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Chemokines and the Tissue-Specific Migration of Lymphocytes

Minireview

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Tissue-selective trafficking of memory and effector T and B lymphocytes is mediated by unique combinations of adhesion molecules and chemokines. The discovery of several related epithelial-expressed chemokines (TECK/CCL25 in small intestine, CTACK/CCL27 in skin, and MEC/CCL28 in diverse mucosal sites) now highlights an important role for epithelial cells in controlling homeostatic lymphocyte trafficking, including the localization of cutaneous and intestinal memory T cells, and of IgA plasma cells. Consitutively expressed epithelial chemokines may help determine the character of local immune responses and contribute to the systemic organization of the immune system.

Nearly 40 years ago, James Gowans demonstrated that circulating lymphocytes enter secondary lymphoid tissues and subsequently return to the circulation through the efferent lymph (Gowans and Knight, 1964). Later data showed that memory T lymphocytes selectively recirculated back through tissues from which they came (including the skin and intestines), and B immunoblasts expressing IgA specifically migrated to mucosal tissues (Cahill et al., 1977; McDermott and Bienenstock, 1979). Together, these findings laid the groundwork for the concept of tissue-specific lymphocyte recirculation, and the idea of specific lymphocyte trafficking has become a fundamental concept in the study of acquired immunity. Physiologically, tissue-restricted recirculation of memory and effector lymphocytes may serve to (1) increase the efficiency and robustness of regional immune responses while decreasing the possibility of tissue antigen crossreactivity; and (2) allow functional immune specialization of particular tissues (e.g., skin versus intestines) or tissue systems (e.g., mucosal epithelial surfaces). Lymphocyte recirculation is tightly regulated by the expression of particular adhesion molecules and chemoattractant receptors on lymphocytes, combined with the spatial and temporal expression of ligands for these receptors by a variety of tissue cells. The present review will examine some fundamental concepts underlying tissue-specific lymphocyte recirculation, focusing primarily on epithelial chemokines with restricted tissue expression as newly appreciated regulators of lymphocyte trafficking through tissues.

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Multiple Steps Promote Specificity in Lymphocyte Trafficking

Lymphocyte recirculation into lymphoid and nonlymphoid tissues was described 10 years ago as a series of adhesive and activation processes that impart specificity while retaining robustness in the face of mutation and regulatory variability (Butcher, 1991). The requirement for multiple protein-protein interactions allows a genetically limited receptor-ligand repertoire to be used combinatorially to control the recirculation of different lymphocyte (and other leukocyte) subsets. Specificity in one step alone can lead to enrichment of the final recruited population, but often several steps in the process are selective for a particular tissue site, resulting in a high degree of specificity.

Recirculation begins with blood lymphocytes interacting transiently and reversibly with the vascular endothelium through villous-expressed adhesion receptors (usually selectins and selectin ligands, but also α_4 integrins) in a process called rolling. Rolling brings lymphocytes into contact with the endothelium where they can sample the surface for activating factors. Activating factors (often chemokines for lymphocytes) bind to pertussis toxin-sensitive G protein-coupled receptors on the rolling lymphocytes, triggering rapid activation of integrins that results in reversible arrest and firm adhesion of the lymphocyte. Additional adhesion molecules, endothelium-associated chemokines, and potentially other signals, then lead the adherent lymphocyte to migrate across the endothelium where tissue-associated chemokine gradients may also direct localization. Because chemokines contribute to both lymphocyte exit from the circulation and localization and retention within tissues, they are important regulators of the lymphocyte recruitment process.

Secondary Lymphoid Organs and Tissue-Specific Lymphocyte Development

Naive T and B cells are preprogrammed to migrate to and recirculate through secondary lymphoid organs (e.g., spleen, lymph nodes, and Peyer's patches) in part through expression of the chemokine receptor CCR7, a ligand for the lymphoid tissue chemokines SLC/CCL21 and MIP-3β/CCL19 (reviewed in von Andrian and Mackay, 2000). Cells encountering antigen in the lymphoid environment undergo reprogramming of homing properties during antigen-driven proliferation and differentiation, allowing their progeny to traffic to extralymphoid tissue sites, although early arising immunoblasts can display substantial promiscuity for different inflamed tissues, perhaps reflecting their return to the blood while still expressing activated integrins. Imprinting, or selection for differential homing properties, is determined by the local lymphoid organ microenvironment (perhaps by dendritic cells and chemokines), and begins almost immediately during the naive-to-memory/effector T cell transition. For example, naive T cells responding to antigen begin to upregulate the intestinal homing receptor $\alpha_4\beta_7$ and responses to the intestinal chemokine TECK/CCL25 (see below) within one to two

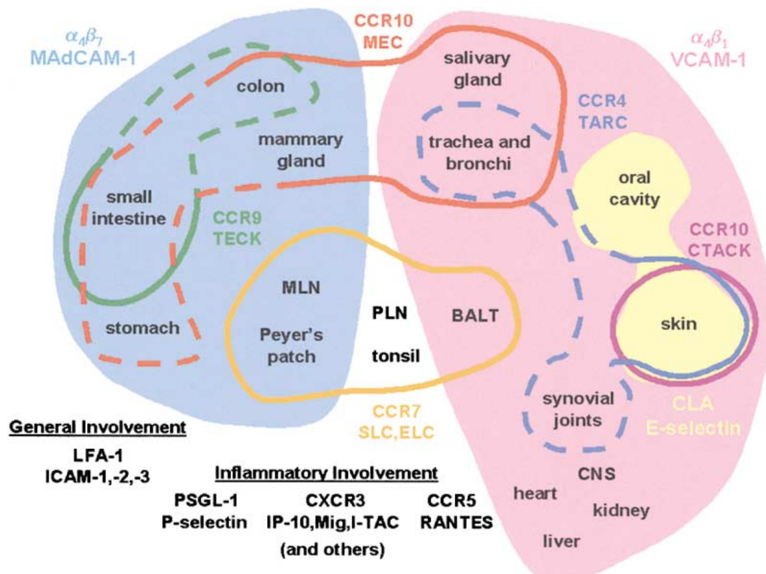

Figure 1. Tissue-Selective Chemokine Expression in the Systemic Organization of the Immune System

In this model, tissue selective chemokines and adhesion pathways control lymphocyte homing while the selectivity of lymphocyte recruitment reflects the combination of vascular adhesion molecules and chemokines expressed in a given tissue site. This schematic diagram groups body tissues according to the predominant constitutive tissue-selective lymphocyte-endothelial adhesion molecule(s) that participates in lymphocyte recruitment (solid colors), and then further groups them by the homeostatic endothelial or epithelial chemokines (and their lymphocyte receptors) associated with each tissue (solid lines; dashed lines show sites where the chemokine is expressed at low levels compared to other grouped tissues, and/or the receptor is only on a subset of lymphocytes). Thus, the lymphoid tissue chemokines SLC/CCL21 and ELC/CCL19 and lymphocyte CCR7 (in conjunction with L-selectin; not shown) help control lymphocyte entry into secondary lymphoid tissues; endothelial TARC/CCL17 and its receptor CCR4 (expressed at high levels by T cells in skin and at lower levels in the lung and joints) along with CTACK/CCL27 and CCR10 (skin; perhaps oral cavity) in conjunction with CLA and E-selectin (skin and oral cavity) and $\alpha_4\beta_1$ and VCAM-1 (nonintestinal sites) control cutaneous memory T cell homing to skin; epithelial TECK/CCL25 (predominantly small intestine) in conjunction with $\alpha_4\beta_7$ and MAdCAM-1 (colon, mammary gland, and small intestine) is implicated in selective trafficking to the small intestine; and MEC/CCL28 (colon, salivary gland, bronchi, and mammary gland) acting either with $\alpha_4\beta_1$ and VCAM-1 (nonintestinal sites) or with $\alpha_4\beta_7$ and MAdCAM-1 (mammary gland and intestines) is proposed to mediate lymphocyte subset recruitment to many mucosal tissues. Some adhesion molecules such as LFA-1 ($\alpha_L\beta_2$) and its ligands (ICAM-1,-2,-3) are involved in lymphocyte homing to most tissues, and inflammation induces the expression of many more chemokines and adhesion molecules (e.g., P-selectin and ligands for chemokine receptors such as CXCR3 and CCR5) that can complement the homeostatic tissue-selective recruitment mechanisms emphasized here. See text for more details.

cell divisions in intestine-associated lymphoid tissues, while conversely, homing properties targeting cells to nonintestinal tissues are upregulated during the initial proliferative response in peripheral lymph nodes (Campbell and Butcher, 2002).

Tissue-Specific Lymphocyte Populations at Epithelial Surfaces

Arguably, the main function of the mammalian immune system is to protect the host from foreign pathogens, and thus evolution would favor the development of lymphocyte trafficking patterns that protect sites of pathogen entry, particularly epithelial tissues (Figure 1). The skin and the gastrointestinal tract are clearly two of the largest epithelial organ systems in contact with the outside world, and accordingly the circulating memory lymphocyte pool contains two clearly separable subsets of lymphocytes with skin versus gut homing potential. Memory for skin-associated antigens is found in a population of skin-homing circulating lymphocytes expressing the cutaneous lymphocyte antigen (CLA), a carbohydrate-defined cutaneous "homing receptor." CLA^+ lymphocytes are highly enriched in cutaneous inflammatory sites and the oral mucosa (which like the skin has a stratified squamous epithelium), but not in other tissues. Conversely, memory for rotavirus (an intestinal epithelial-restricted pathogen) has been localized to a population of circulating lymphocytes expressing high levels of the $\alpha_4\beta_7$ integrin. Lymphocytes that are not in the intestinal $\alpha_4\beta_7^{hi}$ population generally express high levels of the VCAM-1 receptor $\alpha_4\beta_1$ (including many skin-homing lymphocytes), and these $\alpha_4\beta_1^{hi}$ lymphocytes make up a pool of lymphocytes with homing potential to tissues outside the gastrointestinal tract but including nonintestinal mucosal sites such as the bronchi (reviewed in Butcher et al., 1999; Robert and Kupper, 1999; Shaw and Brenner, 1995).

Recent data reveal that chemokines and their receptors also help control the specificity of memory lymphocyte subsets for skin and gut. Two homeostatically expressed chemokines appear to participate in selective T lymphocyte recruitment into the skin. One of these chemokines, TARC/CCL17, is expressed by cutaneous, but not intestinal, endothelium and binds to the chemokine receptor CCR4 expressed at high levels on CLA^+ skin memory lymphocytes (and at lower levels on lung lymphocytes [Campbell et al., 2001]). TARC is hypothesized to trigger the adhesive arrest of rolling lymphocytes in cutaneous venules, especially during chronic inflammation (Campbell et al., 1999). More recently, keratinocytes, the epithelial cells of the skin, have been shown to express the chemokine CTACK/CCL27, which binds to the receptor CCR10 also specifically expressed on circulating skin-homing CLA^+ T lymphocytes (Morales et al., 1999). At least in some settings of inflammation, CCR4 and CTACK/CCR10 have overlapping, redundant roles in cutaneous lymphocyte recruitment since blockade of lymphocyte homing from the blood in a delayed-type hypersensitivity (DTH) model requires simultaneous deficiency or inhibition of CCR4 and CTACK/CCR10 (Reiss et al., 2001). In addition to mediating vascular arrest, CCR4 may be able to support diapedesis and chemotaxis through a gradient of MDC/CCL22 (another CCR4 ligand) secreted by resident activated macrophages. Similarly, in addition to providing an epithelial-derived gradient for diapedesis and recruitment

to the epithelium, CTACK may be transcytosed and presented on the endothelium (Middleton et al., 1997) to support skin-homing lymphocyte adhesion triggering. Importantly, these results demonstrate that inflammatory chemokines cannot substitute for these two skin-selective chemokines in cutaneous lymphocyte recruitment in DTH.

In the intestines, part of the selectivity of lymphocyte homing is due to the expression of the $\alpha_4\beta_7$ integrin ligand MAdCAM-1 on normal and inflamed intestinal endothelium (reviewed in Butcher et al., 1999). Recent results indicate that a specific subset of circulating $\alpha_4\beta_7^{hi}$ lymphocytes is in fact targeted to the small intestine by virtue of expression of the chemokine receptor CCR9 (Zabel et al., 1999). The CCR9 ligand, TECK, is expressed by epithelial cells in the small intestine, especially those in the crypt region most closely associated with the MAdCAM-1$^+$ vessels involved in lymphocyte recruitment (Kunkel et al., 2000; Wurbel et al., 2000). Indeed, TECK is detected on small intestinal endothelium (Papadakis et al., 2000), and may be produced by endothelial cells or simply transcytosed after being secreted by intestinal epithelium. Virtually all T lymphocytes in the small intestine express CCR9 (Kunkel et al., 2000; Papadakis et al., 2000; Zabel et al., 1999), suggesting that CCR9 and TECK play a critical role in lymphocyte biology in this tissue. Even more interesting is the finding that TECK is absent or only weakly expressed in other segments of the gastrointestinal tract (e.g., colon and stomach) and only a small fraction of colon lymphocytes express CCR9 (Kunkel et al., 2000; Papadakis et al., 2000; Wurbel et al., 2000). This finding suggests that even organs thought to be part of a common mucosal immune system may have different lymphocyte homing pathways distinguished by chemokine usage, providing a potential mechanism for further specialization of mucosal immune responses in various intestinal and other mucosal sites.

Mucosal epithelial tissues are also the major site of secretory IgA production by resident plasma cells, and IgA-dependent pathogen neutralization at mucosal sites is critical to host protection. B cell immunoblasts secreting IgA (IgA ASC) also migrate preferentially to the small intestines (and other mucosal sites) (McDermott and Bienenstock, 1979) and, accordingly, express the mucosal homing receptor $\alpha_4\beta_7$ (Butcher et al., 1999). As during T lymphocyte recirculation, these antibody secreting cells likely require a chemoattractant signal for extravasation or tissue localization, and indeed, IgA ASC respond chemotactically to unknown factors present in mucosal epithelial tissues such as the mammary gland (Czinn and Lamm, 1986). Interestingly, splenic, mesenteric lymph node, and Peyer's patch IgA ASC migrate efficiently to TECK, and express CCR9 (Bowman et al., 2001). Thus, epithelial chemokines such as TECK may also participate in ASC localization to epithelial surfaces, in this case the small intestine. Interestingly, while IgG ASC do not respond to TECK (Bowman et al., 2001), they are responsive to other chemokines including SDF-1α/CXCL12 and Mig/CXCL9 (ligands for CXCR4 and CXCR3, respectively) (Bowman et al., 2001; Hargreaves et al., 2001) and most express the integrin $\alpha_4\beta_1$ (Finke et al., 2001). IgG ASC may use these receptors to localize to sites of chronic inflammation. Thus, selectivity in isotype-specific ASC chemokine receptor expression is likely a major determinant of IgA ASC localization to mucosal epithelial surfaces and of IgG ASC localization to systemic sites of chronic inflammation.

Interestingly, the epithelial chemokines TECK and CTACK are very closely related, suggesting that these homeostatic tissue-selective chemokines may have evolved from a common (potentially epithelial) precursor. Indeed, based on sequence homology, a third closely related chemokine was recently discovered (Pan et al., 2000; Wang et al., 2000). MEC (mucosal epithelial chemokine, CCL28) is abundantly expressed by epithelia in the bronchi, colon, salivary gland, and mammary gland, and at lower levels in the small intestine. Importantly, even though MEC is apparently absent from skin, it, like CTACK, is also a ligand for CCR10. Indeed, MEC can attract circulating CLA$^+$ skin-homing lymphocytes, even though these lymphocytes are essentially undetectable in the colon or small intestine. (This can be explained in the context of the combinatorial control of vascular interaction: CLA$^+$ T cells lack the $\alpha_4\beta_7$ integrin expression required for tethering and rolling on intestinal lamina propria venules, and thus are expected to be unable to sample the intestinal endothelium for CCR10 ligand activity.) Nevertheless, CCR10 mRNA is abundantly expressed in the colon and small intestine where MEC is also expressed (Jarmin et al., 2000). Therefore, MEC may serve to recruit, or retain, populations of mucosal lymphocytes (e.g., B or T immunoblasts) that are not well represented in the circulation. Teleologically, the striking overlap of bacterial species in the oral cavity and colon (Kroes et al., 1999), and the obvious relationship between the antigen exposure of the oral cavity and airways, suggests that the unique tissue pattern of MEC expression may serve to unify trafficking of specialized immune cell populations to these physically dispersed but immunologically related mucosal sites.

The evolutionary pressures driving the specialization of lymphocyte subset recruitment to different epithelial organs (manifested in part by differential chemokine expression) are still unclear but likely relate to the type and intensity of antigen exposure in various organs. The specialization between segments of the intestines itself is surprising and may be related to the differing physiological functions and immune requirements of each intestinal segment. For instance, the small intestine is exposed to food-derived antigens to which an immune response is unnecessary and potentially debilitating, as in the case of celiac disease. In contrast, the colon (and oral cavity) are sites of heavy commensal bacterial population. Thus, specialized trafficking mechanisms may allow distinctive immune responses in the small intestine, where immune tolerance to food antigens is important, versus highly colonized sites where constitutive antibacterial immunity or symbiosis is essential. Differences in lymphocyte homing character may also be related to the characteristic segmental involvement of the distal small intestine (ileum) and colon in Crohn's disease and of the colon and rectum in ulcerative colitis (Fiocchi, 1998).

The recent findings reviewed here thus highlight an important role for epithelial cells as active participants in defining the specificity of lymphocyte trafficking and the nature of local immune specialization. Because the role of epithelial cells in the induction and resolution of inflammation, including secretion of inflammatory che-

mokines, is well appreciated (reviewed in Pitman and Blumberg, 2000), a role for these cells in tissue-specific homeostatic lymphocyte recirculation is not surprising. It will be important to define the developmental and regulatory mechanisms underlying the tissue selectivity of endothelial and epithelial expression of homeostatic chemokines (e.g., TARC, TECK, CTACK, and MEC) and to determine if these mechanisms are shared with those controlling expression of vascular addressins (e.g., MAdCAM-1).

While this review has focused primarily on a newly emerging model of epithelial chemokine control of the specificity of lymphocyte homing, significant experimental effort has gone into identifying lymphocyte subsets that specifically traffic through many internal organs, such as the liver, kidneys, and synovial joints (far too much work to adequately cite here). While tissue-specific subsets for these organs cannot be ruled out, the idea of an evolutionary bias toward specific protection of pathogen entry sites may suggest that trafficking of lymphocytes through such "protected" internal organs (in the sense that they are not directly in contact with the outside world) may occur principally under inflammatory conditions, and data from many studies support the hypothesis that lymphocytes infiltrating these organs may rely on a similar handful of widely utilized "inflammatory" adhesion and chemokine receptors (e.g., $\alpha_4\beta_1$ and VCAM-1, P-selectin and PSGL-1, CXCR3, CCR5, etc.) that can likely also complement tissue selective mechanisms in external epithelia-associated tissues in the setting of strong inflammatory insult or tissue damage. A common mechanism of trafficking control in internal organs would not imply that such trafficking is less important or poorly regulated, only that no evolutionary pressure existed to drive the development of internal organ immune response specialization, which would require additional tissue-specific homing mechanisms.

Conclusions

The identification of novel chemokines and chemokine receptors has revolutionized our understanding of tissue-specific lymphocyte recirculation and localization. The differential expression of particular chemokines within epithelial tissues further suggests that organ systems previously thought to be relatively immunologically uniform may have important differences in terms of their immune character (e.g., the small and large intestines), while those thought to be more diverse may be linked in a previously unrecognized way (e.g., the colon, salivary glands and oral cavity, and airways). At the same time, although the discovery of new chemokines and receptors appears to offer answers to long-standing questions in lymphocyte homing, many fundamental questions still remain, including the mechanisms behind the development of tissue-homing subsets, the mechanisms controlling tissue-specific endothelial and epithelial expression of particular chemokines and adhesion molecules, and in some instances the physiological rationale behind the evolutionary separation or unification of particular tissues through lymphocyte recirculation pathways.

Note

Because of the extent and complexity of the lymphocyte homing field, we could not discuss many interesting studies, and we apologize to those whose excellent work could not be cited due to space limitations.

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