Subsets of Migrating Intestinal Dendritic Cells

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Running title:
Migrating Intestinal DC Subsets

Summary
Dendritic cells in the intestine are heterogeneous. Phenotypically different populations of conventional dendritic cells have been identified in the intestinal lamina propria, Peyer's patches, and in the draining mesenteric lymph nodes, to which these DCs constitutively migrate. Markers used to identify these populations include MHC class II, CD11c, CD8α, CD11b, and CD103. Extensive studies in rats, summarised here, which involved collection of migrating DCs by thoracic duct cannulation after mesenteric lymphadenectomy, have clearly demonstrated that the subsets of migrating intestinal lymph DCs have different functional properties. The subsets might play different roles in the induction of oral tolerance and in driving systemic immune responses after vaccination or intestinal stimulation with TLR ligands. The use of these surgical techniques allows investigation of the functions of purified subsets of migrating DCs. However, in the rat these studies are limited by the range of available reagents, and are difficult to compare with data from other species in this fast-moving field. Recent refinements have enabled the collection of migrating intestinal DCs from mice; our initial results are described here. We believe that these studies will generate exciting data and have the potential to resolve important questions about the functions of migrating intestinal DC subsets.

Keywords
Dendritic cells
Cell Trafficking
Lymph Nodes

Introduction to dendritic cells in the intestine

The digestive tract has a surface area nearly 200 times greater than the skin (1), and is an important entry point for pathogenic micro-organisms. It must therefore be protected by effective immune responses. However, these responses must also be prevented from making damaging inflammatory attacks against the benign foreign material to which the intestine is also continually exposed. The dendritic cells (DCs) that continually migrate from the intestine to the mesenteric lymph nodes (MLN) are crucial in balancing immunity and tolerance in the intestine. All the subsets of these migrating intestinal DCs display features that make them uniquely suitable to play the role of sentinels: they patrol intestinal tissues before migrating via lymph to the MLN, transporting antigen and conveying signals to T lymphocytes. These DC-borne signals are critical for directing the T cells to become activated, or to maintain tolerance against specific antigens. Because of these functions, there is intense interest in the role of migrating DCs in controlling intestinal immunity and tolerance.

In the intestine, DCs are present in the organised lymphoid tissues of the Peyer's patches (PP) and isolated lymphoid follicles, as well as interspersed through the lamina propria (LP). Intestinal DCs interact with particulate antigens from the intestinal lumen, either after M cell mediated uptake or by directly extending their dendrites between cells of the intestinal epithelium (2, 3). Even In the absence of any overt inflammatory stimulus, DCs constitutively migrate through the lymphatics to the MLNs (4, 5). In the MLN, DCs present peripherally derived antigen to T cells (6), and are essential for the induction of oral tolerance (7, 8). After stimulation by pathogens or pathogen-derived molecules, they can also stimulate antigen-specific immunity (9-12).

DCs arrive in the MLN from two main sources. Blood-borne precursors enter via high endothelial venules (HEVs), and lymph-borne DCs migrate from the intestine. Under homeostatic conditions populations of DCs migrate continually from peripheral tissues to draining lymph nodes (4), and these DCs have long been recognised to consist of at least two subsets (13, 14). Two approaches have contributed to the study of these migrating DCs; they have been collected by cannulation of the lymphatic vessels of sufficiently large animals (rats, cats, dogs, pigs, sheep or cows), or their functions have been inferred from studies of DC populations purified from tissues. In this review we will focus on the phenotypes and functions of DCs that migrate from the intestine, whether directly observed using purified migrating populations or inferred after purification from tissues.

Populations of DCs in intestinal tissues.

DCs were identified in murine intestinal lymphoid tissues (PP and MLN) in 1973, and were found to comprise approximately 0.2% of total cells in these tissues, after collagenase digestion (15). Subsequently, subpopulations of DCs in the PP, LP and MLN were described.

In the PP, two populations were originally observed, both expressing CD11c. DEC-205$^-$ cells are situated in the sub-epithelial dome, and throughout the follicle, while DEC-205$^+$ DCs are present in the interfollicular T cell regions (16). Subsequently, in the PP the majority of DCs were shown, by flow cytometric analysis of collagenase-digested tissue, to express high levels of CD8α and DEC-205 and low levels of CD11b. (17). In situ immunofluorescence microscopy, however, identified three subsets of CD11c$^+$ DCs in the PP, in different anatomical locations (18). CD8α$^+$ CD11b^{lo} DCs were observed within the

T cell-rich interfollicular regions of the PP, while CD11b^{+} DCs were observed in the sub-epithelial dome (SED) of the PP, adjacent to the specialised epithelial M-cells that sample antigen from the luminal environment. A third "double negative" subset was found in both locations. All the DCs in the interfollicular regions expressed CCR7, while the CD11b^{hi} DC population in the SED was shown to upregulate this chemokine receptor and migrate into the interfollicular region of the PP after systemic stimulation with a microbial product (soluble tachyzoite antigen (STAg), from Toxoplasma gondii). The CD11b^{hi} DC subset, which also expresses CCR6, is absent from the SED of CCR6-deficient mice. These mice are deficient in their intestinal IgA responses to orally administered antigen, and to enteropathic rotavirus infections (19).

In 1996, MHC class II-positive cells with dendritic morphology were described in the intestinal epithelium (20). At least three subsets of CD11c^{+} DCs have subsequently been identified in the LP in mice. The majority of CD11c^{hi} LP DCs are CD11b^{+} CD8α^{-}, while CD11b^{-} CD8α^{+} and CD11b^{-} CD8α^{-} are also found (3). Approximately 70% of the CD11c^{hi} LP DCs express CD103 (a_E integrin) (21). A distinct population of CD11c^{int} MHC class II^{lo} B220^{+} plasmacytoid DCs (pDCs) can also be detected (3). These pDCs depend critically on CCR9 for their migration into the LP (22).

It has been observed *in vitro* that a CD11c-expressing population of cells purified from the LP can insert cellular processes through a monolayer of epithelial cells, to directly sample bacteria (2). Follow-up studies demonstrated that CD11c^{+} cells *in vivo* also produce trans-epithelial dendrites (23), and that the dendrites depend on the cells' expression of CX_3CR1 (fractalkine receptor) (24). While it is clear that these dendrites enable CD11c^{+} CX3CR1^{+} cells to interact with intestinal bacteria, it is not yet known whether these cells are able to migrate from the LP to the MLN and activate naïve T cells. We anticipate that this issue will be resolved in the near future.

A number of populations of 'conventional' DCs have been described in the MLNs of mice in the steady state. Based on their expression of CD8α and DEC-205, three populations were defined as being CD8α^{-} DEC-205^{-}, CD8α^{-} DEC-205^{+}, and CD8α^{+} DEC-205^{+} (25). The steady state MLN DC populations have also been sub-divided on the basis of their expression of CD8α and CD11b, with CD8α^{-} CD11b^{+}, CD8α^{int} CD11b^{-}, and CD8α^{+} CD11b^{-} populations identified (9).

DCs enter the MLN by two routes; from the blood or from the efferent intestinal lymph vessels. In order to understand the functions of the lymph-derived "migratory" DC populations, it is important to differentiate between the DCs that have entered via the two routes. Staining of MLN DCs with antibodies specific for CD8α and the β7-integrin has identified four subsets- two β7^{hi} (CD8α^{+} and CD8α^{-}) populations that have a similar CD8α/β7 phenotype to LP DCs, and two β7$^{int\text{-}lo}$ (CD8α^{++} and CD8α^{-}) populations that are similar to PP DCs. The β7^{hi} subsets are not found in the MLNs of CCR7$^{-/-}$ mice (26), suggesting that they are migratory DC populations. It is also clear that a proportion of the DCs in the MLN express CD103 (α_e-integrin). Detailed phenotyping of these CD103^{+} DCs showed that they have very strong similarities with the CD103^{+} LP DC populations, and that their numbers were also reduced in the MLNs of CCR7$^{-/-}$ mice (21). Labelling studies with BrdU have also shown that the majority of CD103^{+} and CD103^{-} MLN DCs have different developmental origins (27). The developmental relationship between CD103-positive and negative DCs is therefore not clear, although the pace of progress in this area is rapid.

Although not all migratory DCs have been shown to be $CD103^+$, and not all 'resident' DCs are likely to be $CD103^-$ (28), expression of CD103 has proved useful as a tool for differentiating two populations of MLN DCs with different functions and origins. For instance $CD103^+$ MLN DCs from mice, and $CD103^+$ LP-DCs from humans, induce expression of the small-intestinal homing markers CCR9 and integrin $\alpha4\beta7$ on T cells (21) (27). Murine $CD103^+$ MLN DCs but not $CD103^-$ DCs also present orally administered antigens to T cells (27). In addition, $CD103^+$ MLN DCs and LP DCs can induce a population of naïve T cells to express FoxP3 (29) (30). These activities are dependent on the DCs' ability to generate retinoic acid from its metabolites. It should be noted, however, that retinoic acid generated by stromal cells in the MLN might also affect the differentiation of naive lymphocytes (31).

Observations of migrating intestinal DC subsets, collected from lymph

The DCs that sample antigen in peripheral tissues migrate to draining lymph nodes via afferent lymphatic vessels. On reaching the lymph nodes, these DCs interact with T cells, inducing tolerance or activation, as described above. After a few days, the vast majority of DCs die in the lymph nodes; very few migrate out into efferent lymph.

Lymph, containing the migrating DCs, can be collected either by insertion of a cannula into afferent lymphatic vessels, or by placing the cannula in a larger efferent vessel after prior removal of the downstream lymph node. The latter is possible because afferent and efferent lymphatics re-anastamose to allow lymph flow a few weeks after lymph node removal (32). Collection of bona fide migrating DCs by these methods has generated crucial information about their phenotype and functions. DCs are extremely sensitive to changes in their environment (33) (34)). DCs collected by cannulation are therefore stored on ice as soon as they leave the animal, maintaining them as close as possible to their physiological state.

Lymphatic vessels of many species have been cannulated to collect migrating dendritic cells (35). While it is possible to directly cannulate the afferent lymphatics that carry lymph from peripheral tissues to the draining lymph nodes, the small diameter of these vessels and the small volumes of lymph that can be collected, along with the low frequency of DCs in lymph (4) have largely restricted this technique to larger animals (35), (36). Thoracic duct cannulation (TDC) in rats was first developed by Bollman (37). The thoracic duct is one of the largest lymphatic vessels; as well as collecting efferent lymph form the liver and alimentary tract, it collects efferent lymph from other abdominal organs (e.g. pancreas, kidneys, urogenital tract), from the lower limbs, the trunk, and some thoracic organs (38). In order to obtain any significant numbers of DCs using TDC, the downstream lymph nodes must be removed. The lymph that is then collected is known as "pseudo-afferent" lymph. This combination of TDC and lymphadenectomy procedures was first used to study cells in pseudo afferent intestinal lymph (39), and later to study DCs, "non-lymphoid" cells, in the MacPherson laboratory (4).

Direct observations of migrating intestinal DC subsets obtained from rats by thoracic lymph duct cannulation (TDC) after mesenteric lymphadenectomy (MLNX) have revealed much about their functions. These studies are very difficult to perform using DCs purified from either intestinal or lymph node tissues, both because of the extreme difficulty in accurately identifying the migratory cells after extraction from the tissues,

and because their functions are likely to be altered after entering the lymph nodes, and by the necessary physical and enzymatic tissue disruption.
If MLNs are not surgically removed before TDC, only a very small number of DCs (less than 0.015% of lymph cells) can be detected be detected by flow cytometry in thoracic duct lymph ((40)and our unpublished observations). In MLNX rats the frequency of DCs recovered in the thoracic duct lymph increases 50-100 fold. Therefore, the vast majority of lymph DCs (L-DCs) obtained from MLNX rats originate from the intestine. These rat L-DCs have been characterised as veiled cells with high levels of cell-surface expression of CD103 and MHC class II (13). Migration of L-DCs is constitutive; approximately 800,000 intestinal L-DCs migrate each day from a total pool of 7-24 million intestinal $CD103^+$ MHC class II^+ DCs (11). Because they are collected from the lymph L-DCs are, by definition, migratory. L-DCs were first shown to be potent at priming antigen-specific T cell proliferation after intestinal delivery of antigen (6). The developmental origin of intestinal L-DCs is not entirely clear, although they are likely to be ultimately derived from a blood-borne precursor. We have shown that in the steady state at least a small proportion of intestinal L-DCs are derived from $CCR2^{lo}$ CX_3CR1^{hi} blood monocytes (41). Transplantation of small intestines in mice has also shown that LP DCs are replenished from a blood-derived precursor rather than a tissue-derived precursor (27).

Subsets of migrating intestinal lymph DCs in the rat.
Two subsets of migratory intestinal DCs can be identified in he rat by their differential expression of CD4 and CD172a (13). Similar subsets of DCs have also been identified in skin-draining lymph of cattle (42), and in the intestinal lymph of mini-pigs (43). BrdU-labelling experiments in rats suggest that one population L-DCs is not the precursor of the other (13). Both populations of intestinal L-DCs express similar levels of MHC class II, ICAM-1, and CD103. However, the $CD4^{hi}CD172a^+$ L-DC subset (hereafter described as $CD172a^+$) was able to process and present native antigen, and stimulate significantly stronger proliferation from both naïve and sensitised $CD4^+$ T cells in MLR reactions.
While the $CD172a^+$ L-DCs are more potent at stimulating proliferation from $CD4^+$ T (13) (44) and $CD8^+$ T cells (unpublished observations), the $CD172a^{lo}$ L-DCs also have unique properties (45). For instance, they contain cytoplasmic apoptotic DNA, epithelial cell–restricted cytokeratins, and nonspecific esterase (NSE)-positive inclusions. The electrophoretic variants of the NSE from the $CD172a^{lo}$ L-DCs were also observed in intestinal epithelial cells and in DCs in the MLN, intestine, and PPs, but not in other cells or tissues. Histological studies of MLNs from germ free rats showed that DCs in these animals also contained apoptotic DNA suggesting that this occurred in the absence of bacterial stimuli. Thus, this L-DC population constitutively endocytoses and transports apoptotic intestinal epithelial cells to the MLNs, even in the absence of bacterial stimuli. These DCs were therefore described as being involved in the induction and maintenance of peripheral self-tolerance to intestinal antigens. However, at the time these experiments were performed, reagents were not available to determine whether T cells were actually tolerised after interacting with the $CD172a^{lo}$ DCs.
Using the MLNX and TDC techniques it is possible to investigate the functions of L-DC subsets in the steady state, as above, and after administration of substances likely to alter the behaviour of intestinal DCs. For instance, intravenous injection of LPS was shown to drive increased numbers of L-DCs to migrate from the intestine, by a TNF-α-dependent

mechanism (46). However, it did not induce a significant increase in the expression of the costimulatory molecules CD80 or CD86 in these L-DCs. Furthermore, both L-DC populations were shown to be unresponsive to stimulation with LPS *in vitro* (47). However, follow-up experiments revealed that L-DCs do respond to other intestinally-delivered pathogen-associated molecular patterns, such as those provided by TLR2 and TLR9 agonists (48).

Identification of a third L-DC subset

Advances in flow cytometry and the increased availability of fluorochrome-labelled rat-specific monoclonal antibodies have enabled more detailed analysis of the L-DC populations. Simultaneous staining of $CD103^+$ MHC class II^+ cells for CD172a and CD11b (OX42) reveals that the intestinal L-DCs in fact comprise three subsets (49). The $CD172a^+$ subset contains both $CD172a^{hi}$ $CD11b^{lo}$ ($CD172a^{hi}$), and $CD172a^{int}$ $CD11b^{hi}$ ($CD172a^{int}$) cells. In the steady state, all three populations are able to take up orally administered fluorescently-labelled proteins (unpublished observation). In addition, all three subsets induce proliferation and stimulate production of IFN-γ from naïve allogeneic $CD4^+$ T cells, although both $CD172a^+$ subsets produce quantitatively stronger responses than $CD172a^{lo}$ DCs, as described above (44). Interestingly, the $CD172a^{hi}$ DC subset is largely absent from pseudoafferent lymph that drains the liver, after removal of the celiac lymph nodes (49).

The three subsets of intestinal L-DCs respond differently after oral delivery of immuno-stimulatory substances. For instance, if rats are fed the small-molecule agonist of TLR7/8, R848, the output of all three subsets of L-DCs increases 30 to 50-fold within 10 hours. A significant proportion of the increased migration is driven by TNF-α, while the activation of L-DCs is largely dependent on IFN-α/β produced by pDCs (50). After feeding R848, both CD172a-expressing L-DC subsets upregulate expression of CD25, the most sensitive marker of activation of DCs in rats. Unlike the $CD172a^-$ L-DCs, these two CD172a-expressing L-DC subsets express TLR8, and secrete pro-inflammatory cytokines when stimulated with R848 *in vitro*. There are, however, subset-specific differences between the $CD172a^+$ L-DCs subsets in their responses to R848. For instance, the $CD172a^{hi}$ L-DC population is the first to be released from the intestine, it contains the highest proportion of CD25-positive DCs, and it is the only L-DC subset that secretes IL-6 and IL-12p40 directly ex vivo (49). These subset-specific differences are not due to differential TLR7 or TLR8 expression (49), but may be the result of differential localisation within the intestine, or differential expression of currently-unidentified receptors.

In contrast to the response seen after feeding R848, administration of the oral adjuvant E. coli heat labile enterotoxin (Etx) does not stimulate migration of increased numbers of L-DCs (11). It does, however, have profound effects on the $CD172a^{hi}$ L-DC subset, causing these cells to express CD25, and increasing their ability to stimulate naïve T cell proliferation in MLR cultures. Feeding Etx also enables increased numbers of DCs in the $CD172a^{hi}$ subset to acquire co-administered fluorescently-labelled ovalbumin and stimulate stronger proliferative responses from OVA-primed $CD4^+$ T cells.

Taken together, these data support the hypothesis that the $CD172a^{hi}$ ($CD11b^{lo}$) subset of L-DCs is the most 'immunogenic'. Not only does this subset migrate most quickly after R848 stimulation, it also produces the highest concentrations of inflammatory cytokines,

and stimulates proliferation of naïve T cells most strongly. Conversely, while the CD172a^{lo} L-DC subset is the only one shown to acquire apoptotic enterocytes in the steady state, and is always less potent at stimulating responses from CD4$^+$ T cells, we have generated no data that demonstrate a qualitative difference in the T cell responses stimulated by any of the three L-DC subsets. Indeed, the same proportion of CD25$^+$ FoxP3$^+$ T cells proliferate in response to each of the three L-DC subsets (44). At this time, while it may exist, these data do not point to the existence of a truly tolerogenic subset of intestinal L-DCs. We hope that continuing experiments will shed further light on this issue.

In the rat, plasmacytoid DCs (pDCs) do not migrate in intestinal lymph. Not only can cells of the appropriate phenotype not be detected in thoracic duct lymph of MLNX rats, but MHC-II$^+$ non-T and B cells from this lymph are not able to upregulate IFN-α/β mRNA levels or secrete IL-12p40 after TLR9 or influenza virus stimulation - a hallmark of pDCs (51). These data led us to conclude that pDCs were not able to migrate from peripheral tissues to the draining lymph nodes via the lymph. However, this issue has become more complex with the recent publication of data demonstrating both that pDCs (interferon-producing cells with plasmacytoid morphology) can be identified in steady-state lymph draining the skin in sheep, and in mini-pigs (52), and that pDCs in mice can migrate, via blood, from a cardiac allograft to peripheral lymph nodes (53). These data indicate that pDCs might migrate in afferent lymph from certain tissues or in certain species, and also that they might migrate from peripheral tissues to lymph nodes via the blood circulation.) Future investigations may reveal further details about the migration of pDCs.

Subsets of migrating intestinal DC subsets in mice- CD103, CD11b and CD8α

The DCs that migrate from the intestine are required for the maintenance of tolerance against benign intestinal antigens in mice (8). The phenotypes and functions of these cells are, therefore, the subject of intense interest. In particular, as described above, a CD103$^+$ subset of DCs has been identified in steady state MLN and PP. The CD103$^+$ DCs have unique functions and CD103 is considered to be a useful marker for cells that have migrated to the MLN from the LP. However, it is still not clear whether all migratory intestinal DCs are CD103$^+$, or whether all "MLN-resident" DCs are CD103$^-$.

It has been clearly shown in many species that migrating intestinal DCs may be divided into functionally-different subsets. Without direct observations, it is difficult to investigate these subsets in mice. However, a number of investigators have used innovative approaches to tackle this problem.

For instance, careful phenotyping of DCs in the LP and MLN, and study of these populations in CCR7$^{-/-}$ mice where migration in afferent lymph should be significantly impeded, indicated that two populations (CD8α^+ and CD8α^-) of migrating intestinal DCs exist, and these expressed high levels of β7 (26). Both of these 'migratory' DC subsets contained similar numbers of cells and constituted roughly 40% of the total DCs found in the MLN. Recent data, generated using CX3CR1-GFP mice, show that the largest population of CD11c$^+$ cells in the small intestine, which express CD11c and CX_3CR1, are not able to migrate to the MLN (Agace, Society for Mucosal Immunology 2009). Therefore, the migrating intestinal DCs must originate from the other, CX3CR1-negative populations of CD11c$^+$ cells in the intestine.

On the other hand, investigations using a lysozyme M-Cre reporter system have suggested that, in the absence of an inflammatory stimulus, migrating intestinal DCs do not form a major population of MLN DCs (54). These data are difficult to reconcile with those above, especially as neither CD103 nor and β7 were analysed in the latter study. The situation is further complicated by the fact that the lysozyme M enzyme was not expressed during the development of all transgenic DCs, and that the investigators did not examine the possibility that a subset of migrating intestinal DCs might express CD8α.
In the spleen, DCs expressing CD8α are specialised for the uptake of apoptotic material and Fcγ-R-independent cross-presentation of this material (55). Similarly, $CD8\alpha^+$ DCs purified from the pancreatic lymph nodes have been shown to be responsible for generating cross-tolerance to pancreatic antigens in a transgenic system (56). Also, of the skin-derived DC subsets, $CD103^+$ DCs may be best able to present Herpes Simplex virus-derived antigens to naïve $CD8^+$ T cells after natural infection (57). One might speculate therefore, that if a $CD8\alpha^+$ subset of migrating intestinal DC were to exist, it might be specialised for the presentation of exogenously-derived antigens to $CD8^+$ T cells, perhaps in both tolerogenic and immunogenic situations.

Migrating intestinal DC subsets- MLN, LP and PP subsets

To account for the fact that migrating intestinal L-DCs appear able to induce both T cell tolerance and inflammatory responses, it has been proposed that subsets of DCs migrating from the intestine to the MLN may differ under steady-state and inflammatory conditions. Indeed, these populations may have different functions because they are derived from distinct cellular precursors or are resident in the intestine for different periods (54, 58). For instance, cells that remain in the LP for longer periods may become conditioned by epithelially-secreted factors e.g. TGFβ and thymic stromal lymphopoietin (TSLP), to induce tolerogenic responses from responding T cells (59). Although TSLP has been shown to condition human monocyte derived DCs (59), MLN DCs from TSLPR-deficient mice have the same capacity to induce $FoxP3^+$ T cells *in vitro* as MLN DCs from wild type mice; TGFβ and RA were found to be responsible for DC conversion (60). Alternatively, intestinal DCs may be able to induce pro-inflammatory responses after stimulation with TLR agonists. For instance, we and others have shown that, despite the unresponsiveness of intestinal DCs to the TLR4 agonist LPS, intestinal DCs retain the capacity to respond to other TLR agonists (48, 61, 62). These apparently paradoxical findings are a clear indication that the immunological consequences of TLR engagement or inflammatory stimulation on subsets of intestinal DCs merit further study.
While it has been clearly demonstrated that DCs migrate from the intestine to the MLNs in the steady state, it is still not clear whether these migratory DCs are derived from the LP alone or whether PP DC subsets also migrate to the MLN. A study in Göttingen minipigs, comparing the phenotypes of DCs in intestinal tissues and in pseudoafferent lymph, concluded that migratory DCs were largely derived from the LP (43). Also, when rats are dosed orally with the TLR7/8 ligand R848, which induces the migration of all detectable DCs out of the LP into the lymph, the numbers of DCs in the T cell areas of the PPs are unaffected (50). *A priori*, to argue that DCs were able to migrate in large numbers out of the PP, one would have to maintain that the PP were specialised secondary lymphoid organs- DCs do not migrate out of other lymph nodes, even under inflammatory conditions (our unpublished observations).

Conversely, other data indicate that DCs might, in fact, migrate from the PP to the MLN. Elegant experiments were performed in which two types fluorescently-labelled antibiotic-resistant bacteria were delivered orally or injected into intestinal loops (63). The fluorescent bacteria were observed in the PP, but not in the LP. Also, CCR7-dependent accumulation of antibiotic-resistant bacteria was observed in the MLN after intragastric delivery, largely within $CD11c^+$ cells. These data indicate that the majority of DCs migrating from the intestine are, in fact, derived from the PP, not the LP. In the future, direct analyses of migrating intestinal DCs in experimental animals will help to resolve this issue.

Migrating intestinal DCs in mice.

We have recently refined the MLNX and TDC procedures and applied them to the study of migrating intestinal DCs in mice. Several groups have previously collected thoracic duct lymph from mice (64), (65), (66), but only Joan Rhodes has combined this with mesenteric lymphadenectomy to isolate murine intestinal DC (67-69). To our knowledge, this technique for collecting mouse DCs is not currently in use except in our laboratories. The availability of mouse-specific immunological reagents, and of mice with targeted genetic alterations, now enables us to answer important questions relating to DC functions, both in the steady state and under inflammatory conditions. Alternatively, it is also possible to collect very small volumes of lymph containing migrating intestinal DCs by directly sampling afferent intestinal lymphatic vessels. The advantages of this technique are that DCs collected in this way are obtained from animals with intact mesenteric lymph nodes, using a one-step procedure. The disadvantage is that the number of DCs obtained from each animal is small; large numbers of animals are therefore needed to generate sufficient DCs for functional analyses.

Our initial experiments using TDC of MLNX mice have clearly demonstrated that, as in the rat, these DCs contain at least two subsets. Surprisingly, both $CD8\alpha^+$ and $CD11b^+$ DCs are found, in similar proportions, in steady-state mouse thoracic duct lymph (Figure 1). The presence of migrating $CD8a^+$ DCs was somewhat unexpected, as other authors have suggested that $CD8a^+$ DCs are unlikely to migrate via lymph from peripheral tissues (57) (54). All $CD8\alpha^+$ and 90% of $CD11b^+$ L-DCs express CD103. These observations are consistent, for instance, with a report that describes migrating intestinal DCs in the LP and MLN as containing $CD8\alpha^+$ and $CD8\alpha^-$ DC subsets expressing CD103 (26). However, we reproducibly find that 10% of our $CD11b^+$ L-DCs do not express CD103. Future experiments will examine the functional properties of all three L-DC subsets and determine whether $CD103^-$ DCs represent a functionally distinct subset of migrating DCs.

Conclusions

The use of surgical techniques to collect DCs in the act of migrating from the intestine of rats has revealed that these DCs are made up of at least three subsets, and that these subsets have different functional properties. These studies have been limited, however, by the lack of immunological reagents available for use in rats. The recent development of techniques, by ourselves and others, to acquire bona fide migrating DCs from mice will therefore enable us to deepen our understanding of the functions of these important DC subsets. In particular, we hope that three fundamental questions may soon be answered: Do intestinal DCs migrate from the lamina propria, and/or the Peyer's patches? How do

the DCs that migrate from the intestine in the steady state contribute to the maintenance of oral tolerance? Finally, how do the properties of migrating intestinal DCs change after stimulation (in intestinal pathology, or after adjuvant or pathogenic stimulation), to induce systemic immune responses against intestinal antigens? These are areas of intense current interest, and we anticipate that the next few years will see rapid progress in this field.

Figure Legend

Figure 1. Dendritic cells purified from mouse lymph. L-DCs from MLNX mice. (A) L-DCs express high CD11c. (B) L-DCs comprise two subsets ($CD8\alpha^+$ (blue) and $CD11b^+$ (red)). (C) A population of $CD103^-$ $CD8\alpha^-$ DCs is routinely observed. (D) Both subsets express similar levels of CD4. Overlays of expression of CD4 and CD8 compared with lymphocytes (green).

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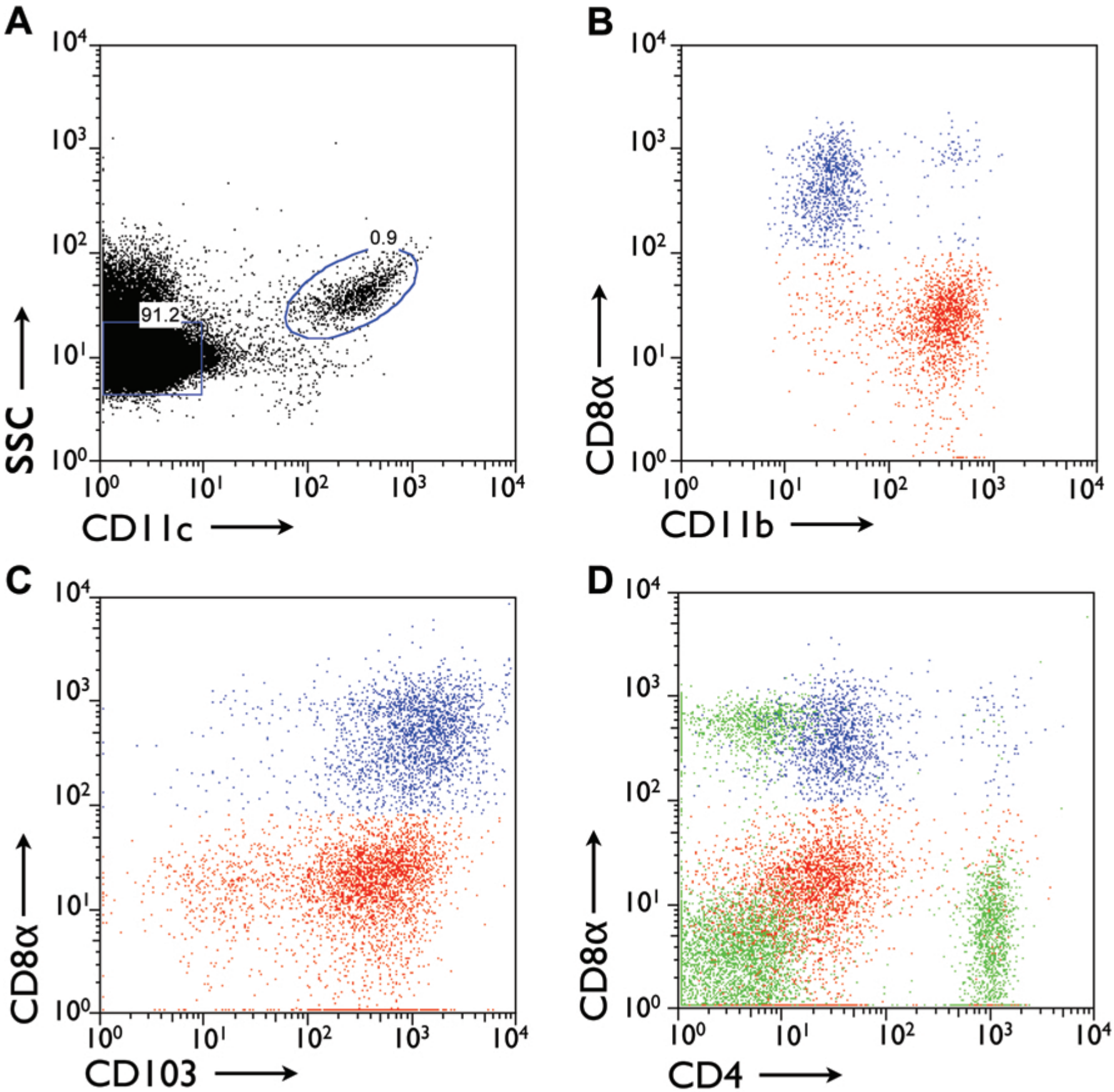
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