Chemokines and the Homing of Dendritic Cells to the T Cell Areas of Lymphoid Organs

By Jason G. Cyster

A quarter of a century ago, it was proposed that veiled cells in the lymph were antigen-bearing Langerhans' cells (LCs) en route to the LN T cell area (1, 2). Extensive investigations have since established that LCs are immature dendritic cells (DCs) and that insults to the skin—including exposure to contact sensitizers, bacteria, or UV light—cause many of these cells to enter lymphatic vessels and travel to LN s (3). During transit the cells undergo a program of maturation events that take them from being poorly immunogenic to being the most potent of all APCs (3). Rapid transit of maturing DCs from the site of infection to the draining lymphoid tissue is likely to be critical for quick initiation of the adaptive immune response. But how do these cells migrate to lymphatics and subsequently into the LN T zone? A flurry of recent studies (4–9) have implicated chemokines and chemokine receptors in directing DC migration, and now a study reported in this issue provides strong evidence that one chemokine, secondary lymphoid tissue chemokine (SLC), plays an important role in DC migration in vivo to T cell zones of LN s and spleen (10).

Chemokines are small basic proteins that engage seven transmembrane receptors on responsive cells and promote chemotaxis (11). First characterized for their role in attracting cells to sites of inflammation, chemokines have more recently been found to direct cell movements within lymphoid tissues. Two chemokines that have been suggested to serve a homing function in the T cell compartment are SLC/6Ckine (12–16) and EBV-induced molecule 1 ligand chemokine (ELC)/macrophage inflammatory protein (MIP)-3β (17–19). SLC and ELC are structurally related chemokines and both bind the receptor CCR7 (20, 21). SLC is expressed at high levels by high endothelial venules (HEVs) in LN s and at lower levels by a poorly defined population of stromal cells in T cell areas of LN s, spleen, and Peyer's patches (13, 15, 16). ELC is made by a subset of DCs, and possibly by other nonlymphoid cells, in T cell areas of lymphoid tissue (19). Both chemokines are efficacious attractants of T lymphocytes (19, 21) and both can promote integrin activation on rolling lymphocytes (13, 22). Together these findings have led to the notion that SLC functions in recruitment of T cells across HEVs into LN s and more generally in promoting T cell migration into lymphoid T zones. ELC may work with SLC in recruiting cells into the T zone and in the next step, in promoting encounter between T zone DCs and T cells.

Mice carrying the paucity of lymph node T cells (plt) mutation, a spontaneous mutation that arose on the DDD/1 strain background, have a defect in T cell homing into LN s and splenic white pulp (23, 24). The expression pattern and properties of SLC led Gunn et al. to consider it a candidate for the plt gene, and this idea received a boost when mapping studies placed the plt mutation on a region of mouse chromosome 4 syntenic to the region of human chromosome 9 that contains the linked SLC and ELC genes (12, 17, 24). Gunn et al. have now demonstrated that expression of SLC is defective in plt mice (10). This finding and the prior T cell trafficking studies by Nakano et al. (23, 24) together provide strong evidence that SLC is necessary for homing of naive T cells across HEVs and into lymphoid T cell areas (10). Expression of the potentially closely linked ELC gene was also reduced in plt mice, although only partially and possibly as a secondary effect of the defective SLC expression (10). However, despite the mapping data and absence of SLC mRNA at levels detectable by Northern blot, sequence analysis of the SLC gene from plt mice has failed so far to uncover a mutation that could be responsible for the loss of SLC expression (10). Mutation of a distant regulatory region remains a likely possibility, but until such a mutation is found one must be cautious in concluding that defects in plt mice reflect solely a deficiency in SLC.

In situ hybridization analysis in wild-type mice demonstrated that lymphatic endothelial cells in many tissues make SLC (13). Taken together with its expression in LN T cell areas, this finding suggested that SLC might have a role in homing of DCs from peripheral tissues to lymphoid T zones. Support for this possibility came in several important studies over the last year showing that maturing DC upregulate expression of CCR7 and chemotactically respond to ELC (4, 6–9). At the time these studies were performed, it had not been reported that SLC was a ligand for CCR7. In vitro studies have since shown that transfection of cells with CCR7 is sufficient to confer chemotactic responsiveness to SLC as well as ELC (20, 21), making it likely that CCR7-expressing DCs migrate towards both chemokines. By studying DCs in plt mice, Gunn et al. have provided in vivo evidence of a role for SLC in directing DC migration (10).

The number of DCs in the LN s of plt mice is reduced approximately threefold compared with wild-type animals, consistent with a DC homing defect (10). 1 d after skin
painting with the contact sensitizer FITC, the frequency of FITC-bearing DCs in plt LN s was fourfold lower than in control LN s, providing evidence that SLC is needed for DC migration from skin to LN s via afferent lymphatics (10). The frequency of LCs in skin was indistinguishable in plt and wild-type mice, so the next question was whether SLC was required for DCs to enter lymphatic vessels. The small size of mice makes it difficult to cannulate afferent lymphatic vessels and perform the direct measurement of veiled cell frequencies done so elegantly in larger animals (1, 2). However, a method of tracking LC migration into lymphatics has been developed in mice where ears are split in half and incubated in vitro until many LCs begin to mature and migrate into dermal lymphatic vessels (25, 26). LCs were found to enter the dermal lymphatic vessels of plt mice with an efficiency that was indistinguishable from controls (10). These studies provide evidence that SLC is needed for efficient passage of DCs from lymphatic vessels into LN T zones, but not for entry into the lymphatic ves-

DCs are members of a family of tissue DCs, and almost every tissue contains sentinel DCs (3). Although differences between immature tissue DCs in different locations have been reported, most tissue DCs have in common the propensity to emigrate to draining lymphoid tissues in response to LPS, TNF, or IL-1 (3). All the DC types so far tested upregulate CCR7 upon stimulation, making it likely that they all use this receptor in order to migrate to lymphoid T zones (4–9). It remains to be investigated whether the same directional cues are also involved in the homeostatic flux of DCs from tissues to LN s that occurs in the absence of stimulation (3). A subset of DCs in peripheral lymphoid tissues, including lymphoid lineage DCs (27), may not derive from peripheral tissues but instead may enter directly from the blood (3). Some insight into the behavior of these cells in plt mice is provided by findings in the spleen. Wild-type mouse spleen contains a population of DCs in the T zone that express high levels of DEC205 and that are thought to be mostly of lymphoid lineage, and a population of myeloid lineage DCs in the marginal zone that express little DEC205 (3). Exposure to LPS causes marginal zone DCs to migrate rapidly into the splenic T zone (3). In plt mouse spleen, the distribution of DCs is altered, with fewer cells located inside white pulp cords (10). In addition, staining for the T zone DC marker DEC205 is substantially reduced, suggesting either that the number of lymphoid lineage DCs is reduced or that DEC205 expression is dependent on normal organization of cells in a T zone. LPS treatment of plt mice failed to cause DCs to congregate in areas thought, by their proximity to arterioles, to be T zones (10). These findings provide evidence that SLC is needed for homing of multiple types of DC s to lymphoid T cell areas.

SLC shares the CCR7 receptor with ELC, and an important issue still to be addressed is the relative contribution of these two chemokines to DC homing to T cell areas. ELC expression in plt mice is approximately threefold lower than in wild-type controls (10). At least a fraction of the ELC produced in lymphoid tissues comes from T zone DCs (19), making it possible that the reduced ELC expression in plt LN s and spleen is secondary to lower numbers of T zone DCs. Normal interactions between T cells and ELC-producing cells, which are likely to be disrupted in plt mice, might also be important in maintaining ELC expression. However, the possibility that the plt defect directly affects ELC expression has not been ruled out. Whatever the explanation, the reduced ELC levels may contribute to the phenotype of plt mice. Reciprocally, the continued expres-

A model of the events in LC migration to the LN T zone that incorporates the recent findings on chemokine and chemokine receptor expression is presented in Fig. 1. Immature DCs express a variety of inflammatory chemokine receptors, including CCR1, CCR5, CCR6, CXCR1, which may participate in DC recruitment to inflamed tissues (4–9). Differential chemokine receptor expression may contribute selectivity in the recruitment process, since CCR6, the MIP-3α receptor, is expressed at high levels by lung DCs and DCs derived in vitro from CD34+ cord blood precursors but not by monocyte-derived DCs (4, 7, 28). It seems likely that some chemokines often thought of as inflammatory—as well as others still to be characterized—help recruit immature DCs to become sentinels in noninflamed tissues, a possibility supported by the finding of constitutively expressed MIP-3α in liver and lung (29). Monocyte chemotactic protein 1 (MCP1) is expressed constitutively in many tissues (30), and although its cognate receptor, CCR2, is not highly expressed by immature DCs, it is strongly expressed by monocytes (31). A recent finding indicates that monocytes may differentiate into DCs during migration into lymphatic vessels (32), MCP1 and CCR2 may make an important contribution to DC trafficking.

The rapid upregulation of chemokine expression that occurs at sites of inflammation should help recruit more DC precursors to the site, but might also be expected to interfere with the ability of antigen-bearing DCs to emigrate. Hence, the recently observed rapid decrease in chemokine receptor function during DC maturation, either by direct downregulation (4–9) or by functional modulation as a result of intrinsic expression of chemokine (7), is likely to be important in allowing the cells to move from the site (Fig. 1). Adhesion molecule changes may also be important for emigration, including reduced expression of E-cadherin, activation of α6 integrins, and switching of CD44 isoforms (3). In addition to these changes, it would seem likely that
attractant cues are needed to guide DCs to the lymphatic vessels, and the expression of SLC in many lymphatic vessels (13) suggests it has a role at this site. The failure to detect any effect of the plt mutation on DC entry into dermal lymphatics does not yet exclude a role for SLC, as the plt mutation may be in a regulatory region of the SLC gene and so may not fully disrupt SLC expression at all sites. The possibility that ELC is made by lymphatic endothelium in the skin has yet to be explored.

Once DCs have entered lymphatic vessels and become veiled cells, how do they then move out into the T zone parenchyma to become interdigitating DCs (Fig. 1)? CCR7, SLC, and ELC can now be said to have a role and CXCR4/stromal cell factor 1 (SDF1) might also contribute (5–7), but what other molecules are needed for the cells to move from the subcapsular sinus? Cells lining the sinus are part of a larger network of fibroblastic reticular cells that form cords and channels through the LN parenchyma (33). What role does this network play in presenting chemokines and other guidance cues to the migrating DCs? Within lymphoid tissues, even within the T zone itself, DCs are not evenly dispersed. What additional cues contribute to subcompartmentalization of the cells? Finally, as DCs themselves are being found to express an increasing array of chemokines, to what extent do they contribute to the overall organization of the lymphoid tissue? Could migrating DCs provide a homeostatic link between the severity of a peripheral infection and the magnitude of increased lymphocyte retention that occurs in the draining lymphoid tissue? As an understanding of the factors regulating DC migration to the T cell areas of lymphoid tissues has important implications for many aspects of immunobiology, including the development of new adjuvants and immunosuppressants, we can be sure that answers to many of these questions will soon be unveiled.

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