Mol. Nutr. Food Res. 2009, *53*, 947 – 951 DOI 10.1002/mnfr.200800192

Review

Antigen presenting cells and T cell interactions in the gastrointestinal tract

Thomas T. MacDonald, Anna Voessenkamper and Antonio Di Sabatino

Centre for Infectious Diseases, Institute of Cell and Molecular Science, Barts and the London School of Medicine and Dentistry, London, UK

Dendritic cells make up less than 1% of the cells in lymph nodes and tissues, but they are critical in initiating and directing T cell immune responses. In the gut, with exposure to myriad food and bacterial antigens, they probably control T cell unresponsiveness to food antigens and T cell hypersensitivity in disease situations. The need for the immune system to 'know' gut luminal antigens is demonstrated by the fact that dendritic cells send processes through the epithelium and directly sample antigens in the gut lumen. We present evidence that type I INF made by plasmacytoid dendritic cells may be important in coeliac disease.

Keywords: Cell-mediated immunity / Cytokine / Dendritic cells / Gut / Peyer's patch / Th1

Received: May 16, 2008; revised: August 16, 2008; accepted: January 8, 2009

1 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 DCs 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 costimulatory 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 and infectious agents, bacterial and viral products signal *via* pattern-recognition receptors to increase costimulatory molecules, and the DC then processes and presents peptide ligands on MHC. The DCs then leave the tissue and migrate *via* afferent lymph to the T cell zones of draining lymph nodes where they initiate T cell responses (Fig. 1). Even in nodes, however, they make up less than 1% of total mononuclear cells [2].

DCs also control T cell responses both quantitatively and qualitatively. Immature DCs presenting antigens to T cells tend to lead to T cell anergy rather than activation because of the lack of the costimulatory signals to the T cell. The profile of cytokines made by DCs also controls Th1/Th2 and Treg differentiation. Thus, DC which make IL-12 (the major Th1-inducing cytokine in man) promote INF-γ secreting T cells and DC which make IL-10 promote Treg cells or Th2 cells. It is probably simplistic to assume that all immature DCs tolerise T cells and all DCs in lymph nodes are mature, since there is ample evidence that immature DCs can prime T cells and that lymph node DCs are immature.

DCs are also very heterogeneous. Broadly speaking in man they can be subdivided into two main categories [1]. Plasmacytoid DCs 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 DCs are closely related to monocytes and when cultured with GM-CSF, and IL-4, blood monocytes become myeloid DCs. Skin Langerhans cells are the prototypic myeloid DC, expressing CD11c, and CD11b, but lacking CD123. Interstitial DCs are also myeloid DCs but are found in lymph nodes and tissues. One of the major differences between plasmacy-

Correspondence: Professor Thomas T. MacDonald, Centre for Infectious Diseases, Institute of Cell and Molecular Science, Barts and the London School of Medicine and Dentistry, 4 Newark St, London E1 2AD, UK
E-mail: t.t.macdonald@qmul.ac.uk
Fax: +44-0207-882-2185

Abbreviations: DCs, dendritic cells; **FAE,** follicle associated epithelium; **IBD,** inflammatory bowel disease; **PP,** Peyer's patch

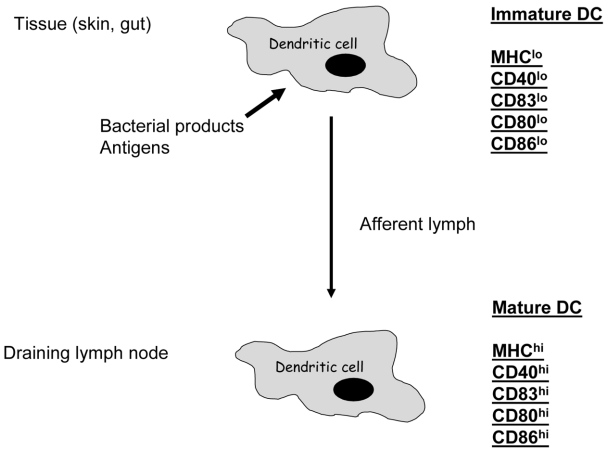

Figure 1. Immature DCs in the tissues can capture antigens but are poor at presenting peptides to T cells because they express low levels of costimulatory molecules and T cells. However, on exposure to bacterial products such as LPS, they increase expression of costimulatory molecules and move into draining lymph nodes where they can efficiently present peptides to T cells.

toid and myeloid DCs is that the former make abundant INF-α which functions as an antiviral molecule but is also a potent inducer of Th1 cells in man.

Although there is a large literature on *in vitro* generated DCs, and blood DCs, much less is known about DCs in the gut. Technical difficulties make studying gut DCs 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, DCs are exquisitely sensitive to LPS, present in abundance in the gut lumen from commensal bacteria. Exposure of immature DCs to very low levels of LPS rapidly increases costimulatory molecules and cytokine production [3]. In rats it is possible to study the DCs draining from the gut very close to their natural state by cannulating the thoracic duct of mesenteric lymphadenectomised rats, so that DCs can be collected in 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 DCs in human gut and sample the rodent literature where there are important lessons to be learned.

2 What are the types of DCs in the human gut?

Two techniques have been used to look at DCs in normal human gut, immunohistochemistry and flow cytometry. The first has the advantage of looking at cells *in situ*, but is nonquantitative, 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 DCs [5, 6]. There is essentially no staining for CD123 or other markers of plasmacytoid DCs in the lamina propria of the lower bowel (MacDonald and Pickard, personal observation). By flow cytometry, CD11c+ cells can be identified in cell suspensions of colonic mucosa and they express low levels of CD80, CD83 and CD86, so they are probably immature DCs [7]. In gut inflammation, as in inflammatory bowel disease (IBD), some workers have shown higher expression of costimulatory molecules on DCs [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 DCs 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 (PP) and solitary lymphoid follicles in small and large bowel, are the inductive site of mucosal immune responses and are probably where DCs 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 DCs are found in the T cell zones, but CD11c+ myeloid DCs are present primarily below the FAE (Fig. 2 and [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 coreceptor for HIV [9]. In rodents, the DCs in the dome of PP are immature and are attracted to this site by the chemokine CCL20, produced by FAE and binds to CCR6 on the immature DCs [10].

3 Animal studies which reveal novel insights into mucosal DCs

DCs play at least two 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 DCs preferentially induce expression of α4β7 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 DCs preferentially metabolise vitamin A (retinol) to retinoic acid which also induces the α4β7 integrin on T cells [12]. Thus, the gut homing tropism of PP-derived T cells is probably controlled by DCs.

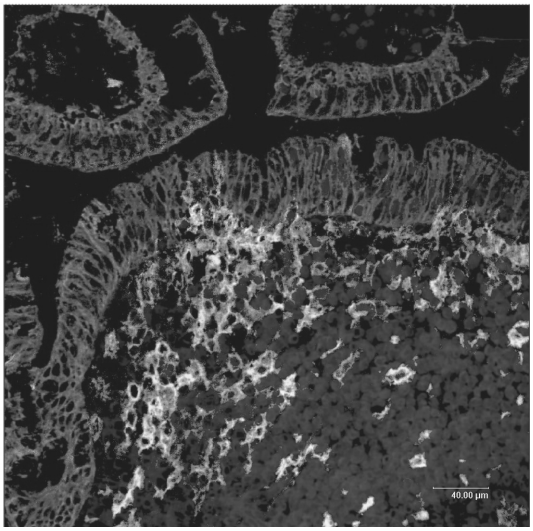

Figure 2. Confocal image of myeloid DCs in a normal human PP. The tissue section is stained with CD111c-FITC. Note the accumulation of DCs just below the FAE (original magnification 400 ×).

A second novel function is the ability of DCs in the lamina propria to reach through the epithelium and sample antigens from the lumen (Fig. 3). This was first observed *in vitro* in cocultures of DCs 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, DCs also send processes into the lumen [14]. More recently, mice have been produced which express the fractalkine receptor in DCs, linked to GFP, so that DCs can be visualised in real time *in vivo* [15]. These mice demonstrate that DCs in the villus cores reach through the epithelium and sample commensal microbes. There is also evidence that DCs transport luminal commensals to the mesenteric lymph nodes [16].

4 DC 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 DCs in the gut in health and disease. More data derives from mice.

In PP, two populations of DCs in the subepithelial dome produce different cytokines. The CD11b+/CD8α– population make IL-10 and drive Th2 cells whereas the $CD11b^{low}$/CD8α population make IL-12 and drive Th1 responses [17]. It is not clear how these two populations interact to prime particular T cell responses in PP tissue. In the normal lamina propria of the mouse, where these two populations are also present, the predominant cytokine produced appears to be IL-10 [18].

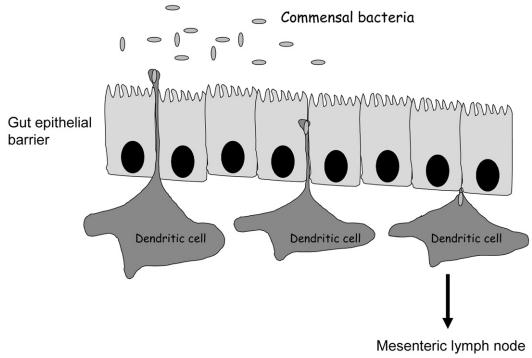

Figure 3. DCs in the gut lamina propria reach through the epithelium to capture luminal commensals. They then probably migrate to the mesenteric lymph nodes.

In man, there is very little data on DCs in PP in terms of cytokine production. T cells from normal human PP are strongly Th1 polarised with abundant production of INF-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 DCs) 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 DCs is increased [20]. Using flow cytometry, very few DCs in normal colon make IL-6, IL-10 or IL-12. In Crohn's disease, both IL-6 and IL-12 containing DCs increase with no significant increase in IL-10 containing DCs [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 DCs. 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.

5 Do gut DCs play a role in human gut disease?

Cross-sectional studies looking at DCs 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 DCs 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 as in coeliac disease, do gut DCs present gluten peptides to CD4 Th1 cells and cause coeliac disease; or do gut DCs drive the Th2

responses responsible for over-expression of IL-4 and switching to IgE which underlies immediate hypersensitivity responses to food antigens? We previously identified a patient whose coeliac disease appeared to be triggered by treatment with type 1 INF and also showed type 1 INF in coeliac tissue, of interest because type 1 INF is a potent inducer of Th1 cells in man [25]. Likewise we also demonstrated that addition of type 1 INF to antiCD3 activated T cells in organ cultures of human foetal gut produced a Th1 immune response and a flat mucosa [26]. We have identified increased numbers of plasmacytoid DCs in the duodenum of patients with active coeliac disease [27]. Quantitative PCR of these cells showed that they produced IL-18, but not IL-12 or IL-23. However, they do express INF-α and a neutralising anti-INF-α antibody partially inhibits the Th1 response to gluten. So it does appear that type I INFs from DCs may be important in generating the Th1 response in active coeliac disease.

One area which has provoked some thought is the claim that gliadin, a food protein, has direct effects on DCs. Peptic fragments of gliadin added to human monocyte-derived DCs induce phenotypic maturation and secretion of cytokines, particularly IL-6, IL-8 and TNFα [28]. It needs to be determined if the same effect is seen with gut DCs, 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 DCs; a reverse of the current position of most immunologists who see the main determinant of food hypersensitivity as the host immune response and genetics.

6 Concluding remarks

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

The authors have declared no conflict of interest.

7 References

[1] Pulendran, B., Maraskovsky, E., Banchereau, J., Maliszewski, C., Modulating the immune response with dendritic cells and their growth factors, *Trends Immunol.* 2001, *22*, 41 – 47.

[2] Reis, E., Sousa, C., Dendritic cells in a mature age, *Nat. Rev. Immunol.* 2006, *6*, 476 – 483.

[3] Suri, R. M., Austyn, J. M., Bacterial LPS contamination of commercial collagen preparations may mediate dendritic cell maturation in culture, *J. Immunol. Methods* 1998, *214*, 149 – 163.

[4] Liu, L. M., MacPherson, G. G., Antigen acquisition by dendritic cells: Intestinal dendritic cells acquire antigen administered orally and can prime naïve T cells *in vivo*, *J. Exp. Med.* 1993, *177*, 1299 – 1307.

[5] Silva, M. A., Lopez, C. B., Riverin, F., Oligny, L. *et al*., Characterization and distribution of colonic dendritic cells in Crohn's disease, *Inflamm. Bowel Dis.* 2004, *10*, 504 – 512.

[6] Ikeda, Y., Akbar, F., Matsui, H., Onji, M., Characterisation of antigen-presenting dendritic cells in the peripheral blood and colonic mucosa of patients with ulcerative colitis, *Eur. J. Gastroenterol. Hepatol.* 2001, *13*, 841 – 850.

[7] Bell, S. J., Rigby, R., English, N., Mann, S. D. *et al*., Migration and maturation of human colonic dendritic cells, *J. Immunol.* 2001, *166*, 4958 – 4967.

[8] MacDonald, T. T., Monteleone, G., Immunity, inflammation and allergy in the gut, *Science* 2005, *307*, 1920 – 1925.

[9] Jameson, B., Baribaud, F., Pohlmann, S., Ghavimi, D. *et al*., Expression of DC-SIGN by dendritic cells of intestinal and genital mucosae in humans and rhesus macaques, *J. Virol.* 2002, *76*, 1866 – 1875.

[10] Iwasaki, A., Kelsall, B. L., Localisation of distinct Peyer's patch dendritic cell subsets and their recruitment by chemokines macrophage inflammatory (MIP)-3α, MIP-3β, and secondary lymphoid organ chemokine, *J. Exp. Med.* 2000, *191*, 1381 – 1394.

[11] Mora, J. R., Bono, M. R., Manjunath, N., Weninger, W. *et al*., Selective imprinting of gut-homing T cells by Peyer's patch dendritic cells, *Nature* 2003, *424*, 88 – 93.

[12] Iwata, M., Hirakiyama, A., Eshima, Y., Kagechika, H. *et al*., Retinoic acid imprints gut-homing specificity on T cells, *Immunity* 2004, *21*, 527 – 538.

[13] Resigno, M., Urbano, M., Vazasina, B., Francolini, M. *et al*., Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria, *Nat. Immunol.* 2001, *2*, 361 – 367.

[14] Becker, C., Wirtz, S., Blessing, M., Pirhonen, J. *et al*., Constitutive p40 promoter activation and IL-23 production in the terminal ileum mediated by dendritic cells, *J. Clin. Invest.* 2003, *112*, 648 – 651.

[15] Niess, J. H., Brand, S., Gu, X., Landsman, L. *et al*., CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance, *Science* 2005, *307*, 254 – 258.

[16] MacPherson, A. J., Uhr, T., Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria, *Science* 2004, *303*, 1662 – 1665.

[17] Johansson, C., Kelsall, B. L., Phenotype and function of intestinal dendritic cells, *Semin. Immunol.* 2005, *17*, 284 – 294.

[18] Chirdo, F. G., Millington, O. R., Beacock-Sharp, H., Mowat, A. M., Immunomodulatory dendritic cells in intestinal lamina propria, *Eur. J. Immunol.* 2005, *35*, 1831 – 1840.

[19] Monteleone, G., Holloway, J., Salvati, V. M., Pender, S. L. F. *et al*., Activated STAT4 and a functional role for IL-12 in human Peyer's patches, *J. Immunol.* 2003, *170*, 300 – 307.

[20] te Velde, A. A., van Kooyk, Y., Braat, H., Hommes, D. W. *et al*., Increased expression of DC-SIGN+IL-12+IL-18+ and CD83+IL-12+IL-18– dendritic cell populations in the colonic mucosa of patients with Crohn's disease, *Eur. J. Immunol.* 2003, *33*, 143 – 151.

[21] Moneleone, G., Biancone, L., Marasco, R., Morrone, G. *et al*., Interleukin 12 is expressed and actively released by Crohn's disease intestinal lamina propria mononuclear cells, *Gastroenterology* 1997, *112*, 1169 – 1178.

[22] Hart, A. A., Al-Hassi, H. O., Rigby, R. J., Bell, S. J. *et al*., Characteristics of intestinal dendritic cells in inflammatory bowel diseases, *Gastroenterology* 2005, *129*, 50 – 65.

[23] Gionchetti, P., Rizzello, F., Venturi, A., Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: A double-blind, placebo-controlled trial, *Gastroenterology* 2000, *119*, 305 – 309.

[24] Ulisse, S., Gionchetti, P., D'Alo, S., Russo, F. P. *et al*., Expression of cytokines, iNOS, and MMPs in pouchitis: Effect of probiotic treatment, *Am. J. Gastroenterol.* 2001, *86*, 2691 – 2699.

[25] Monteleone, G., Pender, S. L. F., Alstead, E., Hauer, A. C. *et al*., Role of interferon-α in promoting T helper cell type 1 responses in the small intestine in coeliac disease, *Gut* 2001, *48*, 425 – 429.

[26] Monteleone, G., Pender, S. L. F., Wathen, N. C., MacDonald, T. T., Interferon-α drives T cell-mediated immunopathology in the intestine, *Eur. J. Immunol.* 2001, *31*, 2247 – 2255.

[27] Di Sabatino, A., Pickard, K. M., Gordon, J. N., Salvati, V. *et al*., Evidence for the role of interferon-α production by dendritic cells in the Th1 response in celiac disease, *Gastroenterology* 2007, *133*, 1175 – 1187.

[28] Palova-Jelinkova, L., Rozkova, D., Pecharova, B., Bartova, J. *et al*., Gliadin fragments induce phenotypic and functional maturation of human dendritic cells, *J. Immunol.* 2005, *175*, 7038 – 7045.

