Dendritic cell traffic control by chemokines

Federica Sallusto, Alfonso Martín-Fontecha and Antonio Lanzavecchia

Institute for Research in Biomedicine, Via Vincenzo Vela 6, CH-6500 Bellinzona, Switzerland

Introduction

Dendritic cells (DCs) are widely accepted as the most potent and versatile antigen-presenting cells. They have an extraordinary capacity to acquire and process antigens for presentation to T cells and to express high levels of major histocompatibility complex (MHC) molecules and co-stimulatory molecules that drive naïve T cell activation. In addition DCs produce cytokines, primarily IL-12, which contribute to shape the quality of the T cell response generated. The capacity to migrate to sites of inflammation and from there to the T cell areas of secondary lymphoid organs is a fundamental aspect of DC biology. It has become apparent that the large families of chemokines and chemokine receptors provide a flexible code for regulating DC traffic and positioning in both homeostatic and inflammatory conditions.

Dissemination of DC precursors and immature DCs under steady state and inflammatory conditions

Under steady state conditions immature DCs seed into all bodily tissues where they reside as "sentinels" ready to react to incoming pathogens, a state that is defined as immature [1]. Langerhans cells (LCs) are a subset of immature DCs resident in epithelia and characterised by a relatively slow turnover [2]. LCs contain characteristic endosomal structures, called Birbeck granules, organised by a LC-specific lectin (Langerin) and are anchored to epithelial cells through E-cadherin. LCs express CCR6, the receptor for CCL20, a chemokine which is produced constitutively by keratinocytes [3]. Human monocytes cultured in the presence of TGF-β acquire some of the cardinal features of LCs, such as expression of Langerin [4], raising the possibility that LCs differentiate from peripheral monocytes under the aegis of local cytokines.

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Immature DCs are also present in the dermis and in all parenchyma. These cells have a turnover of approximately 2–4 days and need to be continuously replenished by precursors derived from the blood [2, 5]. The precursors of tissue DCs have not been fully characterised. They may be circulating immature DCs [6], which are present in low numbers in peripheral blood, or monocytes. The latter represent an abundant source of DC precursors that can be recruited at sites of inflammation or infection where they rapidly differentiate to DCs [7, 8]. Monocytes express CCR2 that promotes extravasation into inflamed tissues and migration towards a gradient of inflammatory chemokines. Mice deficient in CCR2 or in its ligand MCP-1 have impaired immune responses that appear to be due to defective monocyte migration both in the afferent and efferent phase of the immune response [9, 10]. Monocytes also express CCR5, a receptor for inflammatory chemokines and a co-receptor for HIV, and CXCR4, the receptor for CXCL12. It is possible that CXCR4 may be involved in the constitutive traffic of monocytes and DCs into certain tissues including tumours where hypoxia induces high levels of CXCL12 production.

In mice peripheral blood monocytes are a heterogeneous population comprising at least two functional subsets: a short-lived CX_3CR1^{lo} $CCR2^{+}$ $Gr1^{+}$ subset that is actively recruited to inflamed tissues and a CX_3CR1^{hi} $CCR2^{-}$ Gr1- subset characterised by CX_3CR1-dependent recruitment to non-inflamed tissues [11]. Both subsets have the potential to differentiate into DCs *in vivo*. The level of CX_3CR1 expression also defines two major human monocyte subsets, the $CD14^{+}$ $CD16^{-}$ and $CD14^{lo}$ $CD16^{+}$ monocytes, which share phenotype and homing potential with the mouse subsets [12]. Recently a subset of circulating monocytes, identified as $Gr1^{int}$, has been identified that selectively expresses CCR7 and CCR8 [13]. These monocytes may be disposed to become lymphatic-migrating DCs. When these monocyte-derived DCs exit skin to emigrate to lymph nodes, they may use not only CCR7, as it will be described below, but also CCR8.

A distinct subset of DCs, called plasmacytoid DCs (pDCs) or interferon-producing cells (IPCs) has been described in humans [14–16] and, more recently, in mouse [17]. Although IPCs are capable of presenting endogenous antigen to $CD8^{+}$ T lymphocytes [18], their hallmark function is the production of high amounts of type I IFN following viral infection. Immature IPC precursors circulating in peripheral blood express CXCR3 and CXCR4 as well as L-selectin and E/P-selectin ligands (PSGL-1 and CLA). This pattern of expression would be consistent with the capacity of these cells to migrate both to inflamed lymph nodes and peripheral tissues where CXCR3 and CXCR4 ligands are displayed on endothelial cells. Indeed, IPCs are typically localised around high endothelial venules (HEV) in inflamed lymph nodes and in some inflamed tissues [19–21]. Migration of IPCs require the coordinate action of CXCR3 and CXCR4, possibly through a mechanism that entails features of haptotaxis, i.e., dependency on chemokine immobilisation, and chemorepulsion, i.e., movement away from highest chemokine concentration [21, 22].

DC maturation: effects on chemokine receptor expression and chemokine production

The DC maturation process can be induced by a variety of stimuli. The most effective are microbial products that trigger specific Toll-like receptors (TLRs) on DCs. Interestingly human myeloids DCs (mDCs) and IPCs express complementary sets of TLRs and consequently respond to different agonists [23, 24]. In particular, mDCs express TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, and TLR8 whereas IPCs express TLR7 and TLR9. Thus, while risiquimod (a synthetic compound that triggers in humans TLR7 and TLR8) triggers both DC types, LPS (a TLR4 agonist) selectively triggers mDCs and CpG (a TLR9 agonist) selectively triggers IPCs. In addition, DC maturation can be induced by inflammatory cytokines, such as IL-1 and TNF, as well as by endogenous "danger signals" released by necrotic cells, such as heat shock proteins and urate crystals [25]. CD40L is also a potent DC maturation stimulus but since it is delivered by activated T cells it acts primarily as a secondary stimulus that enhances cytokine production initially elicited by microbial stimulation [26, 27].

Using a global gene expression approach it has been recently shown that the maturation program induced by TLR triggering involves the coordinate regulation of approximately 8,000 genes that control several DC functions ranging from antigen capture and presentation to co-stimulation, cytokine production and chemokine expression and responsiveness [28]. While most of the genes appear to be triggered by almost all stimuli a few genes have a high activation threshold. Indeed genes involved in the differentiation of Th1 and inflammatory T cells, such as IL-12, IL-23 and Delta-4, have been found to be elicited only in response to combinations of selected TLR ligands which act in synergy [28].

In response to microbial products DCs produce high amounts of inflammatory chemokines, up to the extraordinary amount of 2 pg/cell of CCL4 [29]. These chemokines, which include CCL2, CCL4 and CCL5, are produced very rapidly but only for a limited period of time and may play two distinct functions: first they attract DC precursors at sites of antigen exposure; second, by inducing a rapid and complete internalisation of the cognate receptors on maturing DCs allow these cells to exit the tissue. Indeed, CCR1 and CCR5 disappear within 1 h from the surface of maturing DCs while they remain detectable intracellularly for several days [29]. Eventually, however, these receptors are downregulated at the mRNA level. At later time points following induction of maturation DCs express CCL17 and CCL19 that attract CCR4 and CCR7 positive cells, respectively, and may thus favour interaction with naïve and activated T cells [30].

A common feature of maturing DCs and IPCs is the upregulation of CCR7, the receptor for CCL19 and CCL21. CCL21 is constitutively expressed in lymphatic endothelial cells and high endothelial venules and is involved in the recruitment of maturing DCs and other $CCR7^+$ cells at these sites [31]. CCL21 is expressed together with CCL19 by stromal cells in the T cell areas in a lymphotoxin β-dependent

fashion. CCL19 is also produced by maturing DCs at late time points after stimulation and is therefore expected to be released primarily in the lymph node. CCR7 expression and responsiveness gradually increased in maturing DCs. This receptor also shows a striking resistance to ligand-induced downregulation, indicating that DCs can sustain the response to CCL19 and CCL21 throughout the maturation process. The transcriptional regulation of the CCR7 gene has not been characterised. In general CCR7 expression is induced by stimuli that induce upregulation of MHC and co-stimulatory molecules. However, there are examples of maturation stimuli that do not induce CCR7 expression and stimuli that induce CCR7 expression independently of maturation. An example of the latter is the uptake of apoptotic cells by human monocyte-derived DCs that induces CCR7 expression and DC chemotaxis in response to CCL21, but results in downregulation of HLA-DR and CD86 [32].

DC traffic from sites of antigen capture to sites of antigen presentation

Priming of naïve T cells requires the encounter with antigen-presenting DCs in the specialised T cell areas of secondary lymphoid organs (Fig. 1). In certain experimental conditions it has been shown that intact antigen present in peripheral tissues can be transported to lymph nodes through the lymph. There it can be captured and presented by lymph node resident DCs that, under steady state condition, represent an extensive network of poorly stimulatory cells still endowed with antigen capturing capacity [33, 34]. The major route of antigen delivery to the lymph node is represented by peripheral tissue-resident DCs that migrate to the draining lymph nodes. For instance, maturing DCs that have taken up antigen in the skin and have been stimulated by microbial products migrate into lymphatic vessels and localise to the T cell areas of the draining lymph node. Similarly, splenic immature DCs which are present in the marginal zone and are exposed to blood-borne antigens rapidly mature and migrate to the T cell area following intravenous injection of microbial products. Both these processes are dependent from CCR7 upregulation in mature DCs.

CCR7-deficient mice have a major defect in DC migration from tissue to lymph nodes and from the marginal zone to the T cell zone of spleen [35]. Adoptive transfer experiments formally demonstrated that CCR7-deficient DCs do not migrate when injected to normal CCR7-expressing hosts [36]. Two recent lines of evidence suggest that the CCR7-dependent pathway of migration can be boosted by inflammatory mediators. First, the lipid mediators cysteinyl leukotrienes and prostaglandin E2 enhance the sensitivity of CCR7 [37, 38]. Second, inflammatory cytokines such as TNF and IL-1 increase expression of CCL21 on lymphatic endothelial cells [36]. Both mechanisms enhance the entry of maturing DCs into lymphatic vessels and the migration to lymph nodes.

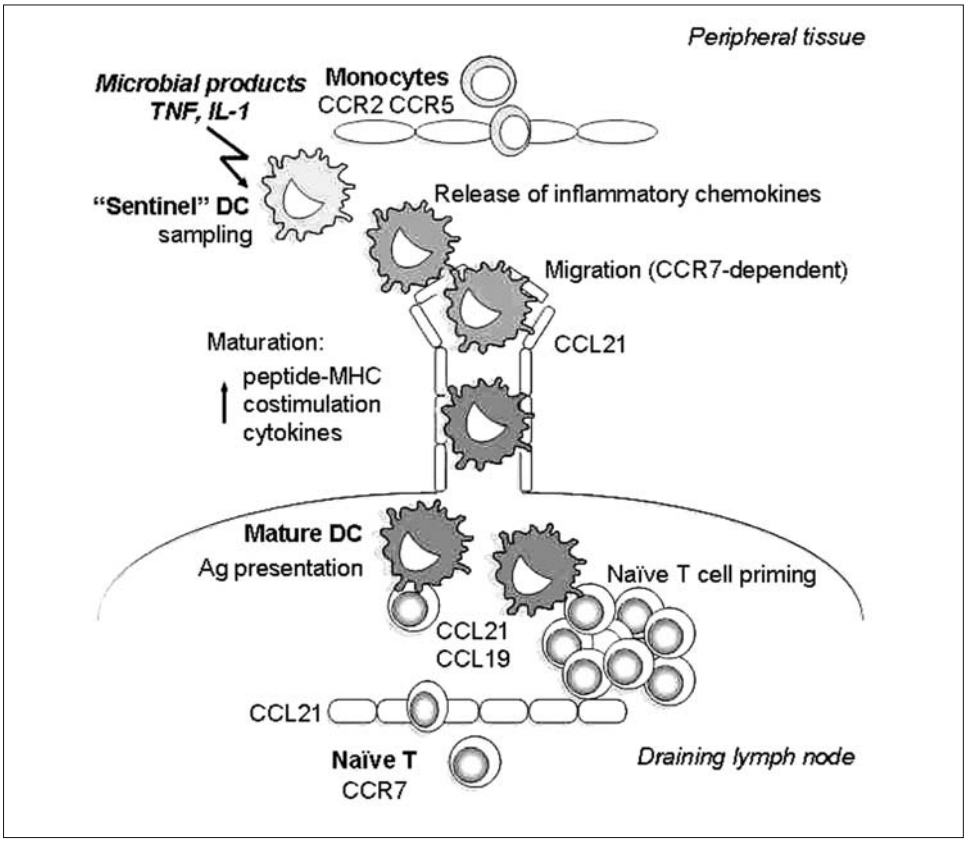

Figure 1
Immature "sentinel" DCs triggered by microbial products and inflammatory cytokines in peripheral tissues release inflammatory chemokines thus attracting DC precursors (monocytes) from the blood, and migrate in a CCR7-dependent fashion into lymphatic endothelial vessels. Maturing DCs upregulate MHC and co-stimulatory molecules and produce cytokines and chemokines, thus acquiring T cell priming and polarising capacity. Mature DCs localise in the T cell area where they present antigen to naïve T cells that home to the T cell area through a CCR7-dependent mechanism and induce their proliferation and differentiation to effector cells. Additional molecules, such as selectins and integrins, participate in these processes which are not depicted in the scheme.

Besides its role in driving the migration of antigen-carrying mature DCs in the course of an immune response, CCR7 appears to control the migration of DCs to lymph node in the steady state, a phenomenon that is much less understood. Mice lacking the adaptor molecule DAP12 present a homeostatic accumulation of DCs in

peripheral sites, raising the possibility that a DAP12 linked receptor such as TREM-2 may play a role in controlling DC migration in homeostasis [39, 40].

Recent *in vivo* analysis using green fluorescent protein (GFP)-tagged cells revealed relevant differences between LCs and dermal DCs. After skin immunisation both LCs and dermal DCs migrate to the lymph node but the latter appear to migrate more rapidly, to colonise different areas, to express higher levels of co-stimulatory molecules and to be more capable of eliciting T cell responses [41]. Indeed, deletion of LCs did not impair the triggering of hapten-specific T cells.

DCs play an important role in the gut where they scan an enormous and continuously exposed surface. Mucosal DCs present in the lamina propria express CX_3CR1 which is required to form transepithelial dendrites, which enable DCs to directly sample luminal antigens, and commensal and pathogenic bacteria [42, 43]. These cells conditioned by local cytokines (for instance TGF-β) or T cells may regulate gut homeostasis, immunological tolerance and inflammation in the gut.

Impact of DC maturation and migration on T cell priming in physiological and vaccination settings

There is now abundant evidence that maturation state of antigen presenting DCs dictates the outcome of the T cell response. The most striking example is provided by the findings that in mice targeting of soluble antigens to lymph node resident immature DCs leads to an abortive T cell proliferation and establishment of tolerance whereas in the presence of a DC maturation stimulus, in the form of CD40 antibodies, the same antigen leads to effective T cell priming and generation of effector and memory cells [44].

In addition to the maturation state, the absolute number of antigen presenting DCs that migrate to the draining lymph node has a profound impact on the magnitude of the T cell response. This is particularly relevant in immunisation protocols in which antigen-loaded DCs are injected subcutaneously as cancer vaccines. In these protocols, human immature DCs are generated *in vitro* from haematopoietic progenitors or monocytes, pulsed with antigen in the forms of protein, peptide or mRNA, and induced to mature by stimulation with microbial products or inflammatory cytokines before injection [45]. In preclinical mouse systems subcutaneously injected mature DCs migrate to the lymph node in a CCR7-dependent fashion where they elicit T cell responses. In this setting the magnitude and quality of $CD4^+$ T cell response was proportional to the number of antigen-carrying DCs that reached the lymph node and could be boosted up to 40-fold by pre-injection of TNF that conditioned the tissue for increased DC migration by increasing the expression of the CCR7 ligand CCL21 in lymphatic endothelial cells [36]. Thus, lymphatic drainage of mature DCs can be manipulated to increase DC vaccine efficacy.

In mice mature DCs migrating to the draining lymph nodes rapidly recruit in a CCR7-independent, CXCR3-dependent manner natural killer (NK) cells, which are normally excluded from lymph nodes [46]. NK cell depletion and reconstitution experiments show that NK cells provide an early source of IFN-γ that is necessary for optimal Th1 polarisation. These results show that DCs can influence Th1 differentiation not only by elaborating Th1 promoting factors, such as IL-12, but also by recruiting to lymph node, through a yet undefined mechanism, NK cells that in some systems represent an essential source of IFN-γ for T cell polarisation.

Another factor that may influence T cell fate is the kinetics of DC activation. Recently migrated DCs actively produce Th1 polarising cytokines and effectively prime Th1 responses [47]. In contrast at late time points the same cells exhaust the IL-12 producing capacity and although still retaining T cell stimulatory capacity promote T cell proliferation without differentiation. Thus while "active" DCs induce differentiation of effector T cells, exhausted DCs may induce the development of memory T cells [48].

Conclusions

Gaining a better understanding of the migratory pathways of DCs in physiological settings will be essential for future advances in using DCs as a means to fine-tune immune responses in clinical settings such as in cancer, autoimmunity and transplantation. In the case of induction of anti-tumour response, strategies are being evaluated aiming at increasing the delivery of antigen-carrying mature DCs to lymph node to enhance the efficacy of the vaccine [49]. In other cases, such as in autoimmune disorders and transplantation, it may be beneficial to deliver to the lymph node immature tolerogenic DCs to dampen the immune response and induce and/or maintain peripheral tolerance. Interfering with the migration of DCs in the context of transplantation, i.e., blocking the reverse transmigration of donor DCs from the transplanted organ to the blood [50], is presently more difficult because the molecular mechanisms controlling this event are still poorly defined. Nonetheless also this approach holds promises as a yet another way to modulate the immune response by targeting DC migration.

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