's increase with no significant increase in IL-10 containing DC's (22).

The probiotic cocktail VSL#3 has been successfully used to treat pouchitis in man (23). When added to lamina propria cells, it increases production of IL-10 by DC's. Likewise in patients, giving VSL#3 increases IL-10 in mucosal biopsies (24). Although IL-10 is generally thought of as being important in Th2 or Treg generation, its main function is to inhibit antigen presentation by accessory cells, which would have the net effect of decreasing T cell activation.

Do gut dendritic cells play a role in human gut disease?

Cross-sectional studies looking at DC's in diseased versus normal gut can identify differences in DC maturation state and phenotype but they cannot make mechanistic associations. For example in Crohn's disease, where IL-12 producing dendritic cells probably drive the T cell response to

antigens of the commensal flora which cause the disease, it is not known if this is a primary or secondary response. Likewise in adverse responses to food proteins, do gut DC's present gluten peptides to CD4 Th1 cells and cause celiac disease; or do gut DC's drive the Th2 responses responsible for over-expression of IL-4 and switching to IgE which underlies immediate hypersensitivity responses to food antigens? The answers to these 2 questions are not known. Surprisingly, in celiac disease there is no information of dendritic cell function and it is not known which cytokines drive the Th1 response in the lamina propria characteristic of the disease. We previously identified a patient whose celiac disease appeared to be triggered by treatment with type 1 interferon and also showed type 1 interferon in celiac tissue, of interest because type 1 interferon is a potent inducer of Th1 cells in man (25). Likewise we also demonstrated that addition of type 1 interferon to anti-CD3 activated T cells in organ cultures of human fetal gut produced a Th1 immune response and a flat mucosa (26). However these data remain circumstantial until we can determine if DC's in celiac mucosa make type 1 interferons.

One area which has provoked some thought is the claim that gliadin , a food protein, has direct effects on dendritic cells. Peptic fragments of gliadin added to human monocyte-derived DC's induce phenotypic maturation and secretion of cytokines, particularly IL-6, IL-8 and TNF α (27). It needs to be determined if the same effect is seen with gut DC's, and the receptors to which the fragments are binding need to be identified, but it does raise the interesting possibility that food proteins may have direct effects on DC's; a reverse of the current position of most immunologists who see the main determinant of food hypersensitivity as the host immune response and genetics.

Concluding remarks

Despite their undoubted importance in immune responses, studies of dendritic cells in the gut are in their infancy. Functional studies in mice indicate that the relationship between DC's and the antigens of the gut flora are much more dynamic than previously considered with DC's reaching through into the lumen and capturing antigens. However whether these DC's present gut antigens to mucosal T cells is not well understood, and overall the role of DC's in gut immunity deserves greater attention.

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Legends for Figures

Figure 1.

Immature dendritic cells in the tissues can capture antigens but are poor at presenting peptides to T cells because they express low levels of co-stimulatory molecules and T cells. However on exposure to bacterial products such as lipopolysaccharide, they increase expression of costimulatory molecules and move into draining lymph nodes where they can efficiently present peptides to T cells.

Figure 2.

Confocal image of myeloid dendritic cells in a normal human Peyer's patch. The tissue section is stained with CD111c-FITC. Note the accumulation of dendritic cells just below the FAE (original magnification x400).

Figure 3.

Dendritic cells in the gut lamina propria reach through the epithelium to capture luminal commensals. They then probably migrate to the mesenteric lymph nodes.