Commentary

New Mechanisms and Pathways for Monocyte Recruitment

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A great deal of recent research has identified chemoattractants and cellular activators responsible for neutrophil trafficking into inflamed tissues, as well as for lymphocyte homing to secondary lymphoid organs in the steady state and into foci of chronic inflammation (1–5). Considerably less is known about the molecules regulating the trafficking of monocytes, particularly the constitutive trafficking of monocytes through tissues in health and the recruitment of monocytes to lymph nodes in disease. Two articles in this issue of The Journal of Experimental Medicine (6, 7) and one in a recent issue (8) shed some light on this subject and also prompt some questions for future investigation.

Under steady-state conditions in mice about half of the circulating monocytes leave the bloodstream each day (9, 10). Effete monocytes are destroyed in the spleen, but a considerable fraction of circulating monocytes enter the tissues of the body, differentiating into tissue macrophages (9, 10) or dendritic cells (DCs; references 11 and 12). The lifespan of individual tissue macrophages is controversial, but the permanence of tattoos attests to the ability of a stable or self-renewing population of macrophages to be maintained in place for the lifetime of the individual. In contrast, immature DCs within the tissues are able to leave via afferent lymphatic vessels for the draining lymph nodes, where they mature, present antigen to T cells, and die within a few days of arrival. Thus, a large fraction of monocytes can potentially be cleared as a byproduct of immune surveillance.

In mice responding to an inflammatory challenge, the number of monocytes leaving the circulation per day is at least double (10). The half-life of circulating monocytes in humans is about three times longer than in mice (13), but the thousandfold greater monocyte mass in humans means that ~340 million monocytes leave the circulation each day.

Monocyte Recruitment into Tissues. While chemokines such as monocyte chemotactic protein (MCP)-1 (CCL2) have been demonstrated to recruit monocytes into foci of active inflammation (14–16), it has not been clear whether monocytes use the same molecular signals to emigrate into tissues as part of the constitutive or steady-state efflux from blood. Prerequisites for a molecule that recruits monocytes into healthy tissues should include (i) constitutive expression of the chemoattractant by cells of that tissue (i.e., epithelia or stroma), (ii) preferential or selective response of monocytes to this molecule, and (iii) the ability to recruit monocytes into tissue without prematurely stimulating their respiratory burst or genetically programmed effector functions. The recent paper by Kurth et al. (8) describes a new chemokine pathway for monocyte recruitment during inflammation. This same pathway, however, might be used for the constitutive recruitment of monocytes to skin and gut.

Kurth et al. (8) provide evidence that breast and kidney expressed chemokine (BRAK, CXCL14) is selectively chemotactic for monocytes activated by prostaglandin E_2 (PGE_2). During culture in the presence of PGE_2 and possibly other mediators capable of raising intracellular cAMP, monocytes become markedly more responsive to BRAK, while losing chemotactic responsiveness to traditional monocyte chemokines MCP-1, regulated on activation, normal T cell expressed and secreted (RANTES), and stromal cell–derived factor 1 (CCL2, CCL5, and CXCL12, respectively) (8). Monocytes respond to BRAK through an unknown receptor in a pertussis toxin-sensitive manner. BRAK mRNA is expressed constitutively by a variety of epithelia including the basal keratinocytes and dermal fibroblasts of skin, and cells in the lamina propria of gut (8). The authors propose that once monocytes enter tissues in response to local inflammation, PGE_2 at the site renders them responsive to the high levels of BRAK in these tissues, attracting them to the subepithelial locations where they mature into macrophages.

The findings reported in this paper imply a possible role for BRAK in the constitutive emigration of monocytes into these tissues as well. A fraction of monocytes present in peripheral blood are intrinsically responsive to BRAK in the absence of exogenous activation. Under baseline conditions ~1/5 as many monocytes migrate in response to BRAK as under optimal PGE_2 activation. It is exciting to speculate that this subpopulation of monocytes might be constitutively recruited to BRAK-expressing tissues by BRAK bound to and presented by endothelial cell heparan sulfate. This would allow monocytes, but not lymphocytes or neutrophils, to leave the circulation and enter these tissues in the absence of inflammation.

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Monocyte Recruitment into Lymph Nodes During Inflammation. Macrophages are omnipresent constituents of lymph nodes, where they are generally found along the sinusoidal vessels and in the medulla. However, they are not normally noticeable in the lymphocyte-rich cortex. While macrophages are plentiful in normal lymph nodes, their precursors, monocytes, are not. This is particularly true in the cortex where recirculating T cells come into contact with antigen presenting cells and immune responses are initiated. On the other hand, macrophages can become prominent in inflammatory lymphadenitis of a variety of etiologies, and in some cases of sinus histiocytosis become a predominant cell type. This rapid expansion of mononuclear phagocytes in the lymph node implies recruitment from outside the lymph node.

Cells resembling monocytes have been described to enter lymph nodes by squeezing between the endothelium of the high endothelial venules (HEVs), but only under inflammatory conditions (17). In the in vitro Stamper-Woodruff assay, monocytes and monocyte cell lines will bind to HEV draining sites of inflammation, but not HEVs in normal lymph nodes (18). This suggests that monocytes can be recruited across HEVs in vivo. Recruitment of monocytes to the lymph node cortex via HEVs could provide an important source of effector cells to defend against microorganisms or malignant cells that may have gained access to the lymph node via afferent lymphatic channels. It could provide a potential source of immature DCs as well. However, the signals regulating this influx and the anatomic pathways taken have not been defined.

Studies by Gretz et al. (19) provided some insight into how monocytes could be recruited into lymph nodes draining sites of inflammation. They demonstrated that chemokines and other small solutes arriving via afferent lymphatics travel through the lymph node via conduits that link the subcapsular sinus with the basal surface of HEVs. These conduits radiate out from the HEVs toward the lymph node capsule. Anatomically, the conduit consists of the space between the reticular fibroblasts and the collagen fibers that support them. In this way, they hypothesized, inflammatory agents, including chemokines that reach lymph nodes draining a site of inflammation would be shunted directly to the HEVs (bypassing the lymph node parenchyma) and aid in the recruitment of leukocytes into the nodes. Chemokines can be transcytosed from the basal to the apical surface of endothelial cells, where they can be presented for activation of leukocyte adhesion (20).

Other studies demonstrated that chemokines secreted in or injected into skin arrive in the draining lymph node where they can be presented on the apical surface of HEVs for recruitment of lymphocytes. This has been demonstrated with secondary lymphoid tissue chemokine (SLC, CCL21; reference 21) and Epstein-Barr virus–induced molecule 1 ligand chemokine (ELC, CCL19; reference 22). In principle, this system could be used to recruit monocytes to lymph nodes via HEVs.

Two articles in this issue demonstrate how inflammatory chemokines originating in the skin can indeed stimulate the influx of circulating blood monocytes into the draining lymph nodes via HEVs. Palframan et al. (6) show that MCP-1 produced at a cutaneous site of inflammation and carried via afferent lymphatics to draining lymph nodes can recruit monocytes into those lymph nodes via HEVs. This work takes advantage of transgenic mice in which CX3CR1-expressing cells constitutively express green fluorescent protein (23). (CX3CR1 is the fractalkine receptor and is expressed predominantly by monocytes, but also by subsets of natural killer cells, DCs, and microglia). This allows the workers to selectively track the relatively small population of monocytes in the murine circulation. Janatpour et al. (7) show that the monokine induced by IFN-γ (MIG, CXCL9) is expressed on a subset of HEV draining sites of inflammation, and that this chemokine is also capable of recruiting monocytes selectively to these HEVs. MIG mRNA was detected in the lymph node, implying local production of MIG protein, but the authors did not address whether MIG was also produced at the site of inflammation and carried in via draining lymphatics. The important point made by both groups is that inflammation at a distance stimulates the de novo synthesis of inflammatory chemokines that are presented on HEVs of the draining lymph node, where they recruit monocytes by "remote control" (6).

Both papers use similar methods to induce inflammation. Palframan et al. (6) used an intracutaneous injection of keyhole limpet hemocyanin in complete Freund's adjuvant in the flanks or lateral thorax. Janatpour et al. (7) used a subcutaneous injection of complete Freund's adjuvant alone in the footpads. In both cases draining lymph nodes were harvested. Both groups found an increase in MCP-1 protein in lymph nodes. Janatpour et al. expected MCP-1 to be the major chemokine working to recruit monocytes in their system, but found it not to be expressed on HEVs by immunofluorescence. On the other hand, MIG, the levels of which were increased even more than MCP-1 in lymph nodes, was expressed on HEVs. The Palframan study did not directly localize MCP-1; they measured it by ELISA of lymph node homogenates. However, there could be technical reasons to explain the differences in their findings. The presence or absence of KLH at the injection site might affect the cytokine and chemokine response. Furthermore, the relative roles of chemokines may change over the time course of the inflammatory response. In the Janatpour study most data were collected at 3 d, while the Palframan study concentrated on 5–7 d. Both studies used mice of the C57Bl/6 strain (although the number of backcrosses from the 129Sv background for the knockout mice could potentially affect the response). However, variables as seemingly innocuous as differences in the environment at the animal housing facility can affect the inflammatory response. Carefully controlled experiments demonstrated that MIG and MCP-1, respectively, were the main chemokines responsible for recruiting monocytes in those studies. There is every reason to be-

lieve that both studies are correct; several chemokines are capable of recruiting monocytes to sites of inflammation, and the predominant chemokine may differ depending on the inflammatory stimulus, timing, genotype, and presence of other inflammatory agents.

Monocyte Subsets? Inflammatory chemokines recruited the monocytes to the draining lymph nodes rather efficiently. Palframan et al. calculate that the rate of recruitment of monocytes to the nodes is ~30% the rate of recruitment of naive T cells to the same lymph node (6). Nevertheless, the monocytes recruited to the lymph node HEVs in both studies were a fraction of the circulating monocyte pool. Although exact numbers are difficult to obtain, Palframan et al. calculate that ~1 in 6 monocytes that passed through the HEVs were recruited into the lymph node in response to MCP-1. Janatpour et al. calculate that ~2% of the circulating monocytes cross HEVs in response to MIG. Are these cells representative of the majority of circulating monocytes, or do they represent an important subset? One would expect that these cells would be equipped with chemokine receptors and cell adhesion molecules to facilitate their binding to and migration across HEVs. In fact, the investigators found that these cells expressed L-selectin (CD62L; reference 6) critical for rolling on HEVs and CXCR3, the receptor for MIG (as well as for the other IFN-γ–inducible cytokines, IP10 and I-TAC, CXCL10, and CXCL11, respectively) (7). While CD62L is expressed by most monocytes, CXCR3 is not. Janatpour et al. claim that a small percentage (<2%) of circulating $CD14^+$ monocytes in mouse blood expressed CXCR3, which matches the proportion seen normally on circulating human monocytes. Thus, the cells migrating into inflamed lymph nodes in their study presumably represent a subset of monocytes primed to respond when MIG presented on the luminal surface of HEVs. Since most monocytes express CCR2, the receptor for MCP-1, it is possible that the monocytes recruited so efficiently in the Palframan study represent a subset primed to respond to MCP-1 in the context of other signals from the HEVs.

A known subset of circulating "monocytes" that is recruited to lymph node HEVs under inflammatory conditions are the plasmacytoid cells (formerly called plasmacytoid T cells and plasmacytoid monocytes) now more properly termed plasmacytoid DCs (24). Plasmacytoid cells have been shown to circulate in human peripheral blood at very low frequency and, upon stimulation with viruses or CD40 ligation, produce very large amounts of IFN-α (25, 26). These same cells can then differentiate into DCs (24, 27). Plasmacytoid cells accumulate around HEVs in certain types of inflammatory lymphadenitis (see reference 28 for a short series of these reports.) Human plasmacytoid DCs lack CD14 and CD11b, in contrast to monocytes, but do express both CD62L and CXCR3 (25), just as the migrating cells in these papers (6, 7). Do the HEV-homing cells reported by these groups represent the murine equivalent of human circulating plasmacytoid cells? Or do they merely share some important markers that are necessary for homing to lymph node HEVs under inflammatory conditions? There is, of course, no a priori reason why plasmacytoid cells in humans and mice must bear exactly the same markers. A decisive test would be to determine whether these cells produce large quantities of IFN-α when stimulated by viral infection or CD40 engagement (25, 26).

The mononuclear cells that home to lymph nodes under inflammatory conditions may represent subset(s) of circulating monocytes. The monocytes homing to lymph nodes in response to MIG (7) likely represent a different group than those homing to lymph nodes in response to MCP-1 (6), since in each case the ability to block homing with specific antibody was almost complete. This brings up larger questions: do specific subsets of monocytes home to distinct sites, e.g., skin or lymph nodes, the way subsets of memory lymphocytes do? If so, do they leave the bone marrow primed to enter these tissues by virtue of expressing the receptor for BRAK or CXCR3, respectively? Or is expression of these receptors stochastic and determined at the time of the inflammatory response by the particular stimuli? Are there other subsets of monocytes bearing chemokine receptors and adhesion molecules that target them to venules in other tissues such as lung, gut, and brain, or to mucosal or mesenteric lymph nodes? Are the monocytes that home to a tissue "constitutively" the same group that homes there when that tissue is inflamed?

Cells originally described as "lymphocytes" based on their appearance in peripheral blood smears are now known to be comprised of many physiologically distinct subsets. If we had the molecular markers, flow cytometry capabilities, and insight 50 years ago that we have today, these cells might have received different names. Similarly, the term "monocytes" may describe a heterogeneous group of cells with similar appearance but different roles in the immune system. Whether monocytes are predetermined to home to specific tissues, and if so, when and where they gain the chemotactic receptors and adhesion molecules that facilitate their entry into these tissues will constitute important and interesting questions for future research.

In the meantime, the papers discussed here (6–8) represent significant advances in our understanding of monocyte trafficking by providing important new insights into the mechanisms and receptors used to selectively recruit monocytes from the circulation into specific tissues. The ability of BRAK to selectively draw monocytes into skin and gut may allow these sites to regulate the local production of macrophages (8) in the absence of a general inflammatory infiltrate. The absence of other leukocytes or particulate antigens might influence the balance of differentiation of monocytes into DCs or macrophages (12, 29). The selective recruitment of monocytes to lymph nodes draining inflamed tissues (6, 7) could potentially provide cells to aid in both the afferent and efferent arms of cell-mediated immunity. Although the monocytes recruited to lymph nodes might theoretically differentiate into antigen-presenting DCs, most lymph node DCs ap-

pear to enter the lymph node via afferent lymphatics. An exception to this could be the plasmacytoid DCs (see above) that are efficiently recruited to inflamed nodes, although the true fate of these cells in vivo is presently not known. More likely, monocytes recruited across HEVs provide a rapidly-mobilized source of effector cells to a zone of the inflamed lymph node where monocytes/macrophages are normally scarce.

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