Commentary

Mobilizing Dendritic Cells for Tolerance, Priming, and Chronic Inflammation

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Migration of Dendritic Cells from Blood to Tissues. A fundamental aspect of dendritic cell (DC) function is their capacity to migrate. It allows them to exert a continuous surveillance for incoming antigens in almost all body tissues and a prompt report to T cells in secondary lymphoid organs. Under steady state conditions, the rate of DC migration from blood to tissues and from tissues to lymph nodes is probably very low, and most of these DCs reside in the tissues in a dormant state ready to be activated by pathogens. However, in inflammatory conditions the rate of DC migration can be considerably increased to meet the increased requirement for antigen presentation. Several recent papers shed new light into the mechanisms that control the various steps that DCs have to undergo to perform their function in the induction of tolerance, priming, and chronic inflammation.

To exit from the blood stream DCs, like other leukocytes, first need to tether to the endothelium (Fig. 1). This process, which is essential for the subsequent steps of firm adhesion and extravasation, is mediated by selectins that bind to specific carbohydrates on specialized cell surface molecules (1). In this issue, Robert et al. demonstrate that DCs that circulate in peripheral blood express a glycosylated form of P-selectin glycoprotein ligand (PSGL)-1 that binds to P- and E-selectins, which are expressed at low levels on endothelial cells and are upregulated by inflammatory stimuli (2). Using an in vivo imaging system, they show that DCs tether and roll on P- and E-selectins expressed by capillary endothelial cells and preferentially extravasate at sites of inflammation. This novel finding indicates that blood-borne DCs are poised to exit blood at inflammatory sites, thus allowing rapid recruitment of these APCs where their surveillance function is most needed.

Recruitment of leukocytes from blood to tissues is regulated at the level of the endothelial cells, where inflammatory cytokines increase the expression of adhesion molecules and chemokines and induce the formation of Weibel-Palade bodies. These represent storage granules for P-selectin (3, 4) and, as shown in two recent papers, also for IL-8 (5, 6). The content of the Weibel-Palade bodies can be rapidly mobilized by stimulation of endothelial cells with histamin or thrombin. This mechanism can be viewed as a form of "memory," since it allows endothelial cells that had been exposed to inflammatory stimuli to respond to a new challenge rapidly and without need for new protein synthesis, which has clear physiological as well as immunopathological significance.

A long-standing question has been the nature of the circulating cells that give rise to tissue DCs. Are these the few cells with DC phenotype characteristics that are present in the blood stream, or are there more frequent precursors that rapidly develop into DCs after entry into tissues? Monocytes have been shown to exit blood (7), and represent nonproliferating precursors that differentiate in vitro to immature DCs in cultures supplemented with GM-CSF and IL-4 (8). The factors that may promote the monocyte to DC transition in vivo still need to be defined. Recently, Randolph et al. showed that in an in vitro culture system, human peripheral blood monocytes cultured with endothelial cells differentiate into DCs within 2 d, particularly after phagocytosing particles in subendothelial collagen (9). These DCs migrated back across the endothelium as they

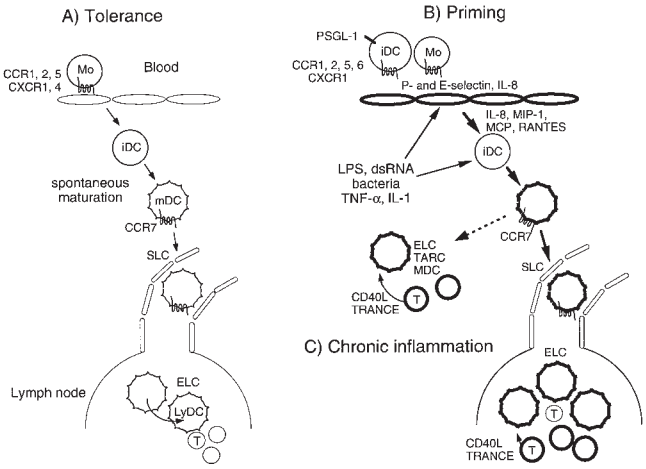

Figure 1. Mobilization and activation of DCs control the induction of tolerance, priming, and chronic inflammation. (A) Under steady state conditions, the recruitment of DC precursors into tissues and the migration of mature DCs to lymph nodes occur at low rates. Tissue antigens carried by short-lived migratory DCs may be transferred to resident lymphoid DCs that induce T cell tolerance. (B) Activated endothelial cells recruit DCs at higher rates. Immature DCs are attracted by inflammatory chemokines towards the site of inflammation, where bacterial and viral products induce DC maturation and activation. In the lymph node, activated T cells can further enhance DC activation and survival via CD40L and TRANCE. Because many and highly activated DCs are present, a productive T cell response is induced. (C) Mature DCs that fail to migrate to lymph nodes may serve as nucleation sites for chronic inflammatory reaction. Chemokines produced by these DCs attract maturing DCs as well as recently activated T cells, that maintain the inflammatory reaction. Thick lines indicate activated cells. Mo, monocytes; iDC, immature DCs; mDC, mature DCs; LyDC, lymphoid DCs.

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/99/02/611/04 $2.00
Volume 189, Number 4, February 15, 1999 611–614
http://www.jem.org

would do during entry into lymphatics, while those that remained in the subendothelial matrix became macrophages. These results suggest that the interaction with endothelial cells or extracellular matrix may be sufficient to drive differentiation of monocytes to either DCs or macrophages, each possessing inherently different migratory capacity. It will be interesting to apply the in vivo imaging system used by Robert et al. (2) to trace monocyte migration and differentiation in vivo.

The Role of Chemokines in the Multistep Navigation of DCs. Chemokines play a role not only in the process of extravasation, but also in the subsequent process of migration within the tissues to the final target. Different chemokines provide codes for areas that are undergoing different types of inflammatory reactions or are sites of constitutive traffic (10, 11). A current view holds that the whole migration process occurs in distinct steps, each driven by a particular chemokine–chemokine receptor pair (12). DCs provide a striking example of this so-called multistep navigation, since they use different sets of chemokine receptors to migrate from blood to inflamed tissues and from there to the lymphatics by which they reach their final destination in the T cell areas of lymph nodes (Fig. 1).

Monocyte and monocyte-derived immature DCs express receptors for inflammatory chemokines such as CCR1, CCR2, CCR5, and CXCR1, and are therefore attracted to inflammatory sites where cognate ligands such as IL-8, macrophage inflammatory protein (MIP)-1α, monocyte chemotactic protein (MCP)-1, and regulated upon activation, normal T cell expressed and secreted (RANTES) are produced (13, 14). Monocytes also express CXCR4, which may be responsible for low level constitutive recruitment by stromal cell–derived factor (SDF)-1 (13). In addition, immature DCs present in tonsils and in cultures derived from $CD34^+$ progenitors express CCR6, a receptor specific for liver and activation-regulated chemokine (LARC)/MIP-3α, which is produced in the crypts of tonsils (15). Altogether it appears that inflammatory chemokines guide the first step of DC migration from blood into tissues and within tissues to inflammatory sites where antigens must be captured.

Besides being attracted by inflammatory chemokines, DCs also produce these chemokines in large amounts. The production of inflammatory chemokines by DCs is rapidly triggered after exposure to various maturation stimuli, and has two major consequences. First, it enhances recruitment of immature DCs, which is important to sustain antigen sampling at later time points. Second, it downregulates the expression of the cognate receptors on maturing DCs, thus allowing them to leave the inflamed tissues (13).

The second step of DC migration, from inflamed tissues into lymphatics and from there to T cell areas of lymph nodes, is regulated by a different set of chemokines. Maturing DCs upregulate receptors for constitutive chemokines such as CXCR4, CCR4, and especially CCR7 (13–15). CCR7 recognizes secondary lymphoid tissue chemokine (SLC), produced by lymphatic endothelial cells (16), and Epstein-Barr virus–induced molecule 1 ligand chemokine (ELC), produced in the T cell areas by mature DCs (13, 15, 17), and therefore appears to be a key receptor for this second step of directional migration (Fig. 1).

Although at early time points after induction of maturation DCs are abundant sources of inflammatory chemokines, at later time points mature DCs produce high levels of constitutive chemokines such as thymus and activation-regulated chemokine (TARC), macrophage-derived chemokine (MDC), and ELC (F. Sallusto, unpublished data). This allows mature DCs, once they reach the T cell areas, to attract other maturing DCs as well as naive and recently activated T cells that express the cognate receptors CCR7 and CCR4 (for a review, see reference 18). Therefore, chemokine production by mature DCs plays an important function in organizing T cell areas and lymph node structure.

Migration of Stimulatory or Tolerizing DCs. Antigen presentation can lead either to full activation and priming of effector and memory T cells or to an abortive stimulation, resulting in functional inactivation and T cell tolerance. Finding the factors that regulate the balance between tolerance and response is now considered the holy grail of immunology. The current view is that the critical factor lies in the nature of the APC: mature DCs prime, whereas resting B cells and tissue cells tolerize. This notion has been recently challenged by Kurts and colleagues (19). These authors show that, in the absence of inflammation, a model antigen expressed in tissue cells is presented by bone marrow–derived APCs on class I molecules in the draining lymph node. Strikingly, this presentation results in transient CD8 T cell proliferation, which leads to tolerance. These observations imply that migratory, bone marrow–derived APCs (most likely DCs) pick up antigen in noninflamed tissues and migrate at low rate to the lymph nodes to induce tolerance to tissue antigens. At odds with the current view, the findings by Kurts et al. suggest that DCs are responsible not only for priming, but also for tolerance.

Two mechanisms may account for this striking finding. The first possibility is that the tolerogenic DCs represent a specialized lineage. A likely candidate is the lymphoid DC described by Shortman and colleagues, which can stimulate T cells but appears to be unable to support IL-2 production (20). If distinct DC subsets are involved in induction of tolerance or response, it will be important to assess whether they are recruited in response to different stimuli. For instance, tolerogenic DCs may be mobilized constitutively, whereas the stimulatory DC lineage may be preferentially recruited under inflammatory conditions. Recently, Inaba and colleagues showed that antigens carried by short-lived migratory DCs can be transferred to lymph node–resident, most likely lymphoid DCs (21; Fig. 1). They suggested that, in the absence of inflammation, this mechanism may be responsible for the induction of tolerance to tissue antigens, thus accounting for the observation of Kurts et al. (19).

The second possibility is that the same DC type is responsible for inducing either tolerance or response, depending on several factors such as the number of DCs that reach the lymph node, their life span, and the nature and amount of costimulatory molecules and cytokines they ex-

press. These factors will be determined by the context in which DCs are stimulated to mature. Bacterial and viral products such as LPS and double-stranded (ds)RNA recruit large numbers of DCs and activate them to a high stimulatory status, whereas endogenous inflammatory cytokines are much less effective (22–24). CD40L and TNF–related activation-induced cytokine (TRANCE) represent additional endogenous stimuli by which T cells can enhance DC stimulatory capacity (25–27) and viability. According to the second DC activation hypothesis, tolerance or priming will be determined by how far pathogens and/or memory T cells raise the activation state of maturing DCs, i.e., high activation state and high numbers will favor priming, whereas low activation and low numbers will induce tolerance or ignorance (Fig. 1). The regulatory role of pathogens, antigen dose, and kinetics of presentation has been amply documented (28). There is now growing evidence that most of this regulation is taking place primarily at the level of DCs.

A Role for DCs in Chronic Inflammation. A long-standing observation has been that tissues undergoing chronic inflammatory reactions contain infiltrates of lymphocytes that are organized in lymph node–like structures. TNF-α and lymphotoxin (LT) are required for this lymphoid neogenesis (29), and recent data suggest that DCs might also play a role in maintaining chronic inflammation via de novo formation of local lymphoid tissue. Ludewig et al. showed that repetitive immunization with DCs carrying a diabetogenic peptide induced chronic inflammation and lymphoid neogenesis, demonstrating that DCs presenting self-antigens are not only potent inducers of autoreactive T cells, but also help to maintain a peripheral immune response locally (30). It will be important to consider this as a potential hazard in DC-based antitumor therapies.

Based on the central role played by chemokines, it is tempting to suggest that DCs that mature in inflamed tissues and fail to migrate to the lymph nodes may act as nucleation sites to organize a lymphoid structure that sustains the chronic inflammatory reaction (Fig. 1). Indeed, mature DCs produce constitutive chemokines such as ELC, TARC, and MDC which attract recently activated T cells that have upregulated CCR7 and CCR4 as well as maturing DCs that may sustain the process of lymphoid neogenesis. This mechanism may explain why sites of chronic inflammation such as the rheumatoid arthritis synovia can act as sinks for recently activated T cells such as those that are chronically activated by endogenous antigens, for instance EBV (31). Although these cells are not autoreactive, they may nonetheless aggravate the chronic inflammatory process. For example, they could be activated in an antigen-independent fashion by certain cytokine combinations present in the microenvironment (32), and in this way may provide to DC survival and activation signals (via CD40L and TRANCE) that may be important to sustain the inflammatory process.

As our understanding of DC physiology improves, we realize that we cannot look at these cells simply as the "good guys." Because of their fundamental role in initiating T cell responses, it is perhaps not surprising that they are involved in many aspects of immune regulation, including tolerance and autoimmunity. Understanding the control of DC traffic and activation will provide new avenues for therapeutic intervention.

We thank K. Hannestad for critical reading and comments.

The Basel Institute for Immunology was founded and is supported by Hoffmann-La Roche, Basel, Switzerland.

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Received for publication 6 January 1999.

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