

The Role of Dendritic Cells in Immunity and Tolerance in the Intestine

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Anatomical specialization of the mucosa and associated lymphoid tissue suggests that induction of a mucosal immune response will have certain unique features that affect the success of oral vaccines and therapeutics.¹ In particular, the initiation of an effective immune response to oral pathogens vs the induction of tolerance to food antigens is likely to be controlled by signals delivered in mucosal tissues. The failure to rapidly develop an immune response to an intestinal pathogen would have devastating consequences for an infected host.² Conversely, the inappropriate development of an inflammatory response to food antigens or normal commensal bacteria could severely hinder nutrient uptake by the intestine and also have life-threatening consequences for the host.³ Thus, rapid discrimination between harmful pathogens and harmless bacteria or food in the mucosa is essential to host survival.

Antibodies directly recognize intact nonself proteins or organisms and can target their removal from host circulation. In marked contrast, T cells recognize small peptide fragments in the context of major histo-compatibility (MHC) molecules on the surface of antigen-presenting cells. Dendritic cells are highly specialized antigen-presenting cells that can capture foreign or self proteins from peripheral tissues and quickly deliver this material to naïve T cells in draining lymph nodes.^{4,5} Intestinal dendritic cells can initiate an inflammatory response by directly recognizing microbial products, and also can degrade and present antigen to naïve pathogen-specific T cells in mucosal lymphoid tissues.⁶⁻⁸ Dendritic cells therefore represent a critical interface between innate and adaptive immunity in the intestine.

Indeed, our laboratory recently demonstrated that intestinal dendritic cells are absolutely required to develop a cellular immune response to oral infection.⁹ Bone-marrow chimeras were developed from CD11c-DTR transgenic mice that express the diphtheria toxin (DT) receptor under the control of the CD11c promoter.¹⁰ Thus, CD11c⁺ dendritic cells express the DT receptor, while other cell types do not. When mice are injected with diphtheria toxin, dendritic cells are rapidly depleted from all secondary lymphoid tissues, including intestinal Peyer's patches and mesenteric lymph nodes (Fig 1).⁹⁻¹¹ In the absence of this critical cell population, pathogen-specific T cells fail to respond to oral infection,⁹ demonstrating a requirement for dendritic cells in intestinal T-cell activation.

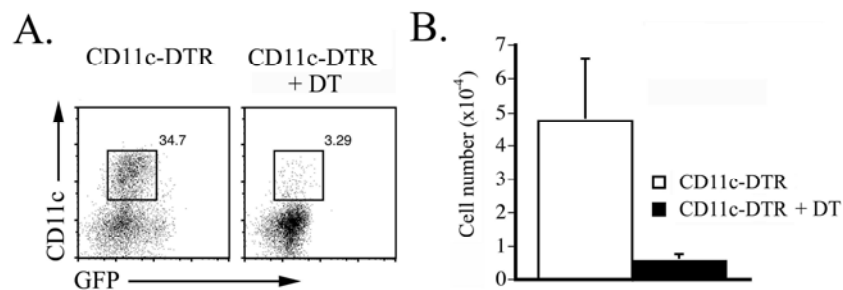


Fig 1. Depletion of CD11c⁺ intestinal DC. CD11c-DTR BM chimeras were injected with DT (4 ng/g body weight) and PPs harvested 24 hours later. Plots show (A) PP MHCII⁺ cells from CD11c-DTR DT-treated and untreated chimeras and (B) the mean number \pm SD of DCs in the PPs. (DT = diphtheria toxin, DCs = dendritic cells, PPs = Peyer's patches)

Although dendritic cells can be subcategorized in a variety of ways, recent data suggest that chemokine receptor expression might be a useful marker to define subpopulations in the intestine.^{6,12} For example, chemokine receptor 6 (CCR6) expression defines a subset of dendritic cells that are located in the subepithelial dome region of Peyer's patches.^{13,14} Our laboratory recently demonstrated that this population migrates toward the infected follicle-associated epithelium immediately following *Salmonella* infection.⁹ Both CCR6⁺ dendritic cell migration

and *Salmonella*-specific T-cell activation were dependent on CCR6 expression,⁹ suggesting that migration of this population is critical for initiation of an adaptive immune response in the Peyer's patches, the major site of bacterial entry.¹⁵ Thus, the CCR6⁺ subset represents a fairly minor fraction of Peyer's patch dendritic cells but appears to be essential for immunity to pathogens (Fig 2).

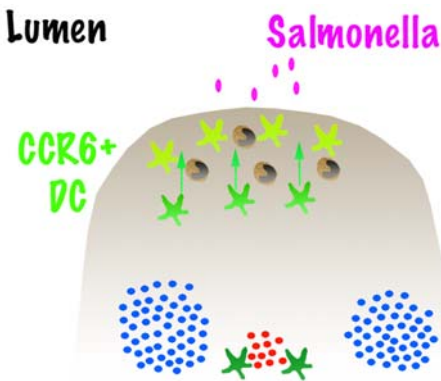


Fig 2. Rapid PP DC migration in response to pathogens. CCR6⁺ DC (green) are found between the T cell area (red) and the outer epithelial layer of the PP (top of dome). Other DCs that do not express CCR6 are found in closer association with the epithelial layer (yellow) or within the T cell area (dark green). B cell follicles are shown in blue and macrophages in brown. Upon detection of *Salmonella* (purple) by epithelial cells, CCR6⁺ DCs rapidly migrate toward the epithelial layer. (PP = Peyer's patch, DC = dendritic cell)

A second population of Peyer's patch dendritic cells express the chemokine receptor CX3CR1 and are found tightly associated with the follicle-associated epithelia.⁹ Other laboratories have shown that close proximity to the epithelial layer predisposes dendritic cells to generate T-cell immune tolerance rather than immunity.^{16,17} Therefore, antigen processing and presentation by different CCR6⁺ or CX3CR1⁺ dendritic cell populations may be the critical for determining whether an immune response is initiated or turned off in the Peyer's patches. Thus,

differential dendritic cell populations defined by chemokine receptor expression may regulate immunity versus tolerance.

Dendritic cells also are found in the intestinal lamina propria, where they do not express CCR6 but do express CX3CR1.^{9,18,19} Lamina propria dendritic cells can extend processes between epithelial cell tight junctions and acquire bacteria directly from the intestinal lumen.¹⁹⁻²¹ The process of dendrite formation requires expression of CX3CR1 *in vivo*.¹⁹ The function of these transepithelial dendrites is not completely clear but can represent a non-Peyer's patch portal of entry for some intestinal pathogens such as *Salmonella* (Fig 3).^{22,23}

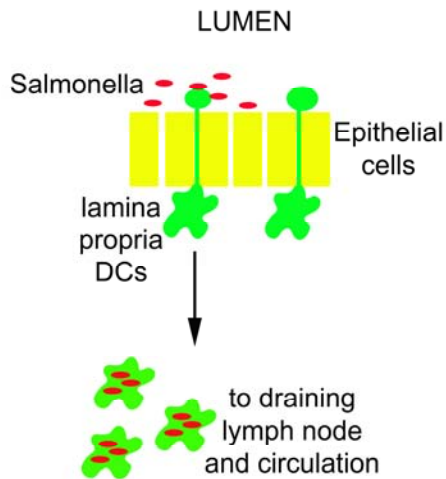


Fig 3. Rapid LP DC migration and bacterial dissemination. CX3CR1⁺ DC (green) are positioned close to the epithelial layer (yellow) in the intestinal lamina propria and extend cellular processes into the lumen to interact with intestinal bacteria. *Salmonella* can exploit this intestinal DC sampling function, rapidly penetrate the host, and gain access to systemic tissues.

However, the homeostatic function of these intestinal dendritic cells may have more to do with inducing or maintaining tolerance to normal bacterial flora than detecting invasive pathogens. A role for CX3CR1⁺ dendritic cells in oral tolerance seems especially likely given

the close association of these cells with epithelial cells which, as previously noted, predisposes dendritic cells to induce T-cell tolerance.^{16,17}

A key feature of dendritic cells in both Peyer's patches and lamina propria is their ability to migrate within, or to, secondary lymphoid tissues so that they can capture antigens and also encounter naïve antigen-specific T cells. However, the bacterial signals that drive intestinal dendritic cell migration remain poorly defined. It is known that some intestinal dendritic cells directly detect pathogen-associated molecular patterns (PAMPS) via innate receptors,²⁴ and our laboratory is examining one of these receptors, TLR5. TLR5 is a receptor that recognizes bacterial flagellins and induces an inflammatory response. Flagellin is the main component of bacterial flagella and is produced in large quantities by flagellated bacteria.²⁵ Bacterial flagellins also are dominant target antigens in mouse and human inflammatory bowel disease,²⁶ where reactivity to flagellins correlates with increasingly severe disease.^{27,28} The conserved structure of bacterial flagellins across several species ensures that TLR5 detects a diverse array of flagellated bacteria including *Listeria*, *Salmonella*, *Legionella*, and *Pseudomonas*. As with other TLR family members, TLR5 signaling induces NF-kappaB activation, inflammatory cytokine production, and increased expression of MHC and co-stimulatory molecules.²⁹⁻³³ Thus, bacterial flagellins are a conserved microbial structure directly recognized by the innate immune system. Our laboratory is examining whether flagellins and TLR5 play a role in directing intestinal dendritic cell migration during immunity and tolerance.

In conclusion, dendritic cells are critical cells that determine whether intestinal immune responses are initiated or whether tolerance to harmless antigens is induced. The role of subsets expressing different chemokine receptors is being examined and may help the development of oral vaccines or future therapies for inflammatory bowel disease.

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Q & A

Q: Dr McSorley, you mentioned oral tolerance and the role of various dendritic cell subsets, but you have not mentioned CD103 and retinoic acid. Can you comment on that?

Dr McSorley: We have not worked on CD103 and retinoic acid. Many other people have.

Q: Can you put your data in the context of that work, then, which is all in vitro?

Dr McSorley: My reluctance to talk about that is partly because we have not defined what these chemokine receptor positive populations CX3CR1 and CCR6 are, and we do not have a good way to fit them into an established model such as myeloid lymphoid plasmacytoid dendritic cells. It would be fairly easy to go back and characterize the dendritic cell populations like Brian Kelsall did [*J Exp Med* 1996;183:237-247; *Mucosal Immunol* 2008;1:460-469], and then fit them out with our chemokine receptors. We think that the CCR6 population in the Peyer's patches are myeloid cells, but I imagine the CX3CR1 population is CD103 positive, migrates to the mesenteric lymph node, and is part of that population. CD103 positive and negative cells are in the mesenteric lymph nodes, and the CD103 positives are supposed to be the cells migrating from the lymph nodes; so I guess our population is CX3CR1. However, we have not researched that or whether CX3CR1 would still be expressed in that population in the mesenteric lymph nodes.

I would like to study whether retinoic acid affects the ability of a cell population to activate our *Salmonella*-specific cells. We have looked for Th17 cells, which are regulated by retinoic acid in the Peyer's patches, and we have not seen any production of IL-17 in our infection model. We also have looked for Treg cells, which are regulated by retinoic acid, and we have not seen much FoxP3 expression either. Maybe it is just our system. Other researchers who have studied those populations have different systems, and perhaps in their studies of oral tolerance

are looking at endogenous responses to gut flora. One would see more of that regulation by retinoic acid of Th17 and Treg cells.

Q: Would you expect the CX3CR1 dendritic cells to produce different levels of retinoic acid compared to the CCR6, given tolerance vs immunity?

Dr McSorley: Yes, that could certainly be a hypothesis. We have not studied that. Some of the participants of this conference probably would be interested in the CX3CR1 population in terms of tolerance, food, and commensals. However, that population is irrelevant to those of us working on infectious disease. We are interested in another population to stimulate an immune response. We primarily think about vaccination and elimination of a pathogen.

Q: Do you need to pretreat your mice with antibiotics when studying *Salmonella* infection? What sort of hygiene do you use in housing your mice?

Dr McSorley: Some researchers who study *Salmonella* infection pretreat with antibiotics, and I believe when they do that they are looking at something completely different from what we see. We just treat our mice orally with *Salmonella*. They have a normal gut flow. They are kept in a fairly clean room, although it is not germ-free, and we see how they respond.

Q: You said you were surprised to see 80% activation of the cells in the Peyer's patches within 6 hours of infection. Why were you surprised? Is it because of the complexity? Yet we know how quickly digestion occurs. Is there something more fundamental we miss in thinking about how these things are processed?

Dr McSorley: Some people have spent their careers studying such things as Peyer's patch infection of M cells by *Salmonella*, or the processing of class II peptides in response to given dendritic cells or antigen-presenting cells, and bacteria or protein antigen. I am surprised that, in all that literature, these complex processes happen in such a compressed time scale. Not only do

the presentation and actual penetration of the bacteria occur quickly, but also the movement of the cell populations to the area where the bacteria are, and subsequently the movement out of that area to find a T cell and actually find it. When we transferred our T cells, we would see about three antigen-specific cells within the Peyer's patches, so the dendritic cells would have to find them in the interfollicular region. It still surprises me that all these events—migration, antigen presentation, and the pathogenesis of the bacteria—occur so quickly.

Q: What strain of *Salmonella* do you use? If you use an attenuated strain, do you see any differences in which dendritic cells will pick it up?

Dr McSorley: It is a wild-type strain. It will kill the mice within about 5 to 7 days, depending on what dose you give them. We also have used attenuated strains, and the same process occurs. With an attenuated strain, we just see less activation. We think that fewer bacteria get into the Peyer's patches quickly with an attenuated strain, even if we adjust the system to give higher doses of bacteria. A small number of bacteria do get in and start to replicate; so over time we get more bacteria. It is roughly the same kinetics with T-cell response, but with a lower percentage. Instead of 80% of the cells being activated, for instance, we may see only 25% or 30%.

Q: But is it the same dendritic cell population, the CCR6?

Dr McSorley: We have not studied that, mostly because of technical reasons. If you have a system in which you can see 85% or 90% of the cells activated compared to 30% activated, you do not spend time working with the one that results in 30% activation.

Q: Did you imply that the CCR6⁻ cells may be primarily responsible for the tolerogenic responses in the control? I also am thinking more broadly about these cells. Possibly they are present earlier in life. Let us say that if there is a predominance of the CCR6⁻ in a young infant,

maybe he or she is effectively becoming tolerized by the presence of the commensal pioneers early in life. And later on, the CCR6⁺ cells come in for defense.

Dr McSorley: That would be an interesting hypothesis. I do not know whether there are any data on this. We do not know about the development of these cell populations, not even where the CCR6 population comes from. Some may come from blood, dendritic cell precursors, but they could come from some kind of inflammatory monocyte. We do not know anything about development in terms of when these cells appear, in mice or in humans.

Q: Are you studying the relevance to humans and whether those CCR6 or CX3CR1 dendritic cells are present in the human gut?

Dr McSorley: I would like to, but as an academic scientist, I work on what they let me work on. I am in the gastrointestinal division; I have good access to patient tissues. If we can funnel money from other projects into that project, we may look into it. Some studies show upregulation of CCR6 and particularly CCL20 expression in patient tissues [Schutyser E et al: *Cytokine Growth Factor Rev* 2003;14:409-426]. Some mRNA studies suggest that the ligand-receptor pair CCL20-CCR6 plays a role in homeostasis, inflammation, and pathology at gut mucosal surfaces.

