

REGULATION OF DENDRITIC CELL RECRUITMENT BY CHEMOKINES

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Dendritic cells (DC) are a heterogeneous family of cells that function as sentinels of the immune system. This article summarizes observations suggesting that inflammatory chemokines secreted at the site of pathogen invasion determine the DC subset recruited and influence the class of the immune response initiated. Langerhans cells are selectively recruited by MIP-3 α /CCL20. In contrast, CCR7 ligands have a key role in the accumulation of antigen-loaded mature DC in T cell-rich areas of the draining lymph node. Improved understanding of the regulation of DC trafficking might offer new opportunities for therapeutic interventions to control immune responses.

LIFE CYCLE OF DENDRITIC CELLS

Dendritic cells (DC) are bone marrow-derived leukocytes that function as sentinels of the immune system (1–3). DC precursors migrate from the bone marrow through the blood stream to almost every tissue, where they eventually become resident immature DC. Langerhans cells (LC) in the epidermis are the best-studied example of immature DC, which show a strong capacity for antigen uptake but a low capacity for antigen presentation. During pathogen invasion, immature DC capture intruder antigens and rapidly leave the epidermis. They crawl through the dermis, cross the endothelium of lymphatic vessels, and migrate to the draining lymph node. During their migration from the peripheral tissues, DC undergo phenotypical and functional maturation. In vitro-generated DC, either from monocytes cultured in the presence of granulocyte-macrophage colony-stimulating factor and interleukin (IL)-4 (4) or from CD34⁺hematopoietic progenitor cells (HPC) cultured in granulocyte-macrophage colony-stimulating factor plus tumor necrosis factor (TNF)- α (5), have provided models for studying DC maturation. After exposure to pathogens or proinflammatory mediators (IL-1, TNF- α), DC lose the ability to capture antigens, translocate MHC class II molecules from the lysosomal compartment to the membrane, and up-regulate the expression of costimulatory molecules (6–8). After reaching the subcapsular sinus of the lymph node, DC move to the T-cell areas through which T cells recruited from the blood percolate. T-cell area DC, known as interdigitating dendritic cells, are actively involved in the presentation of antigen to naive T cells. During interaction with CD4 T cells, stimuli such as CD40 ligand (CD40L) will empower activated DC with the capacity to present antigen to naive CD8⁺ T cells (9, 10). The presentation of antigen to the appropriate T cells seems to be the ultimate mission of the DC recruited from the periphery, as

most of them die in the T-cell areas, most likely by apoptosis. The complex pattern of DC migration favors the presentation of antigen captured at the periphery to the rare antigen-specific T cells and the activation and subsequent clonal expansion of these T cells.

DIFFERENT IMMATURE DC POPULATIONS ARE RECRUITED BY DIFFERENT CHEMOKINES: THE EXAMPLE OF MIP-3 α /CCL20. A SELECTIVE CHEMOATTRACTANT FOR EPITHELIAL DC

Each step of DC trafficking involved in either their “steady state” distribution in peripheral and lymphoid organs or in their recruitment upon inflammation/injury is likely to be controlled, at least partially, by soluble chemotactic factors known as chemokines.

Although many chemokines can induce in vitro DC migration (11–16), each immature DC population displays a unique spectrum of chemokine responsiveness. Among DC populations, LC have a unique anatomical localization on the epithelial surfaces. Our understanding of the elements that control this selective localization is progressing; important factors include the potentially unique origin of LC (17, 18), the chemotactic factors required for LC precursor recruitment (19), an epithelial environment (i.e., transforming growth factor- β) that conditions their ultimate differentiation (20, 21), and homing/adhesion molecules (e.g., CLA, E-cadherin) involved in their residency (22).

MIP-3 α /CCL20 (23–25) seems to be the chemokine that induces the most potent responses of CD34⁺ progenitor-derived CD1a⁺ LC precursors (19). The activity of MIP-3 α /CCL20 has been confirmed in ex vivo-isolated LC (19, 26), and so far only CD34⁺ progenitor-derived DC and ex vivo-isolated LC respond to MIP-3 α /CCL20 consistent with their expression of CCR6, the only known receptor for MIP-3 α /CCL20. Monocytes, MDDC, and ex vivo-isolated blood DC populations do not respond to MIP-3 α /CCL20 (19). However, recently it has been shown that MDDC can express CCR6 and respond to MIP-3 α /CCL20 when cultured with transforming growth factor- β (27), a factor previously reported to support LC differentiation from monocytes (28). Thus, MIP-3 α /CCL20 seems to induce the selective migration of LC and LC precursors, independently of their origin.

In humans, MIP-3 α /CCL20 has recently been reported to be constitutively expressed by keratinocytes in the epidermal layer of the skin (19, 26). In vitro, using a specific ELISA, the expression of MIP-3 α /CCL20 was found to be up-regulated by inflammatory stimuli such as IL-1 and TNF- β , in epithelial cells such as skin keratinocytes (19). In addition, T-cell factors such as CD40L, IL-17, and interferon- γ cooperate to induce strong levels of MIP-3 α /CCL20 production by such

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cells. These observations could be particularly relevant to disease conditions involving epidermal infiltration by immune cells such as observed in psoriasis. Indeed, MIP-3 α /CCL20 and CCR6 are strongly up-regulated in this pathology (19, 29). These observations suggest that, upon inflammation, the up-regulation of MIP-3 α /CCL20 production may play an important role in the recruitment of LC precursors in the skin. However, this would not exclude a role for this chemokine in the constitutive recruitment of LC.

In vivo, MIP-3 α /CCL20 is also expressed at other epithelial surfaces. In mice and humans, MIP-3 α /CCL20 has been shown to be abundantly expressed in gut mucosa (30). In human tonsils (mucosa-associated lymphoid organs), we have observed a very strong expression of MIP-3 α /CCL20 in the epithelial crypt (14, 19). More recent observations have reported that in the gut, MIP-3 α /CCL20 was indeed highly expressed in the epithelium of Peyer's patches (31, 32). The expression of MIP-3 α /CCL20 in this epithelium is associated with CCR6-expressing cells in the close vicinity of epithelial cells (31). These cells very likely include memory B and T cells, known to respond to MIP-3 α and to be located in such areas as tonsils and Peyer's patches. Furthermore, in Peyer's patches, a subset of CD11b⁺ myeloid DC that lines the dome epithelial layer and expresses CCR6 is the only DC population responding to MIP-3 α /CCL20 (31) and is absent in CCR6-deficient mice (32).

Altogether, these observations illustrate the key role of MIP-3 α /CCR6 in the recruitment of LC-type DC and other immune cells at the epithelial surface, the site of antigen/pathogen entry, and suggest a unique role for this chemokine and LC in the regulation of epithelial immunity.

Although many chemokines can induce in vitro DC migration, each immature DC population displays a unique spectrum of chemokine responsiveness. LC, as illustrated above, migrate selectively in response to MIP-3 α /CCL20 (through CCR6), blood CD11c⁺ DC to monocyte chemoattractant protein (MCP) chemokines (through CCR2), and monocyte-derived DC to MIP-1 α - β /CCL3-4 (through CCR1 and CCR5), whereas blood CD11c⁻ DC precursors do not respond to any of these chemokines. These observations support the notion that the different DC populations will probably not be recruited at the same anatomic site during the same response. It is expected that the type of resulting immune response will likely be dependent on the DC subpopulation recruited and thus on the chemokines secreted.

SEQUENTIAL CHEMOKINE INVOLVEMENT IN RECRUITING IMMATURE DC AT THE SITE OF PATHOGEN ENTRY

To reach the site of antigen deposition at epithelial surfaces, DC have to traverse the endothelial barrier, progress through the tissue (dermis), and cross the dermoepithelial junction (basal membrane). In a recent study,¹ we observed that circulating blood DC as well as monocytes express high levels of CCR2 and primarily respond to MCP and not to MIP-3 α /CCL20. In fact, we did not succeed in identifying circulating blood DC or DC precursors expressing CCR6. Furthermore, while the CD34⁺ HPC-derived CD1a⁺ precursors committed to LC differentiation primarily respond to MIP-3 α /CCL20, the HPC-derived CD14⁺ precursors respond

to both MCP and MIP-3 α /CCL20. In concordance with the sequential expression of CCR2 and CCR6, the HPC-derived CD14⁺ precursors initially acquire the ability to migrate in response to MCP-4/CCL13 and subsequently in response to MIP-3 α /CCL20. Lastly, we observed that in vivo, MIP-3 α /CCL20 and MCP-4/CCL13 form complementary gradients in inflamed skin and mucosa.

These observations suggest that recruitment of DC at the site of infection is controlled by the sequential action of different chemokines: (i) CCR2⁺ circulating DC or DC precursors are mobilized within the tissue, via the expression of MCP by fibroblasts or endothelial cells and (ii) these cells traffic from the tissue to the site of pathogen invasion via the production of MIP-3 α /CCL20 by epithelial cells and the up-regulation of CCR6 in response to the tissue environment.

In vivo experiments in mouse tumor models have corroborated this hypothesis.² After intracutaneous injection, in contrast to MIP-3 α /CCL20, MCP-4/CCL13 has the unique capacity to recruit DC in the draining lymph node in vivo and to increase antigen-specific immunity and antitumor immunity. The site of MCP-4/CCL13 injection is characterized by an influx of monocytic cells, although these lack phenotypical features of DC. These results suggest that MCP-4/CCL13 is able to recruit blood monocytes or as yet uncharacterized immature blood DC precursors that promptly differentiate into typical DC, as previously reported in models of reverse endothelial migration (33, 34).

RECRUITMENT OF MATURING DC INTO DRAINING NODE AND INITIATION OF THE IMMUNE RESPONSE

During injury, inflammatory mediators (e.g., IL-1, TNF), infectious agent products (e.g., lipopolysaccharide, CPG, DNA), or T-cell products (e.g., CD40L, interferon- γ , IL-17) will drive DC maturation and induce maturing DC to emigrate out of the inflammatory site. A first consequence of this maturation will be the loss of functions characterizing immature DC, in particular antigen uptake capacity as a consequence of cytoskeleton rearrangement and endocytic receptor down-regulation. Furthermore, the responsiveness to most of the inflammatory chemokines is rapidly lost as a consequence of (i) a desensitization process involving saturation of their receptors by an endogenous production of ligands by activated DC (15, 35) and (ii) a down-regulation of their receptor expression at the mRNA level (14–16). Concomitantly to the loss of the inflammatory chemokine responsiveness, the CCR7 receptor, not expressed by immature DC, is rapidly induced at the cell surface of maturing DC. The known ligands for CCR7 are SLC/6CKine/Exodus-2/CCL21 and ELC/MIP-3 β /Exodus-3/CCL19 (23, 25, 36–38).

In contrast to many CC-inflammatory chemokines, ELC/MIP-3 β /CCL19 and SLC/6CKine/CCL21 have no chemotactic activity on immature DC. Both ligands have been reported by several groups to mediate potent migration of human and mouse mature DC through CCR7 (14–16, 39–42). All human DC subsets (CD34⁺ progenitor-derived DC, MDDC, blood CD11c⁺, CD11c⁻) respond to ELC/MIP-3 β /CCL19 and SLC/6CKine/CCL21 upon maturation (14).

¹ Vanbervliet. Submitted for publication.

² Vicari. Submitted for publication.

Entry into Lymphatic Vessels: A Control Step?

In mice, SLC/6Ckine/CCL21 has been shown to be expressed by endothelial lymphatic vessels draining nonlymphoid tissues (43). Furthermore, exogenous SLC/6Ckine/CCL21 has been shown to increase the yield of DC emigrating out of mouse skin explants (41), while blocking SLC/6Ckine/CCL21 in vivo impaired emigration of DC out of the dermis (42). Finally, we found that, although SLC/6Ckine/CCL21 is constitutively expressed on mouse endothelial lymphatic, its expression is strongly up-regulated a few hours after lipopolysaccharide injection.³ This might suggest that the entry into lymphatics by maturing CCR7⁺ DC is regulated by the level of SLC/6Ckine/CCL21 expressed by lymphatic vessels, which is under the control of inflammatory stimuli. This possibility raises the question of accessibility of draining lymphatics for in vitro-generated DC currently reinfused intradermally in DC-based antitumor immunotherapy clinical trials.

Role of CCR7 Ligands in the Homing of Antigen-Loaded DC in the T-Cell Area

In mouse and human secondary lymphoid organs, SLC/6Ckine/CCL21 is expressed on HEV (43, 44). ELC/MIP-3 β /CCL19 is constitutively expressed by stromal cells scattered within the T-cell areas of different secondary lymphoid tissues (spleen, lymph nodes, and Peyer's patches) (45).

Together, these observations suggest that, after inflammatory stimuli, DC undergoing maturation express CCR7. Concomitantly, SLC/6Ckine/CCL21 is induced on endothelial lymphatics during the inflammatory reaction and triggers the emigration of maturing DC through the lymph stream. Mature DC entering the draining lymph nodes are driven into the paracortical area in response to the production of ELC/MIP-3 β /CCL19 and/or SLC/6Ckine/CCL21 by cells spread over the T-cell zone.

The key roles of SLC/6Ckine/CCL21 and ELC/MIP-3 β /CCL19 in the recruitment of mature DC into the T-cell area of lymphoid organs have been recently demonstrated in naturally SLC/6Ckine/CCL21-deficient mice (46) and in mice deleted for CCR7 (47). In these models, the anatomical structure of lymphoid organs is disorganized, with a strong defect in naive T-cell homing in the T-cell areas. In addition, in both strains, DC fail to accumulate in the lymphoid organs after injection or contact sensitization. As a consequence, these animals have impaired immune responses with severely delayed antibody responses, lack of contact sensitivity and delayed-type hypersensitivity reactions, and a markedly increased susceptibility to infections.

Role of CCR7 Ligands in the Encounter Between Antigen-Loaded DC and Antigen-Specific T and B Cells

The passage of lymphocytes across the endothelium into lymph nodes and Peyer's patches is a multistep process involving a triggering event mediated by chemokines. The best candidate chemokine in this process is the CC chemokine SLC/6Ckine/CCL21 (25, 37, 38, 43, 48), which is active in inducing integrin-mediated adhesion of naive lymphocytes. The central role of SLC/6Ckine/CCL21 in T-cell migration across HEV was suggested by the observation that mice

deficient for SLC/6Ckine/CCL21 (plt mice) or CCR7 have defective T-cell trafficking into lymph nodes (46, 47, 49).

Thus, concomitantly to mature DC homing into the T-cell areas, CCR7-expressing naive T cells will enter lymph nodes through the production of SLC/6Ckine/CCL21 by HEV. The naive T cells may then be driven to the T-cell area through the increased gradient of ELC/MIP-3 β /CCL19. The source of ELC/MIP-3 β /CCL19 in the T-cell area has been proposed to be the interdigitating dendritic cells themselves (45). Because ELC/MIP-3 β /CCL19 and SLC/6Ckine/CCL21 can attract both mature DC and naive T cells, they are likely to play a key role in helping antigen-loaded DC to encounter antigen-specific T cells. The fact that DC themselves are a source of ELC/MIP-3 β /CCL19 might contribute to the accumulation of mature DC at the initial site, allowing T cells undergoing activation to receive prolonged signals, which is a requirement for naive T-cell activation.

Induction of Antitumor Immunity through SLC/6Ckine/CCL21 Expression in Tumors

Considering its central role in regulating mature DC and naive T cell trafficking, we decided to investigate whether the expression of SLC/6ckine/CCL21 in tumors may lead to antitumor immune responses. The C26 colon carcinoma tumor cell line transduced with SLC showed reduced tumorigenicity when injected in vivo, in immunocompetent mice (50). The protection was CD8 dependent and associated with a heavy intratumoral infiltration of DC. Surprisingly, although CCR7 mRNA expression was dramatically increased in SLC-transduced C26 tumors, tumor-infiltrating DC resembled immature DC.

CONCLUSION

DC induce, sustain, and regulate immune responses. Different DC subsets share biological functions or display specific roles such as polarization of T-cell responses towards type 1 or type 2, regulation of B-cell responses, or induction of antiviral immunity. Although DC can be resident cells such as LC in the epidermis, accumulating evidence shows that during inflammatory reactions, they can be rapidly mobilized from blood to the site of injury. Chemokines are important effectors of the regulation of DC recruitment, and, depending on the chemokine gradient released at the site of injury, different DC populations will be recruited. It is expected that the type of resulting immune response will likely be dependent on the DC subpopulation recruited and thus on the chemokines secreted.

Depending on their maturation stage, DC display functional specialization. To accomplish their different functions linked to the stage of maturation, DC traffic in different specific microenvironments. This trafficking is also under the control of chemokines, and different chemokines regulate the recruitment of the precursor, the immature, and the mature DC. At the site of injury, inflammatory chemokines act sequentially in parallel to the differentiation process that occurs during the recruitment of DC precursors from blood to tissues and navigation within tissues (dermis to epidermis). After antigen uptake, inflammatory stimuli turn off the response to the inflammatory chemokines and concomitantly DC acquire CCR7 expression. Then, maturing DC enter the lymph stream, potentially directed by SLC/6Ckine/CCL21

³ Vicari. Unpublished observation.

expressed on lymphatic vessels and leave the inflamed tissue. Mature DC entering the draining lymph nodes will be driven into the paracortical area in response to the production of ELC/MIP-3 β /CCL19 and/or SLC/6CKine/CCL21 by cells spread over the T-cell zone. Concomitantly, CCR7-expressing naive T cells enter lymph nodes in response to the production of SLC/6CKine/CCL21 by high endothelial venules. The newly arriving DC might themselves become a source of ELC/MIP-3 β /CCL19, allowing amplification and/or persistence of the chemotactic signal. Because these two chemokines can attract mature DC and lymphocytes, they are likely to play a key role in helping antigen-loaded DC to encounter specific T cells. After interaction with antigen-specific T cells, DC will produce many chemokines favoring interactions between activated CD4⁺ T cells with CD8⁺ T cells or B lymphocytes.

Thus, the control of DC trafficking is a complex process with the intervention of several chemokines and many other molecules such as selectins, integrins, and proteases. Future investigations will be required to assess the role of chemokines in constitutive DC trafficking versus induced DC mobilization during inflammation. Such approaches might help in our understanding of the role of DC in peripheral tolerance maintenance versus immune induction. Studies of interaction between chemokines, DC, and effector cells in disease conditions might offer new avenues of therapeutic intervention. A better understanding of the regulation of DC trafficking might allow in vivo manipulation of DC to increase (in cancer and infection diseases) or to suppress (in transplantation and autoimmunity) immune responses.

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