Identification and putative roles of distinct subtypes of intestinal dendritic cells in neuroimmune communication : what can be learned from other organ systems?

REVIEW: "Identification and putative roles of distinct subtypes of intestinal dendritic cells in neuro-immune communication: what can be learned from other organ systems?"

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Abstract

The gastrointestinal (GI) tract, just like the skin and the airways, is constantly exposed to both harmless and pathogenic organisms and hence requires a tightly regulated immune homeostasis to function properly. A central role in the regulation of this balance is played by the dendritic cells (DCs), a heterogeneous population of antigen-presenting cells that can be further divided into distinct subsets with different functions depending on the tissue they reside in. In recent years, the DC population in the lamina propria (LP) of the intestine has emerged as a key player in immune surveillance. Given the extensive innervation of the GI mucosa, these DC subsets possibly are also regulated by interactions with neuronal components. Current knowledge, be it still fragmentary, indicates that dysregulation of this neuro-immune communication leads to the onset of pathological disorders. The present review article deals with the identification and interaction of distinct subtypes of mouse intestinal LP DCs with elements of the enteric nervous system (ENS) in normal and inflammatory conditions. Furthermore, the question is addressed whether any parallels can be drawn between intestinal LP DCs and DCs residing in the skin and lung in order to gain a better insight into common or clearly distinct mechanistic pathways and the possible impact of the mucosal components in the microenvironment. The exact way in which the ENS is serving its immunomodulatory roles in the GI tract is still largely unknown, although there are significant indications for a crosstalk between LP DCs and components of the ENS. This review clearly shows that in the three different organ systems the same neurotransmitters (i.e., SP, CGRP and VIP) reoccur, serving similar functions. Mechanistic lessons learned from other organ systems, such as the skin and lung, may be of

substantial help in further exploring the nature of the neuro–immune communication between GI innervation and LP DCs.

Keywords: dendritic cells, subtypes, ileum, neuro-immune modulation

Introduction

In 1868, dendritic cells (DCs) were reported for the first time in the epidermis of the skin, later accordingly named, after their discoverer Paul Langerhans, 'Langerhans cells' (LC) (Langerhans, 1868). Langerhans erroneously classified these cells as neurons because of their staining with gold chloride, an at that time validated method to stain neuronal structures (Langerhans, 1868). LCs remained poorly understood until Silberberg demonstrated in 1973 the involvement of these cells in contact allergy reactions and as such linked this cell type to the immune system (Silberberg, 1973). In the same year, Steinman and Cohn were able to demonstrate a cell type in the murine spleen, Peyer's patches and lymph nodes that morphologically resembled LCs and appeared to differ from macrophages. They termed these cells as 'dendritic cells' because of their tree-like branching appearance (Steinman and Cohn, 1973) and the Steinman lab demonstrated that DCs are key players in the immune response in that they are capable to generate T cell responses (Steinman and Witmer, 1978) and to induce immune tolerance (Steinman et al., 2003). In October 2011, three days after his death, Ralph M. Steinman was officially awarded, together with Bruce Breutler and Jules Hoffmann, the Nobel Prize in Physiology or Medicine for his pioneering research on DC biology. Almost half a century of DC research has made it obvious that these cells constitute a heterogenic group of antigen-presenting cells that can be further subdivided into several subsets based on different expression patterns of cell surface markers, tissue distribution and function. DCs are encountered under steady-state conditions in both lymphoid and non-lymphoid organs, where they play a vital role in the regulation of innate and adaptive immunity (Steinman, 2012). Three large groups of DCs can be discerned: (1) conventional or classical DCs (cDCs), (2) plasmacytoid DCs (pDCs) and (3) follicular DCs (fDCs) (Cerovic et al., 2009). Unlike the two other subgroups, fDCs do not originate from a bone-marrow hematopoietic stem cell and do not express the general leukocyte marker CD45 (Banchereau and Steinman, 1998). pDCs, which are located in peripheral blood and secondary lymphoid organs, are known to secrete large amounts of interferon-α (IFN-α) in response to viral infections, pointing to their importance in establishing a potent immune response (Liu, 2005). fDCs and pDCs will not be further discussed in this review, which will focus on and outline the current views on the interactions between the tissue-resident cDCs in the intestinal LP and the enteric nervous system (ENS) and draws parallels to neurogenic modulation of cDCs in other locations, more particularly skin and lung in order to unravel possible common or clearly discrepant underlying mechanisms since these populations have been studied more intensively so far compared to the ones residing in the gastrointestinal (GI) tract.

Possible involvement of DCs in gastrointestinal pathologies

Given the fact that the GI tract is continuously exposed to an immense number of different antigens, ranging from innocent food antigens to potentially harmful bacteria, viruses and toxins, it is not surprising that the gut is considered to be our body's largest immune organ. In order to cope with this continuous antigen exposure the gut harbors the largest collection of immune cells in the entire body, designated as the 'gut-associated lymphoid tissue' (GALT). Mucosal intestinal DCs, being an important cell type within the GALT, fulfill a central role in maintaining intestinal homeostasis, initiating innate and adaptive immune responses, controlling inflammation and maintaining tolerance (Niess, 2008; Tezuka and Ohteki, 2010). While capable of inducing tolerance against harmless food antigens and commensal bacteria, DCs will also generate a suitable immune response by regulating antigen-specific T cell functions, when they detect a potential pathogen (Mason et al., 2008; Tezuka and Ohteki, 2010). Following pathogen recognition, DCs will undergo a maturation process during which major histocompatibility complex II (MHCII) and co-stimulatory molecules (e.g. CD80, CD86) become upregulated. The activated DCs will migrate to the draining lymph nodes (Cerovic et al., 2009). Any dysregulation of this delicate balance between homeostatic status and active immune response will lead to an aggravated form of intestinal inflammation, possibly resulting in disorders such as inflammatory bowel disease (IBD) (Xavier and Podolsky, 2007), a spectrum of chronic inflammatory GI pathologies that includes Crohn's disease (CD) and ulcerative colitis (UC). IBD has the highest incident rate in developed countries, with a high prevalence in Europe and the United States but there is a steadily increasing number of IBD patients in other parts of the world (Engel and Neurath, 2010; Molodecky et al., 2012). Several studies, as reviewed by Rutella and Locatelli in 2011 and Farache and colleagues in 2013 (Rutella and Locatelli, 2011; Farache et al., 2013b), have suggested that changes in the phenotype and function of intestinal DCs might contribute to the development of IBD. Extensive data on intestinal DCs from IBD patients and murine models clearly show that DCs infiltrate the inflammation site, coinciding with a significant increase in the expression of the co-stimulatory molecules CD80, CD86, CD83 and CD40 on their cell surface (Ikeda et al., 2001; Vuckovic et al., 2001; te Velde et al., 2003). It was further also demonstrated that the toll-like receptors 2 and 4 (TLR2 and TLR4) were upregulated on intestinal DCs from human IBD-patients (Hart et al., 2005). Furthermore, DCs are able to induce tolerance through induction of regulatory T cells (Tregs) from naive $CD4^+$ T cells. Diminished Treg functioning has been shown to be an important factor in IBD pathogenesis (Kuhn et al., 1993; Roers et al., 2004; Maul et al., 2005; Jin et al., 2012). These cellular mechanisms all point once more to the importance of a correct interplay between intestinal DCs and the T cell responses in preventing GI disorders.

Different subtypes of mucosal dendritic cells in the lamina propria of the GI tract.

Different subtypes of DCs, each with its particular markers, are found throughout the GI tract. Our current knowledge on each of these DC subtypes varies considerably. For instance, research on DCs in the stomach has mainly focused on disclosing the role of gastric mucosal DCs in infection with *Helicobacter pylori* rather than on defining possible subtypes. DCs residing in the LP of the esophagus are equally poorly understood. Most research on 'intestinal DCs' has concentrated on the DC subpopulations in the small intestine with specific focus on ileal intestinal DCs, even though ileum, jejunum and duodenum are functionally different. In addition, so far the large intestine has been largely overlooked in this respect.

DC biology is a rapidly evolving field. Only a few years ago, the most widely used and classical marker to define DCs and to exclude them from macrophages was CD11c. However, CD11c has meanwhile proven to be insufficiently specific to identify the intestinal DC phenotypes since this particular marker was also found to be expressed on intestinal macrophages (Hume, 2008; Schulz et al., 2009). Given the eventual conclusion that there is no such thing as a specific DC marker, not to speak of exclusive markers for the distinct subtypes, the indicated approach at present to identify DC subtypes is the specific combination of different surface markers. It should also be noted that the classic myeloid markers F4/80 and SIRPα (CD172a) are also inadequate in unequivocally identifying the different subpopulations of intestinal mononuclear immune cells (Persson et al., 2010). Intestinal DCs are known to be $CD11c^+$ and $MHCII^+$, are scattered throughout the gut and mainly reside in the intestinal mucosa but can also be encountered in the submucosal, muscular and serosal layers (Helft et al., 2010). In addition to the latter two markers, two different subtypes of ileal mouse LP DCs can be distinguished based on the presence of either CD103 (integrin αE) or CX3CR1 (fractalkine receptor) (table 1 and figure 1). These two distinct DC subpopulations arise from different developmental lineages: $CX3CR1^+$ DCs originate from monocytes expressing Ly-6C stimulated by macrophage colony stimulating factor (M-CSF) and Fms-like thyrose kinase 3 ligand (Flt3L), whereas $CD103^+$ DCs develop from pre-DCs under Flt3L control and granulocyte-macrophage colony stimulating factor (GM-CSF) (Bogunovic et al., 2009; Varol et al., 2009). Pre-DCs originate from a common macrophage-DC precursor in the bone marrow (Persson et al., 2010). The difference between the CX3CR1+ and CD103+ subtypes used to be that only the $CD103^+$ LP DCs migrate via the intestinal lymphatic system to the mesenteric lymph nodes (MLN) (Bogunovic et al., 2009; Schulz et al., 2009) where presentation of captured antigens to the T cells occurs. However, recent studies have revealed that $CX3CR1^+$ LP DCs are also able to migrate, to a lesser extent, to the MLN (Cerovic et al., 2012; Diehl et al., 2013). This migration is influenced by microbiota, as discussed below in further detail. It has also been shown that nutrient deficiency, specifically a lack of retinoic acid, can lead to the expression of $CD207^+$ (or $Langerin^+$) DCs in the GALT, a population that is otherwise undetectable in the intestine. This expansion of $CD207^+$ DCs is regulated by CCR7 signaling (Chang et al., 2010; Chang and Kweon, 2010).

$CD103^+$ LP DCs can induce $Foxp3^+$ regulatory T cell (Treg) responses in a reaction that requires retinoic acid and transforming growth factor- β (TGF-β) (Coombes et al., 2007; Sun et al., 2007). Tregs are crucial in neutralizing any detrimental inflammatory responses (Rescigno and Di Sabatino, 2009). $CD103^+$ DCs are further responsible for stimulating B cells into the process of class switching, during which large amounts of IgA can be produced (Rescigno and Di Sabatino, 2009), and for gut homing of lymphocytes (Mcdole et al., 2012). Earlier studies have shown that CD103+ DCs are unlikely to stimulate Th17 cells, a subset of T_{helper} cells producing interleukin-17 (IL-17). The basis for this assumption was that retinoic acid, a vitamine A metabolite, was found to be a key regulator for TGF-β-dependent Treg immune responses that inhibits the development of Th17 responses, as such preventing a pro-inflammatory immune response (Mucida et al., 2007). However, it has also been reported that $CD103^+$ DCs - in response to specific environmental stimuli - do initiate the development of interferon-γ (IFN-γ)-producing T cells (Laffont et al., 2010). The $CD103^+$ DC population can be further subdivided into a $CD103^+CD11b^+CD8\alpha^-$ and a $CD103^+CD11b^-CD8\alpha^+$ DC subset (Bogunovic et al., 2009; Fujimoto et al., 2011; Cerovic et al., 2012). $CD103^+CD11b^-CD8\alpha^+$ DCs constitute a considerably smaller subpopulation in comparison to $CD103^+CD11b^+CD8\alpha^-$ DCs. The immunological functions of both $CD103^+$ DC subpopulations in the intestine were identified based on their expression profiles of TLRs (Fujimoto et al., 2011). The $CD8\alpha^+$ DC subtypes express TLR3, TLR7 and TLR9, while $CD8\alpha^-$ DCs have been found to express TLR5 and TLR9 on their cell surface, demonstrating a different function in active immune responses (Fujimoto et al., 2011). Both $CD103^+$ DC subgroups are probably able to induce inflammatory responses following TLR ligand stimulation (Fujimoto et al., 2011). Importantly, only the $CD8\alpha^-$ subpopulation expresses mRNA of the retinoic acid-converting enzyme Raldh2, indicating that the $CD103^+CD11b^+CD8\alpha^-$ LP DC subset is the only subpopulation responsible for promoting the TGF-β-dependent induction of $Foxp3^+$ Treg cells (Fujimoto et al., 2011). Initially, it was generally reported that $CD103^+$ DCs are involved in maintaining intestinal homeostasis and the induction of tolerance. Since the discovery of the two distinct subsets within the population of $CD103^+$ DCs, it can be concluded that they are also directly involved in mounting Th1 and Th17 immune responses, depending on the type of antigenic challenge.

The other main LP DC subtype consists of $CD103^-CX3CR1^+$ DCs, whose dendrites can extend into the gut lumen and establish tight junctions with the surrounding epithelium, hereby creating a manner to continuously sampling the gut lumen. The exact mechanism underlying this antigen sampling and the subsequent transport of antigens to the MLN is still largely unknown and remains to be elucidated. In our laboratory, using immunohistochemistry and confocal microscopy, we studied the morphology and distribution of $CD11c^+CX3CR1^+$ DCs and $F4/80^+CX3CR1^+$ macrophages in the LP of the mouse ileum using the myeloid marker F4/80 to distinguish between $CX3CR1^+$ DC and macrophage subsets (figure 2 and figure 3). Recent data show that $CX3CR1^+$ mucosal DCs can also sample circulatory antigens from the fenestrated

capillaries, pointing to an important role for these cells in both intestinal and circulatory immune surveillance (Chang et al., 2013). However, recent two-photon microscopic data revealed that $CD103^+CD11b^+$ DCs are also able to develop 'finger-like' protrusions that are able to capture *Salmonella* from the intestinal lumen (Farache et al., 2013a), showing that $CD103^+$ DCs can also actively monitor the intestinal luminal content. To date, the controversy remains whether $CX3CR1^+CD11c^+MHCII^+$ DCs are to be defined as 'true' DCs, or rather as a particular group of intestinal macrophages (Denning et al., 2007; Persson et al., 2010). One of the reasons some authors do not regard $CX3CR1^+$ cells as DCs is because of the lack of active migration to the MLN and their absence in afferent intestinal lymph (Schulz et al., 2009). However, several recent studies do show a migratory capacity of $CX3CR1^+$ cells. Cerovic and colleagues (Cerovic et al., 2012) demonstrated the presence of two distinct $CD103^-$ DC subsets in intestinal lymph and their migration to MLN, among which $CX3CR1^+$ LP cells. In addition, a recent study of Diehl et al. (Diehl et al., 2013) unequivocally showed that $CX3CR1^{high\ (hi)}$ cells are able to migrate to the MLN after capturing *Salmonella*, under conditions of a disturbed microbiotic environment, for example after administration of antibiotics. This migration points to a DC-like function. Some authors make a distinction between $CX3CR1^{intermediate\ (int)}$ and $CX3CR1^{hi}$ cells and propose that the $CX3CR1^{int}$ cell population leans more towards a possible classification as DC or 'DC-like' (Cerovic et al., 2012; Rivollier et al., 2012). $CX3CR1^+$ DCs can either be pro-inflammatory by stimulating the differentiation of $CD8^+$ T cells after antigen cross-presentation (Chang et al., 2013) and assisting in Th17 cell differentiation (Atarashi et al., 2008) or become tolerogenic through the production of IL-10, thereby assisting in the generation of Tregs (Hadis et al., 2011). The existence of these two possible states of $CX3CR1^+$ DCs remains to be further clarified; however, it was proposed that the intestinal microflora might possess modulatory functions (Grainger et al., 2010; Hadis et al., 2011). To our knowledge, there is no definitive consensus to date on whether to classify these $CD103^-$ $CX3CR1^+$ intestinal immune cells as DCs or macrophages. Only the $CD103^+$ DCs have been considered capable of inducing the expression CCR9, a specific gut-homing molecule, on T cells. However, this function is apparently also exerted by the intestine derived $CD103^-$ lymph DC subset (Cerovic et al., 2012). These data, together with the observation that $CD103^-$ DCs produce IFN-γ and IL-17 and migrate steadily from the gut into the MLN, may prompt to reconsider the classification of $CD103^-CX3CR1^+$ DCs as possible intestinal macrophages. It should also be noted, however, that this $CD103^-$ DC migration from the intestinal mucosa to the MLN remains a controversial topic.

Most work in terms of elucidating the phenotype of intestinal DC subsets had been obtained by experiments in the mouse, mostly on the small intestine. When considering the human situation, to date the largest part of information is derived from peripheral blood DCs. The most studied part of the human GI tract is the large intestine, usually a part of the colon that was removed from patients with IBD (Goode et al., 2000; Vento et al., 2001; Hart et al., 2004; ter Beek et al., 2007; Al-Hassi et al., 2013). In the study of

Hart and colleagues (Hart et al., 2004), LP-DCs from colon were identified by multiparametric flow cytometry as $CD11c^{+}HLA\text{-}DR^{+}$ $Lineage^{-}$ (Lin^{-}), with the Lin^{-} antibody cocktail consisting of CD3, CD14, CD16, CD19, CD34 and CD56. A complete overview on the differences in intestinal DCs in mouse and man can be found in the recent and excellent review by Mann et al. (Mann et al., 2013). Both in the mouse and human GI tract, $CD103^{+}$ LP-DCs are present and their functions are maintained in both species (Jaensson et al., 2008). The other LP-DC subset, the $CX3CR1^{+}$ DCs, appears only to be present in minute quantities, when gating for $CD11c^{+}HLA\text{-}DR^{+}Lin^{-}$ human colonic DCs, excluding $CD14^{+}$ cells in the study of Mann and colleagues, as CD14 is a well-known monocytic marker. However, $CD14^{+}HLA\text{-}DR^{+}$ cells also expressed CX3CR1 (Mann et al., 2013).

Current views on distinct dendritic cell subtypes in the mouse dermis and lungs

The cutaneous dendritic cells

The skin functions as a protective barrier to external potentially harmful pathogens and contains different types of antigen presenting cells. For a long time, it was assumed that only the LC, as the unique antigen presenting cell residing in the epidermis, was capable to detect invading pathogens. The LC is characterized by its expression of CD207 (also called Langerin) (Guilliams et al., 2010). However, with the development of a CD207/eGFP (*Lang*-eGFP) knock-in mouse, it became clear that CD207 was not solely specific to denote LCs, but that is was also expressed on thymic DCs and on splenic $CD8\alpha^{+}$ DCs (Kissenpfennig et al., 2005; Idoyaga et al., 2009) and hence no longer suitable to accurately identify LCs having migrated out of the epidermis. On an ultrastructural level LCs can be distinguished by the unique Birbeck granules, that molecularly are composed of CD207 (Valladeau et al., 2000) and that have a 'rod-like' shape (Birbeck et al., 1961) . Nowadays, it is clear that the LC is not the only antigen presenting cell in the skin. In the dermis, several subsets of dermal dendritic cells (DDCs) have been described (Henri et al., 2010). Next to CD207, two additional markers, CD11b and CD103, led to the identification of five different DC subsets in the dermis under steady-state conditions (Henri et al., 2010). From then onwards, it was shown that the epidermal LCs can be identified as being $CD207^{+}CD11b^{int}CD103^{-}$ and the DDC subsets are $CD207^{-}CD11b^{+}$, $CD207^{+}CD103^{+}$, $CD207^{+}CD103^{-}$ and $CD207^{-}CD11b^{-}$ DDCs (table 1 and figure 4) (Guilliams et al., 2010; Henri et al., 2010). Noteworthy is also the fact that LCs and DDCs develop in a different way. LCs are mainly dependent on TGF- β and M-CSF, and independent of Flt3L, while the $CD207^{+}$DDCs require Flt3L (Borkowski et al., 1996; Strobl and Knapp, 1999; Romani et al., 2010). Another difference between epidermal LCs and dermal DDCs is the outcome of the immune response after interaction with T cells. LCs were shown to have tolerogenic properties, leaning towards inducing immune tolerance in the epidermis under steady state conditions provided that the epithelial basement membrane remains intact (Shklovskaya et al., 2011).

Should the invading pathogens be able to reach the dermis, an immediate immune response will be mounted by migratory DDCs (Shklovskaya et al., 2011).

The respiratory tract dendritic cells

Similar to intestine and skin, the airways are constantly exposed to the external environment. Several mechanisms are active in the airways to prevent a disruption in the pulmonary homeostasis. A first protective barrier is also provided by the epithelial layer lining the respiratory airways; additionally, the airways contain several types of immune cells, such as DCs and macrophages, that are able to retain and eliminate invaded particles or pathogens and to further present them into the humoral or cellular specific defence pathways (Lambrecht and Hammad, 2008). Murine lung DCs can be subdivided into 2 groups, i.e., conventional $CD11c^+$ DCs (cDCs) and plasmacytoid DCs (pDCs), which only moderately express CD11c and can be further distinguished on the basis of expression of Siglec-H, Ly6C and B220 (Gill, 2012). Murine lung cDCs can be further subdivided based on the (absence of) expression of CD103 and CD11b (Gill, 2012). This leads to the division of respiratory DCs into 4 different subtypes under steady state conditions: pDCs, $CD103^+$DCs, $CD103^-CD11b^{hi}MHCII^{hi}$ (regarded as $CD11b^{hi}$ DCs) and $CD103^-CD11b^+MHCII^{neg\text{-}med}$ monocytic DCs (table 1 and figure 5) (Hackstein et al., 2012). Further detailed information on the different DC subsets in the murine lung under steady state and during respiratory viral infections can be found in two elegant reviews by the group of Lambrecht (Hammad and Lambrecht, 2011; Neyt and Lambrecht, 2013). In the human lung, not all subsets have been identified so far. However, in humans, the $CD103^+$ alveolar lung DCs were found to resemble LCs (Lambrecht and Hammad, 2009). In mice, it was shown using a mouse inflammation model that $CD103^+$ lung DCs also expressed CD207 (Sung et al., 2006). It was also proven that only during vitamin A deficiency, a clear expansion of $CD207^+$ DCs occurs in the LP of the small intestine of the mouse that is dependent on CCR7 signaling and that is otherwise not detectable (Chang et al., 2010; Chang and Kweon, 2010).

Cross-talk between the immune system and autonomic nerves

Several studies have shown that a multitude of neurotransmitters, e.g. acetylcholine (ACh) (Kawashima et al., 2007), dopamine (DA) (Nakano et al., 2008), norepinephrine (NE) (Maestroni and Mazzola, 2003), serotonin (5-HT) (Katoh et al., 2006), glutamate (Glu) (Pacheco et al., 2007) and vasoactive intestinal peptide (VIP) (Delneste et al., 1999; Delgado et al., 2004), are able to influence DC functions and the concomitant immune responses. Receptors for these neurotransmitters were found to be expressed on different types of immune cells (Pacheco et al., 2010). The presence of the different metabotropic glutamate receptors (mGluR) was described on distinct types of DCs in different species: for example rat

thymic DCs express mGlu2/3R and mGlu4R and show a particular strong expression of mGlu5R (Rezzani et al., 2003), while human monocyte-derived DCs do not express mGlu5R nor mGlu1R (Pacheco et al., 2010). An *in vitro* study focusing on the expression of the metabotropic glutamate receptor, mGlu4R, on cDCs from murine spleen described that activation of this particular receptor had a direct impact on the cAMP concentrations in DCs, thereby inhibiting the generation of a Th17 response to myelin antigen and curbing an inflammatory response. This indicates that glutamate plays a potentially important role in modulating neuroinflammation during multiple sclerosis (Fallarino et al., 2010). Furthermore, human monocyte-derived DCs were shown to express other neurotransmitter receptors, such as the five dopamine receptor subtypes (DARs; D1R to D5R) (Nakano et al., 2008) and a number of serotonin receptors (5-$HTR_{1/7}$) (Katoh et al., 2006). Recently gathered evidence on murine bone marrow-derived DCs (BM-DCs) shows that activation of D5R by DA stimulates the Th17 immune response and that the D5R is downregulated on the DC cell surface after stimulation with lipopolysaccharide (LPS) (Prado et al., 2012). It has also been shown that immune cells, e.g. DCs are able to synthesize and release neuropeptides themselves, hereby enabling a bidirectional neuro-immune interaction. As demonstrated in the aforementioned studies, research on neurotransmitters and neuropeptides and immune cells has mainly focused on *in vitro* experiments. In the following sections, we will compile all current information regarding tissue DCs in skin, lung and intestine.

Langerhans cells and neurogenic modulation

LCs have been reported to play an important role in the interaction between the immune system on the one hand and the epidermal innervation on the other hand (Hosoi et al., 1993; Staniek et al., 1995). The skin, unlike the GI tract, does not have a largely independent intrinsic nervous system; however, there is a bidirectional cross-talk between the CNS and the epidermis, the so-called integrated neuro-immuno-cutaneous system (NICS) (Brazzini et al., 2003), according to which stimuli received in the skin can influence the immune, endocrine and nervous systems at both a local and central level on the one hand and the CNS, on the other hand, can also modulate inflammatory conditions locally induced in the skin. For a more complete overview about the NICS, we refer to (Brazzini et al., 2003; Boulais and Misery, 2008; Ruocco et al., 2013). The skin is highly innervated, and it has been demonstrated that both LCs and DDCs are in close contact with neuronal axons (Hosoi et al., 1993; Sueki et al., 1995; Gaudillere et al., 1996). A recent study by Riol-Blanco et al. (Riol-Blanco et al., 2014) was able to prove that approximately 75% of all DDCs under healthy conditions are in direct contact or are closely located to sensory nerve fibers in the skin. Using an imiquimod-induced cutaneous inflammation mouse model, this study also revealed a neuro-immune interaction between nerve fibers in the dermis and DDCs. The DDCs are specifically controlled by transient receptor potential vanilloid type I channel $(TRPV1)^+$ and $Na_v1.8^+$ nociceptors, hereby inducing interleukin-23 (IL-23) production by the DDCs which in turn leads to an extensive skin inflammation in mice, resembling human psoriasis (Riol-Blanco et al., 2014). The number of contact sites between skin DCs and nerve fibers is

significantly increased after repeated exposure to stress and sensitization with a particular allergen (Pavlovic et al., 2011). LCs have different receptors on their cell surface, for e.g. for calcitonin gene-related peptide (CGRP) and substance P (SP), substances known to be released from somatic and visceral afferents (Hosoi et al., 1993; Asahina et al., 1995; Misery, 2011). Both SP and CGRP are regarded as potent immunomodulatory neuropeptides, and are involved in inflammatory responses in the skin, through mast cell stimulation, which in turn will lead to the release of histamine and serotonin resulting in neurogenic inflammation (Divito et al., 2011). LCs express the neurokinin-1 receptor (NK1R) and SP is able to interfere with the T cell immune response by inhibiting antigen presentation (Staniek et al., 1997). These findings were also corroborated in another study, in which addition of SR140333, a potent agonist of the NK1R, to *in vitro* generated BM-DCs led to a significant decrease in T cell proliferation (Lambrecht et al., 1999). CGRP, just like SP, is also capable of preventing the antigen presentation by LCs and of inhibiting the T cell immune response (Mikami et al., 2011). It was also demonstrated that CGRP can inhibit the expression of CCR7 in Langerin$^+$ DCs, which were stimulated *in vitro* with LPS, impacting the migration of the Langerin$^+$ DDCs to the draining lymph nodes (Mikami et al., 2011). PCR experiments on both purified murine LCs and murine LC-like cell lines revealed that LCs further express mRNA for the VIP receptors, VPAC1 and VPAC2 (Torii et al., 1997; Kodali et al., 2004). Following stimulation of the XS106 LC-like cell line with LPS, VIP stimulated the production of IL-10 and suppressed the production of IL-12 and IL-1β (Kodali et al., 2004). VIP was further found to decrease the expression of CCR7 on BMDCs *in vitro* after stimulation with LPS, be it with a noticeable increase in CCR1 expression (Weng et al., 2007). These findings indicate that VIP has an anti-inflammatory role in that it prevents DC migration and the subsequent inflammatory response (Weng et al., 2007). Studies with mouse immune cells showed that *in vivo* administration of VIP and PACAP ('pituitary adenylate cyclase-activating polypeptide') stimulated a Th2 response and inhibited a Th1 response (Delgado et al., 1999). Adrenalin receptors were demonstrated on both purified murine LCs and the LC-like cell lines XS52-4D and XS106 which expressed mRNA for β1-, β2- and α1- adrenoceptors (Seiffert et al., 2002). This particular study concluded that signaling through adrenoceptors on LCs can have a direct effect, by diminishing epidermal immune responses. It is clear that the intimate interaction between the CNS and the cutaneous immune system constitutes a delicate balance, which is of utmost importance in preventing noxious inflammatory responses.

Lung dendritic cells and neurogenic modulation

As in skin, an extensive neuro-immune communication is present in the lung. Veres et al. (Veres et al., 2007) showed that pulmonary sensory CGRP$^+$ or SP$^+$ nerve fibres can establish close contacts with DCs. Furthermore, a significant increase in neuro-DC contacts was observed under inflammatory conditions; detailed analysis of the distribution of the CGRP-containing nerves revealed that CGRP could enable the further recruitment of DCs to the site of airway inflammation (Veres et al., 2007). It was also shown that

CGRP can influence the migration velocity of DCs. Low concentrations (1 nM) of CGRP steadily increased the motility of the mouse DCs in living lung slices, whereas addition of higher concentrations of CGRP (100 nM) led to DC immobilization (Voedisch et al., 2012). When VIP was applied in low concentrations (0,1 nM), DC motility steadily decreased (Voedisch et al., 2012). Using a combined approach of RT-PCR, immunofluorescence and cAMP assays, Rochlitzer and colleagues reported that murine lung DCs express functional CGRP receptors, that are down regulated under inflammatory conditions (Rochlitzer et al., 2011). Likewise, DC migration was lower and more CGRP-containing nerves were found in an acute model of asthma, in contrast to a more chronic asthma animal model where DC migration rates actually increased and less CGRP-containing nerves were present (Voedisch et al., 2012). Taken together, CGRP is able to influence the migration of lung DCs in vitro in a dose-dependent manner. A study in which the involvement of NK1R was verified in murine models for chronic obstructive pulmonary disease (COPD) and smoke-induced inflammation revealed that NK1R was implicated in the recruitment of different immune cells, leading to increased DC and macrophage numbers in the airways (De Swert et al., 2009). A specific NK1R antagonist, RP 67580, further resulted in a protective effect in a cigarette smoke-induced inflammation model (De Swert et al., 2009). Another study reported DC activation through NK2R signaling, resulting in a modulation of the T cell responses (Kitamura et al., 2012). Higher expression rates of NK2R were also encountered in asthma models with a Th1 response, leading to the hypothesis that neuromodulation in the lung through NK2R is able to increase the activation of DCs, resulting in a distinct Th1 inflammatory response (Kitamura et al., 2012). A profound knowledge of how neuronal mediators and DCs interact as well as detailed information on the expression pattern of the corresponding receptors on the surface of lung DCs could potentially yield important therapeutic benefits.

Communication between the ENS and distinct DC subtypes

The effects of neurochemical substances on the functioning of intestinal DCs *in vivo* or *in vitro* have, somewhat surprisingly, barely been studied, whereas relatively much research has been devoted to cutaneous or pulmonary DC subtypes, as discussed above. Besides DCs, also macrophages can be detected in the GI tract. A large population of macrophages can be detected throughout the mucosa but another macrophage population resides in the smooth muscle layers (Mikkelsen et al., 1985). Recently, a morphological study employing GI whole mounts of rat, was able to show that these smooth muscle macrophages lie in close contact to different components of the ENS indicating that this subpopulation of macrophages possibly exerts neuromodulatory functions (Phillips and Powley, 2012). The ENS is directly involved in different functions of the intestine, such as coordinating motility, monitoring the secretion of gut peptides, regulating epithelial transport, intestinal immune responses and mucosal blood flow (Snoek et al., 2010; Furness, 2012). Defects in the neuro-immune bidirectional communication are to be considered as possible causes of the development of (auto)immune disorders resulting in severe GI

pathologies. The main candidate mediators in this neuro-immune interaction from intrinsic and extrinsic components of the ENS to different effector cells and immune cells are neuropeptides and non-peptidergic neurotransmitters. An important neurotransmitter in the GI tract is VIP, a neuropeptide consisting of 28 amino acids that can be synthetized by enteric nerves as well as by different types of immune cells, such as mast cells (Martinez et al., 1999; Snoek et al., 2010). VIP binds to VPAC1 and VPAC2, which are both expressed throughout the intestine, amongst others on intestinal DCs residing in Peyer's patches (Massacand et al., 2008). Another study using a mouse trinitrobenzene sulfonic acid (TNBS)-induced colitis model showed that the pathological symptoms could be ameliorated following injection of VIP-activated DCs (Gonzalez-Rey and Delgado, 2006). It was also proven on *in vitro* cultured murine MLN DC's that VIP is able to polarize the immune response towards a Th2 profile by reducing the TNBS-induced expression of TLR2 and TLR4, in an attempt to restore intestinal homeostasis (Arranz et al., 2008). Murine BM-DCs that express VPAC1 and VPAC2 have been shown to additionally contain mRNA that encodes for the five muscarinic ACh receptor (mAChR) subtypes (M1 to M5) (Kawashima et al., 2007). Furthermore, these DCs constitutively express six different subtypes of nictotinic Ach receptors (nAChRs), *i.e*. α2, α5, α6, α7, α10 and β2 (Kawashima et al., 2007). *In vitro* experiments with nicotin led to the activation of human monocyte-derived DCs abundantly expressing the α7nAChR, resulting in an enhanced adaptive immune response with increased T cell proliferation (Aicher et al., 2003). Interesting to note is that the same receptor, α7nAChR , is implicated in the vagal cholinergic anti-inflammatory pathway acting through macrophages, as reviewed by Matteoli and Boeckxstaens (Matteoli and Boeckxstaens, 2013). Murine BM-DCs also showed expression of β1-, β2-, α2A- and α2C adrenoceptors at the mRNA level (Maestroni and Mazzola, 2003). In a dextran sulphate sodium-induced colitis model on mice, it became clear that 5-HT can modulate the NFκB activation pathway, by stimulating the production of pro-inflammatory cytokines by DCs (Li et al., 2011). Also, the neuropeptides CGRP and SP have been shown to be implicated in experimental colitis animal models and in human UC and CD (Mazelin et al., 1998; Gross and Pothoulakis, 2007; Engel et al., 2012), demonstrating a potential pathogenic neuro-immune interaction. In TNBS-induced colitis models in rat and guinea pig different tachykinin receptors (NK1R, NK2R and NK3R), were proven to be involved in the inflammatory processes (Mazelin et al., 1998). However, there was a different association to be seen in each species. In rat, NK1R and NK2R are directly associated with intestinal inflammation while in guinea pig, NK2R and NK3R, but not NK1R are involved. By using selective non-peptide antagonists for these tachykinin receptors, it was possible to ameliorate the tissue damage showing a pro-inflammatory role for SP (Mazelin et al., 1998). In studies on biopsies from UC patients, an increased SP expression in colonic nerve endings (Goode et al., 2000; Vento et al., 2001). Similarly, enhanced NK1R mRNA levels were observed in IBD patients compared to controls (Goode et al., 2000). These studies provide significant evidence that SP acts as a pro-inflammatory neuropeptide in both experimental animal models and human pathology. To the contrary, neuronal CGRP expression was

decreased in the colon of UC patients (Koch et al., 1987; Eysselein et al., 1992). These observations are in line with the findings of a later study in a TNBS-induced colitis mouse model, demonstrating that the protective and anti-inflammatory effect of CGRP was blocked by using a selective CGRP-receptor antagonist and mucosal tissue damage was markedly increased (Reinshagen et al., 1998). Also, in an experimental oxazolone-induced colitis model, CGRP was seen to have anti-inflammatory properties as opposed to SP that can exert pro-inflammatory changes (Engel et al., 2012).

Neuropeptide expression by DCs

Currently, only few information is available about the neuropeptide expression by DCs. During inflammation mouse DCs are able to synthesize 5-HT and transmit it during the DC- T cell interaction, hereby regulating T cell activation (O'Connell et al., 2006) . Another study was able to demonstrate that human monocyte-derived DCs can release Glu while undergoing maturation after exposure to staphylococcal enterotoxin A (Pacheco et al., 2006). This shows that DCs are capable of secreting neurotransmitters such as DA, Glu and 5-HT (O'Connell et al., 2006; Pacheco et al., 2006; Prado et al., 2012). Evidence gained by *in vitro* experiments on BM-DCs revealed that DCs possess the ability to actively synthesize, store and afterwards degrade DA (Prado et al., 2012). In this study, it is proposed that DCs can secrete DA in an autocrine manner, hereby influencing the T cell response *in vivo* through stimulation of D5R on DCs (Prado et al., 2012). Another *in vitro* study on human monocyte-derived DCs established that DCs can release Glu in a nonvesicular manner using the cysteine/glutamate antiporter X_c^- system during maturation (Pacheco et al., 2006). Recently however, it was discovered that *in vitro* splenic or thymic DCs are able to secrete Glu in a very fast, Ca^{2+}-dependent manner resulting in a swift modulation of the T cell response (Affaticati et al., 2011). In contrast to DA and Glu, DCs cannot synthesize 5-HT but express 5-HT transporters (SERTs) which enables them to sequester 5-HT from their surrounding microenvironment. Upon DC maturation, after stimulating the DCs *in vitro* with LPS, the ability of DCs to sequester 5-HT increases (O'Connell et al., 2006). Taken together, these studies suggest DCs as a possible source for neuropeptide signaling. Although functional studies have focused on the influence of these neuropeptides on the T cell immune response, it is plausible nerve fibers contacting DCs can also sense this secretion. The functional role of this DC-to-neuron communication remains to be investigated.

Concluding remarks

Research on intestinal DCs has proven to be a difficult and challenging task. A reliable analysis of the exact phenotype of an intestinal DC *in situ* and *in vivo* implies a set of dissociation- and isolation-protocols that might have a negative impact on their morphological and functional characteristics. Such an approach most likely alters the phenotype and presumably causes activation of the dissociated cells, possibly also changing the expression of particular cell surface markers on the DC cell surface (Pabst and Bernhardt, 2010; Cerovic

et al., 2012). In addition, there is also some degree of uncertainty as to the cell type specificity of these isolation methods. There is still much to be learned about the cross-talk between intestinal DCs and neuronal components *in vivo*. The research focusing on *in vitro* experiments on DC types other than intestinal DCs provides an important first point of reference as to the neuropeptides generally involved in the neuro-immune interaction. Aside from this *in vitro* research it is also advisable not to exclusively focus on one particular type and location of DCs, but to compare the DC behavior in different organ systems and attempt to decipher common mechanistic pathways, taking into account possible regional differences. Learning more about the common neurogenic substances involved in other organ systems could contribute to further denominate the most relevant neuropeptides that in turn can be studied in future *in vivo* experiments with intestinal DCs. All three organ systems dealt with in this review (GI tract, skin, airways), interact directly with the external environment and therefore need to provide a barrier to possible invading pathogens, noxious stimuli or influences that could alter the internal homeostatic state. Within these organ systems, as mentioned above, DCs are part of the immunological first line of defense, capable to generate an adequate T cell response. Also, when we look more closely at the different subtypes and cell surface markers that denote the specific DC subsets in each organ, it was demonstrated that there is in fact one particular marker, CD103, that is present in all three organs representing the migratory DC subset. In the skin, CD103 is expressed by a subset of DDCs (Guilliams et al., 2010; Henri et al., 2010). The $CD103^+$ respiratory DCs are able to migrate to the lymph nodes and, hereby stimulating $CD8^+$ T cell immune responses (del Rio et al., 2007). The $CD103^+$ LP DCs in the GI tract are also able to migrate, in this case to the MLNs and they are involved both in maintaining gut homeostasis and mounting necessary immune responses (Coombes et al., 2007; Fujimoto et al., 2011). It would also be interesting to further explore the ontogeny of all DC subtypes in all three aforementioned organ systems. There are parallels in the development of certain DC subtypes that can be noted, i.e. the $CD103^+$ DCs in the dermis, ileum and lung arise from pre-DCs in the bone marrow (Schlitzer and Ginhoux, 2013). Before, there were still unclarities about the exact provenance of the lung cDCs which were believed to originate from monocyte precursors (Jakubzick et al., 2008). Recently, using the dendritic cell natural killer lectin group receptor 1 (DNGR-1) as a marker for the precursor stages of mouse cDCs, the ontogeny of the mononuclear phagocyte system was studied (Schraml et al., 2013). This shows that lung cDCs in fact are also derived from pre-DCs (Schlitzer and Ginhoux, 2013; Schraml et al., 2013). Further research into the ontogeny of the different subtypes with attention to possible parallels between organ systems may lead to new functional insights. There is evidence at hand for an intimate interaction with the nervous system. To enable this bidirectional communication, DCs have to express different neuropeptide/ neurotransmitter receptors on their surface to interact with released neurogenic substances. Disruption of this delicate communication may lead to exaggerated immune responses, resulting in a number of pathologies and contributing to their severity. Several human diseases (e.g. IBD, asthma and urticaria) appear to be characterized by a noticeable

neuronal involvement. It is clear that the neurotransmitters involved in modulating these neuro-immune responses to a large degree are identical in the different organ systems, *e.g.* CGRP, SP and VIP. In the GI tract, CGRP was shown to have opposite immunological properties to SP, acting as an anti-inflammatory agent while SP possesses pro-inflammatory characteristics. It is also clear that VIP can exert important functions in the intestine, as it is able to improve symptoms of tissue damage and severe inflammation. In the skin, it has become clear that the three aforementioned neuropeptides are implicated in several events in cutaneous immunology. It was demonstrated that LCs express receptors for CGRP and SP on their cell surface, substances that are both capable of inhibiting antigen presentation to T cells. Moreover, CGRP is able to inhibit the migration of Langerin$^+$ DCs *in vitro* (Mikami et al., 2011). Overall, the effect of CGRP is dependent on the type of skin inflammation and can lead to different immunological outcomes (Mikami et al., 2011), as opposed to SP that has pro-inflammatory traits in the skin. VIP on the other hand, clearly has an anti-inflammatory role in the skin, able to inhibit LC migration and inhibit detrimental immune responses. As in the skin, it was described that in the lung, CGRP$^+$ or SP$^+$ nerve fibers lie in close contact to immune cells and DCs in particular. In the respiratory tract, it has also been reported that CGRP is able to influence the DC migration velocity in a dose-dependent manner and that CGRP receptors are down regulated during inflammation. This strongly indicates that CGRP has anti-inflammatory properties. In contrast, SP has shown to have a pro-inflammatory effect when blocking its receptor, NK1R, resulted in a protective effect. The exact role of these neuropeptides or neurotransmitters involved as well as of their receptors on the DCs needs to be further elucidated *in situ*. Targeting these receptors may represent an interesting new therapeutic approach to alleviate symptoms of inflammatory disorders in which the etiology comprises a neurogenic alteration of DC functioning.

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Table 1. Summary of all DC subtypes in mouse ileum, lung and skin.

DCs	GUT	LUNG	SKIN
Classical/ conventional DCs (cDCs)	Lamina propria (LP) of the ileum - $CD11c^{+}CD103^{+}CX3CR1^{-}$ LP-DCs - $CD11c^{+}CX3CR1^{+}CD103^{-}$ LP-DCs (Schulz et al., 2009) (Bogunovic et al., 2009) (Cerovic et al., 2009)	Airway mucosa and pulmonary vessels: - $CD103^{+}MHCII^{+}CD11b^{-\text{ to}+}$ DCs Submucosa lung parenchyma: - $CD103^{-}$ $MHCII^{high}CD11b^{\text{med to high}}$ DCs Migratory: - $CD103^{-}CD11b^{+}MHCII^{-\text{ to }med}$ monocytic DCs (Kim and Braciale, 2009) (Hackstein et al., 2012)	Epidermis: - $CD207^{+}$ (= $Langerin^{+}$) $CD11b^{int}CD103^{-}$ LCs Dermis: - LCs in migration to cutaneous lymph nodes - $CD207^{+}CD103^{-}$ DDC - $CD207^{+}CD103^{+}$ DDC - $CD207^{-}CD11b^{-}$ DDC - $CD207^{-}CD11b^{+}$ DDC (Henri et al., 2010) (Guilliams et al., 2010) (Romani et al., 2010)
Plasmacytoid DCs (pDCs)	pDCs always $CD11b^{-}$ (Merad and Manz, 2009) $CD11c^{low}CD45RA^{+}B220^{+}Ly6C^{+}GR\text{-}1^{low}$ (Asselin-Paturel et al., 2003)	pDCs always $CD11b^{-}$ (Merad and Manz, 2009) $CD11c^{int}Siglec\text{-}H^{+}B220^{+}Ly6C^{+}GR\text{-}1^{int}$ (Gill, 2012) Lung: $CD11c^{+}SiglecF^{-}CD45^{+}$ (Hackstein et al., 2012)	pDCs always $CD11b^{-}$ (Merad and Manz, 2009) $CD11c^{low}CD45RA^{+}B220^{+}Ly6C^{+}GR\text{-}1^{low}$ (Asselin-Paturel et al., 2003) Skin: pDCs only infiltrate after viral infection and chronic inflammation (Gros and Novak, 2012)

Table 1. A summary of all the different DC subtypes that are present in the ileum, the lungs and skin of the mouse. A division between classical/ conventional DC's (cDCs) and plasmacytoid DCs (pDCs).

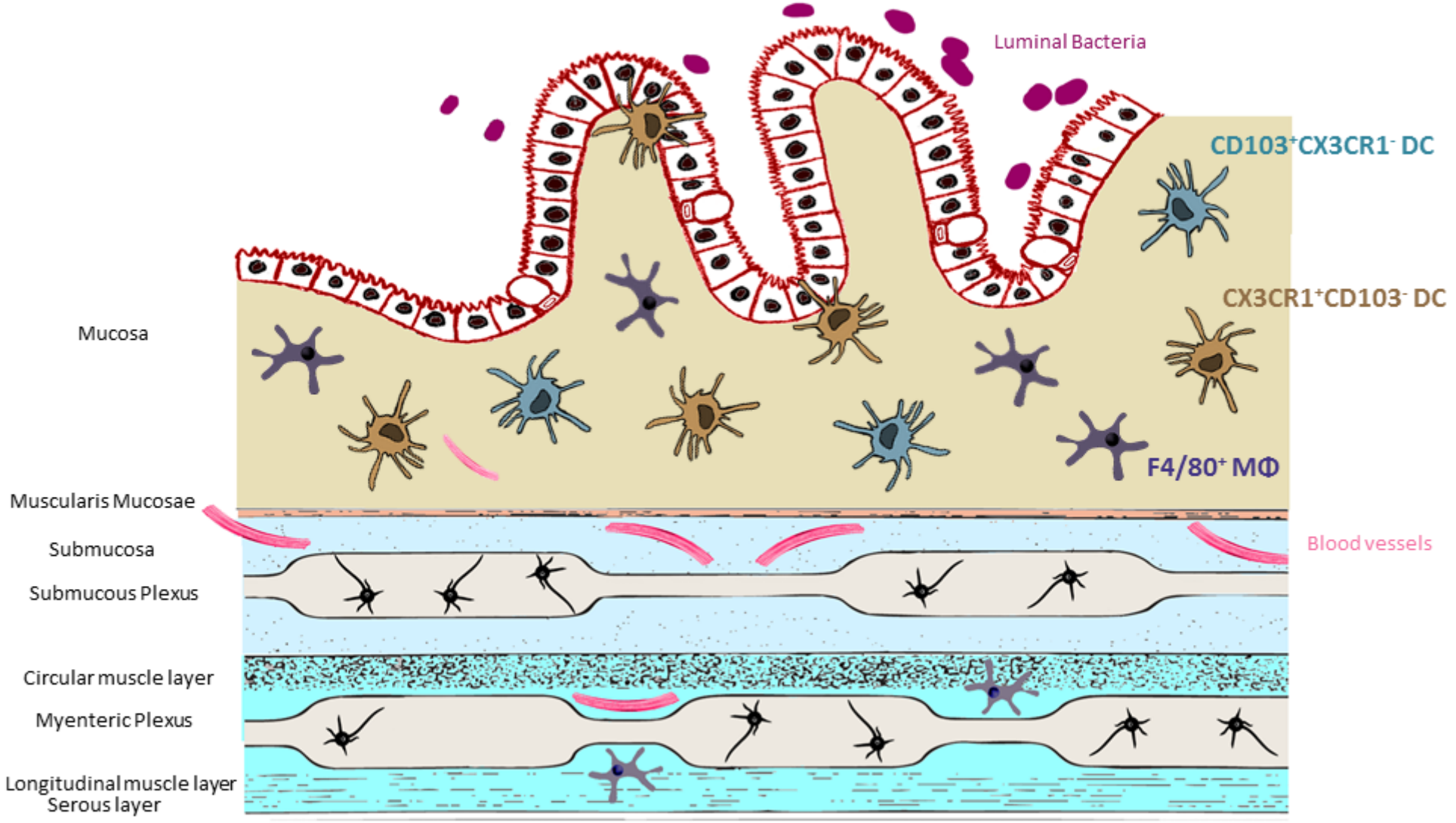
Figure 1. Overview of the different dendritic cell (DC) subpopulations in the ileal lamina propria (LP) of the mouse. The two main DC subtypes are dispersed throughout the LP, i.e. the CD103+CX3CR1- DCs and the CX3CR1+CD103- DCs. Also, in the LP and muscle layer F4/80+ macrophages (MΦ) are present.

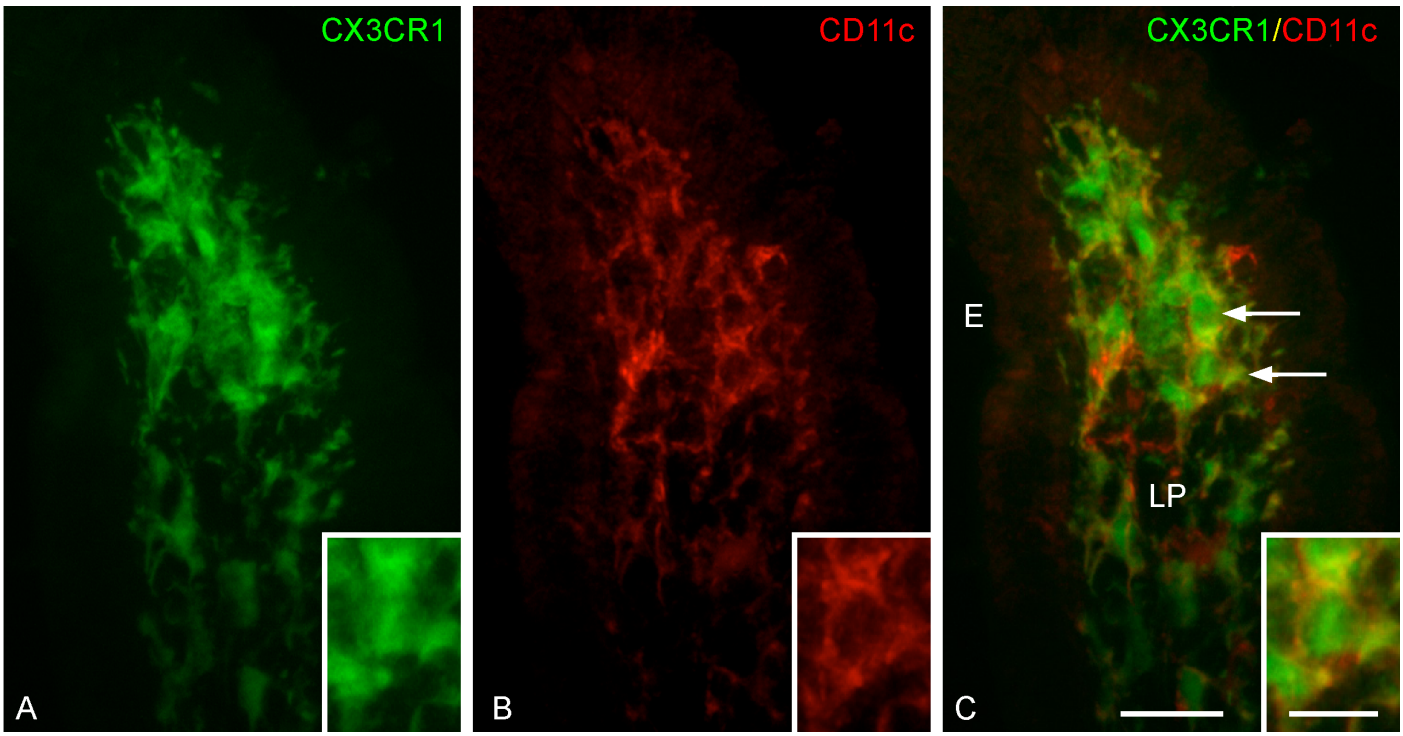
Figure 2. The distribution of CD11c^{+}CX3CR1^{+}F4/80^{-} DCs in the ileum of the CX3CR1$^{+/GFP}$ Bl/6 mouse. Using cryosections and immunohistochemistry, the CD11c^{+}CX3CR1^{+}F4/80^{-} DCs can be visualized throughout the villus (fig c). Scale bar: 20µm.

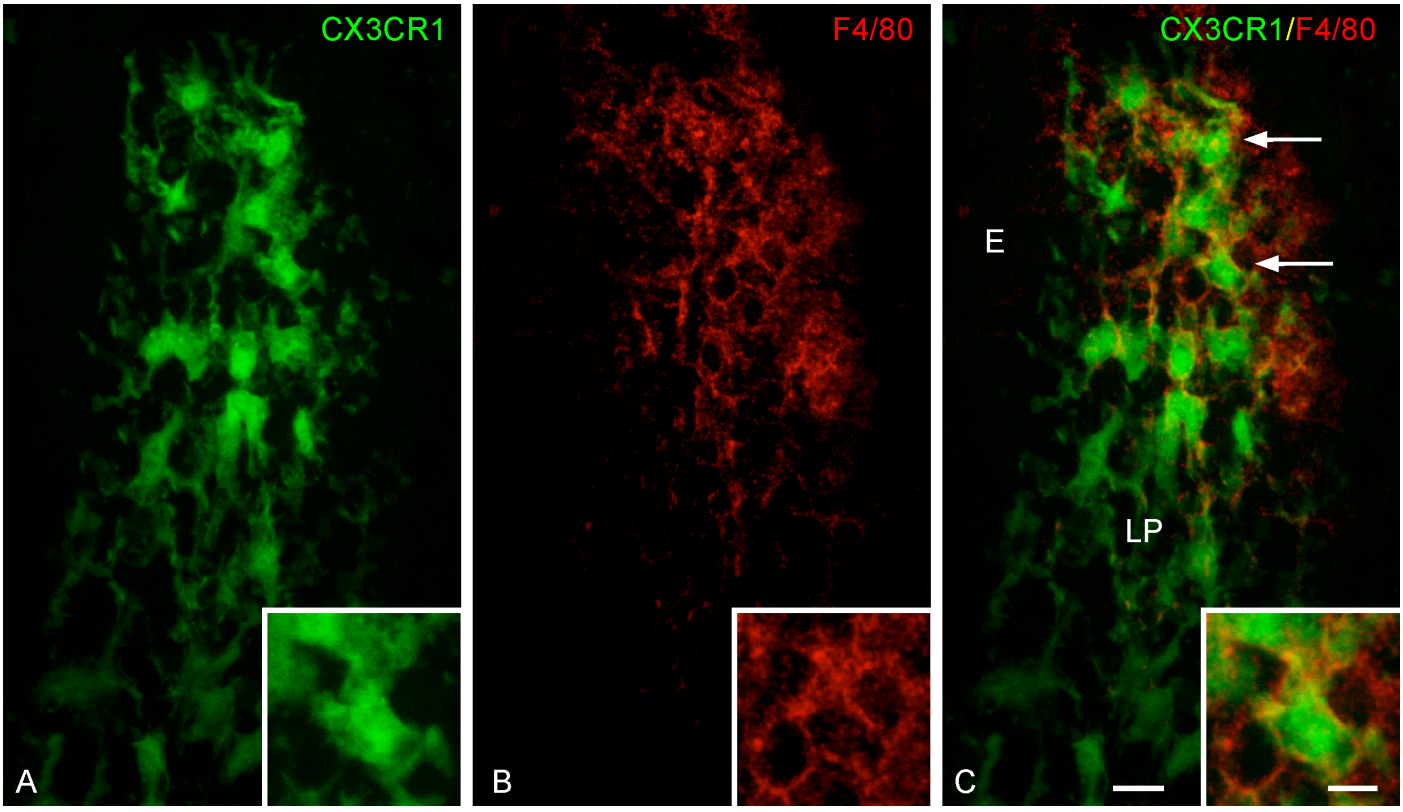
Figure 3. The presence of F4/80^{+}CX3CR1^{+} macrophages in the ileum of the CX3CR1$^{+/GFP}$ Bl/6 mouse. Immunoreactivity of CX3CR1^{+} cells for F4/80 denotes them as 'F4/80^{+}CX3CR1^{+} macrophages. Using cryosections and immunohistochemistry, the F4/80^{+}CX3CR1^{+} macrophages are detected in the lamina propria (LP) of the villus (fig c). Scale bar: 20µm.

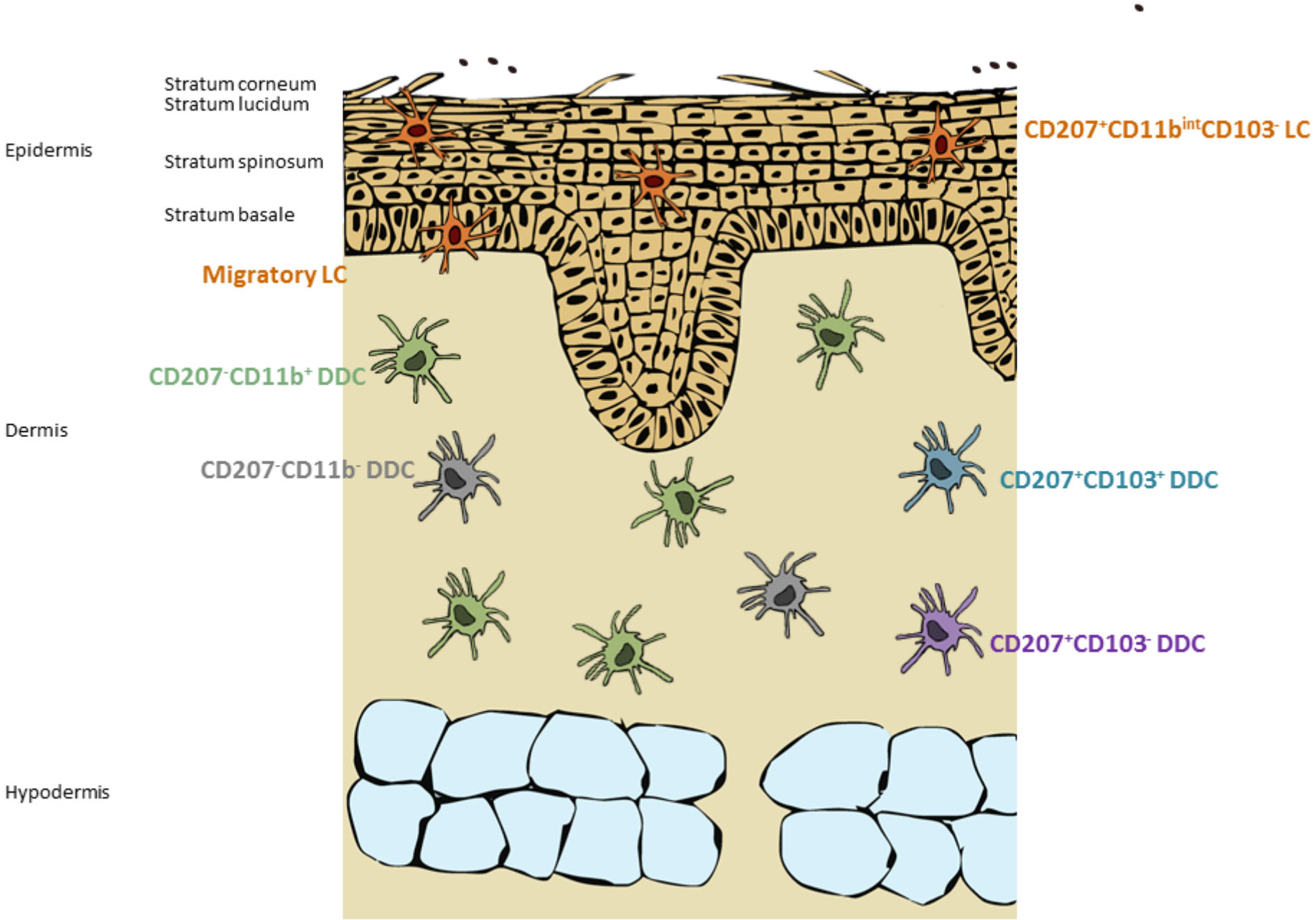
Figure 4. The dendritic cell (DC) subpopulations in the mouse skin. In the epidermis, the Langerhans cells (LCs) are present and can be defined by as CD207^{+}CD11c^{int}CD103^{-}. The dermis hosts the dermal dendritic cells (DDCs) and can also be home to migrating LCs en route to the skin draining lymph nodes. There are different subtypes of DDCs: CD207^{-}CD11b^{+}, CD207^{+}CD103^{+}, CD207^{+}CD103^{-} and CD207^{-}CD11b^{-}.

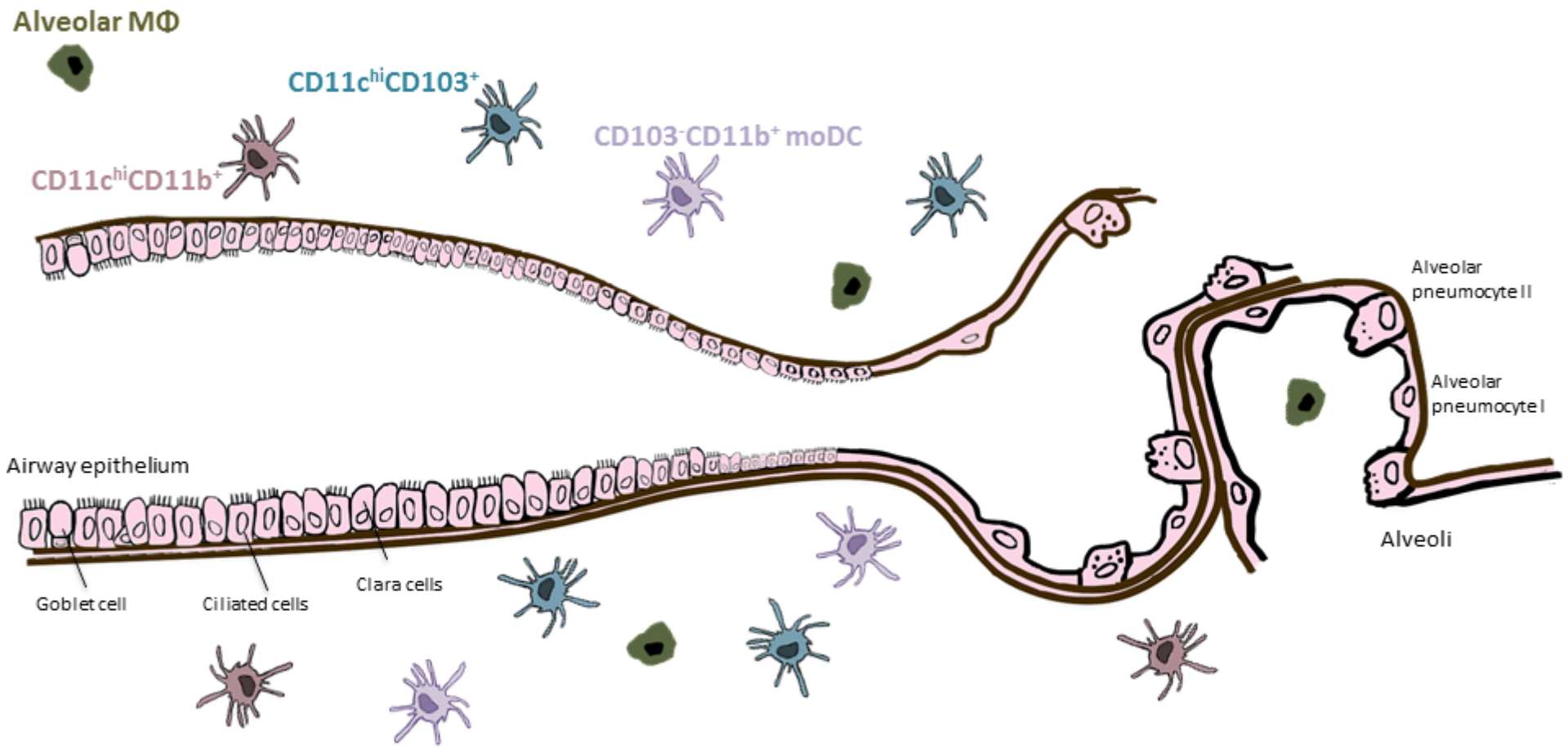
Figure 5. The different respiratory dendritic cell (DC) subtypes. In the respiratory tract of the mouse during steady state conditions, several DC subpopulations are present: CD11c^{hi}CD103^{+} DCs, CD11c^{hi}CD11b^{+} DCs and CD103^{-}CD11b^{+} monocytic DCs. Alveolar macrophages (MΦ) are found throughout the airways.


Luminal Bacteria
CD103⁺CX3CR1⁻ DC
CX3CR1⁺CD103⁻ DC
F4/80⁺ MΦ
Mucosa
Muscularis Mucosae
Submucosa
Submucous Plexus
Circular muscle layer
Myenteric Plexus
Longitudinal muscle layer
Serous layer
Blood vessels


CX3CR1
CD11c
CX3CR1/CD11c
E
LP
A
B
C


CX3CR1
F4/80
CX3CR1/F4/80
E
LP
A
B
C


Stratum corneum
Stratum lucidum
Epidermis
Stratum spinosum
Stratum basale
Migratory LC
CD207+CD11bintCD103- LC
CD207-CD11b+ DDC
Dermis
CD207-CD11b- DDC
CD207+CD103+ DDC
CD207+CD103- DDC
Hypodermis


Alveolar MΦ
CD11c^hiCD103^+
CD103^-CD11b^+ moDC
CD11c^hiCD11b^+
Alveolar pneumocyte II
Alveolar pneumocyte I
Airway epithelium
Alveoli
Goblet cell
Ciliated cells
Clara cells