Lymphocyte homing to the gut: attraction, adhesion, and commitment

Summary: Lymphocytes continuously migrate from the blood into the intestine. Naive lymphocytes leave the blood through high endothelial venules in Peyer’s patches. During the multistep extravasation cascade, they sequentially roll on, firmly adhere to, and transmigrate through the endothelial layer using multiple adhesion molecules and chemotactic signals. In the organized lymphoid tissues of the gut, lymphocytes can become activated, if they meet their cognate antigens transported to Peyer’s patches through the gut epithelium. During activation and proliferation, the lymphocytes become imprinted by the local dendritic cells, so that after returning to systemic circulation via the efferent lymphatic vasculature, they preferentially home to lamina propria of the gut to execute their effector functions. In inflammation, the recirculation routes of lymphocytes are altered, and these may explain the pathogenesis of certain extra-intestinal manifestations of gut infections and inflammatory bowel diseases. The increased knowledge on the mechanisms that regulate lymphocyte homing and imprinting has clear applicability in designing more effective vaccination regimens. A detailed understanding of the mucosal homing has recently led to the development of the first successful anti-adhesive therapeutics in human.

Introduction

Forty years ago, Gowans and Knight (1) isolated lymphocytes from the efferent lymph of normal rats, labeled them radioactively, injected the cells to the blood circulation of recipient animals, and followed their distribution by scintillography and autoradiography. They saw that small lymphocytes distributed themselves to all secondary lymphoid tissues. In contrast, activated immunoblasts selectively migrated into mucosal sites. This finding inspired a series of intelligent in vivo studies by many groups, which all showed that small lymphocytes distribute apparently randomly among different secondary lymphoid organs, whereas activated immunoblasts preferentially migrate back to the tissue type in which they had become activated (2–8). These experiments laid the basis for the concept that we today know as lymphocyte recirculation and homing.
**Lymphocyte recirculation and homing**

During lymphocyte homing, blood-borne lymphocytes make a series of contacts with the blood endothelial cells (9–11). Naive cells preferentially leave the blood in organized lymphoid tissues, in which specialized post-capillary venules lined by high endothelial cells are uniquely specialized for supporting the extravasation (10, 12, 13). The lymphocytes insinuate themselves through the vessel wall and enter the tissue stroma. They then percolate through the connective tissue meshwork in search of their antigens (14). If the lymphocyte does not find its cognate antigen, it leaves the lymphatic tissue via efferent lymphatic vessels that carry the cell back to the systemic blood circulation. Then, the naive lymphocyte is free to continue its odyssey to any other secondary lymphatic organ.

When the naive lymphocyte finds its cognate antigen in the lymphoid tissue, its migratory pattern changes profoundly (9, 10). In this case, the lymphocyte starts to proliferate and differentiate, and the progeny then enters the blood circulation via the same route as naive cells. However, the activated cells have become imprinted in the tissue microenvironment, so that rather than extravasating randomly, they now preferentially leave the blood in the same type of lymphatic tissue in which they became activated. Even more notoriously, they have acquired new properties that allow them to leave the blood in a non-lymphatic peripheral tissue draining into the lymph node in which imprinting took place. Extravasation in the non-lymphoid peripheral tissues takes place via the normal flat-walled endothelium in post-capillary venules.

Currently, two main migratory routes under normal conditions have been described (9, 10). One involves the skin peripheral lymph node (PLN) axis and the other the mucosa-associated lymphatic tissues (Fig. 1).

The anatomical organization of lymphocyte recirculation allows highly efficient contacts between the lymphocytes and antigens. The antigens are concentrated from the periphery, as free antigens or inside activated dendritic cells (DCs), to the local organized lymphatic tissues via the lymphatic vasculature (15, 16). At the same time, naive lymphocytes are delivered continuously to the same organs via the blood supply (10). Thereby, in comparison with stochastic processes, lymphocyte recirculation allows much more efficient triggering of productive immune responses on antigen challenge.

**The multistep adhesion cascade**

The leukocytes leave the blood via a multistep process (9–11, 17–19) (Fig. 2). At the first step, the cells make transient tethers with the vascular endothelium, which can lead to subsequent rolling of the cells. At this step, the cell is moving along the endothelial lining at a reduced velocity to the direction of the blood flow in a shear-dependent manner. If the rolling cell receives appropriate signals from the endothelial cells, it becomes activated. It can then firmly bind to the endothelial lining in a shear-resistant manner. Thereafter, the cell continues locomotion on the endothelial cells in search of a suitable site, where it can penetrate through the endothelial lining and then extravasate through the other layers of the vessel wall. There is evidence that granulocytes may preferentially use the inter-endothelial junctions for the emigration, whereas at least certain lymphocytes appear to be able to be transcytosed through the intact endothelial cells (13, 20, 21).

Several classes of adhesion and activation molecules are involved in securing the different steps of the extravasation cascade (Fig. 2). Hence, selectins and their oligosaccharide-based ligands are pivotal during the transient and reversible contacts at the tethering and rolling steps (22, 23). Thereafter, chemotactic molecules presented by the glycosaminoglycan molecules on endothelial cells are thought to be critical in triggering the activation of rolling cells (24–26). The activatory signals are transmitted through the serpentine receptors on...
leukocytes, which are coupled to G-protein–linked signaling pathways. These signals ultimately trigger changes in the affinity and avidity of leukocyte integrins via conformational changes and clustering (27, 28). The activated integrins in turn bind to the adhesion molecules expressed on the vascular endothelium that often belong to the immunoglobulin (Ig) superfamily to mediate firm binding. These same molecular devices are then utilized during the transmigratory process, along with several other members of the Ig superfamily, and other types of adhesion molecules and proteinases (11, 29, 30).

Mucosal homing

Mucosal homing encompasses recirculation to organized lymphoid tissues (such as Peyer’s patches) and to the non-organized lymphatic structure, the lamina propria, which are two different operational units (31) (Figs 1 and 3). Naive cells mainly leave the circulation in Peyer’s patches through high endothelial venules (HEVs). When they meet their cognate antigen in Peyer’s patches, they start to differentiate and proliferate in that productive microenvironment. The mucosal immunoblasts then enter the efferent lymphatics of the gut, which drain into the mesenteric lymph node (MLN), in which further maturation takes place. Thereafter, the cells are transported into the efferent lymphatics, which drain into the thoracic duct and other major collecting lymph vessels and are finally released into the systemic circulation at the large venous trunks. When these cells then get back to the mucosa via the systemic circulation, they now preferentially leave the blood via the flat-walled venules of the lamina propria (Fig. 1). Some of the lymphocytes that enter the lamina propria are further attracted all the way to the epithelium of the gut (Fig. 3). They constitute the unique population of intraepithelial lymphocytes (IELs) that is characteristic of the mucosal sites. Thus, Peyer’s patches and PLNs can be thought to function similarly as the primary target organs for the entry of naive lymphocytes, and lamina propria and skin as the ‘peripheral’ target organs for activated lymphocytes.
It has been argued for a long time that gut and other mucosal sites (lungs, lacrimal glands, genitourinary tract, etc.) comprise a common mucosal recirculation system (32). There is indeed ample evidence supporting the concept that cells activated at certain mucosal sites (e.g. by vaccination) are able to migrate to anatomically separate mucosal surfaces. However, there also appears to be a subtler compartmentalization of the mucosal immune system (33). Thus, lymphocytes stimulated in a certain part of the gut show preferential reappearance in the same segment of the intestine (34). On the other hand, at least certain lymphocyte types isolated from the upper aerodigestive tract home poorly to gut (35). The expression of unique adhesion and activation molecules in certain areas of the mucosal system may partly account for this phenomenon (33, 36) (see below), but the molecular determinants responsible for the generation of this fine specificity remain mostly unknown.

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**Which molecules determine the organ specificity of mucosal homing?**

Efficient homing to mucosal sites requires proper functioning of organ-specific homing-associated molecules together with non–tissue-specific adhesion molecules. These molecules are presented below and shown in Fig. 4. Moreover, several animals lacking these genes have been produced, and their mucosa-related phenotypes are listed in Table 1.

**Adhesion molecules**

Although there are a multitude of molecules involved in lymphocyte interaction with vascular endothelial cells at different steps of the adhesion cascade, only a few of them are responsible for mucosal specificity of the homing process. Most notably, lymphocytes need to have α4β7 integrin on their surface to be able to interact with its ligand, mucosal

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### Table 1: Molecules involved in lymphocyte extravasation to the gut

<table>
<thead>
<tr>
<th>Adhesion molecule/chemokine</th>
<th>Family</th>
<th>Ig family member</th>
<th>GAG</th>
<th>Selectin</th>
<th>Sialomucin</th>
<th>Chemokine</th>
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<td>-</td>
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**Fig. 4. Molecules involved in lymphocyte extravasation to the gut.** The most relevant endothelial glycoproteins mediating lymphocyte–endothelial cell interactions and chemokines and their best-characterized lymphocyte receptors are shown as receptor-ligand pairs. Their molecular families are also indicated as well as the relative contribution of each receptor-ligand pair (the size of the pink dot) in lymphocyte–endothelial cell interactions. In addition to the molecules presented in this figure, there are individual reports describing involvement of many other molecules in the extravasation process. Ig = immunoglobulin; GAG = glycosaminoglycan. See the text for abbreviations.
addressin cell adhesion molecule (MAdCAM-1), that is present on HEVs in Peyer’s patches and flat-walled venules in lamina propria and lactating mammary gland (31). MAdCAM-1 belongs structurally to the Ig superfamily. It has a unique functionally important modification on HEVs in MLNs and in Peyer’s patches, because it can be decorated with carbohydrates present on PLN addressins (PNAd) and thus serve also as a ligand for L-selectin (37, 38). α4β7-MAdCAM-1 interaction can mediate both lymphocyte rolling and firm adhesion to the vascular wall (39).

During the embryonic development and early childhood, MAdCAM-1 is present also on post-capillary venules in PLNs, whereas it is practically absent from normal adult PLNs. Moreover, when present in PLNs, it is fully active and mediates binding of lymphocytes (40, 41). Widespread expression of MAdCAM-1 may be functionally important in the development of the immune system, because it allows lymphocytes activated at mucosal sites to disperse throughout the body and maximize effective immune responses.

In addition to the studies inhibiting the function of α4β7 with antibodies, the importance of α4β7 in lymphocyte homing to mucosal sites is clearly demonstrated by the β7-knockout mice (Table 1). These mice are healthy, viable, and develop normally, but they have dramatically hypocellular Peyer’s patches that contain only rudimentary follicles. Moreover, the number of lymphocytes in lamina propria is significantly reduced. In short-term in vivo assays, migration of β7 integrin−/− lymphocytes into Peyer’s patches is severely reduced, lymphocyte migration into MLNs is diminished, but migration into PLNs is normal (42). However, efficient homing to Peyer’s patches requires both α4β7 and L-selectin, because it is more impaired in double-knockout mice lacking both L-selectin and α4β7 than in mice lacking only one of these molecules (43).

To our knowledge, there are no published reports regarding MAdCAM-1-knockout mice. Mice lacking the transcription factor NKX2.3 also lack MAdCAM-1, indicating that MAdCAM-1 expression is under the control of NKX2.3. Peyer’s patches of NKX2.3-deficient mice are considerably reduced in size and lack the ordered tissue architecture. T and B cells are misplaced within MLNs and fail to segregate into the appropriate T- and B-cell areas. These aberrations may be assumed to be dependent at least partly on the lack of MAdCAM-1 (44).

When the lymphocytes arrive in lamina propria, a subset of them migrates to the epithelium. αEβ7 (CD103) is upregulated on CD8+ T cells subsequent to their entry into the small intestinal epithelium. There, CCL25 chemokine enhances αEβ7-mediated lymphocyte adhesion to epithelial E-cadherin. This finding clearly demonstrates that besides attracting the lymphocytes toward the epithelium, chemokines are also able to modulate interaction between lymphocytes and epithelial cells at mucosal surfaces (45). αEβ7-knockout animals display reduced number of lymphocytes, not only in epithelium but also in lamina propria, while Peyer’s patches are normal. Therefore, it is highly likely that αEβ7 has other ligand(s) than E-cadherin in lamina propria (46).

Chemokines and their receptors

From the large group of chemokine receptors (24–26), CCR9 can be considered to be relatively mucosa specific. Although it contributes to lymphocyte migration to the thymus, this process takes place during early T-cell development. Mature lymphocytes carrying CCR9 are guided to the small intestine (47). The intestinal ligand of CCR9 is CCL25, and it is mainly expressed in mucosal epithelium. However, it has been demonstrated to be present also at vascular surfaces, thus attracting cells at the entrance site and helping them to further localize within the intestine (48). Mice lacking the CCR9 chemokine receptor show a mild impairment of early T- and B-cell development and a reduction in the number of T-cell receptor γδ− and αβ+ gut IELs (47, 49) (Table 1). Moreover, in

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Mucoideal phenotype</th>
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<tbody>
<tr>
<td>L-selectin</td>
<td>Reduced lymphocyte homing to Peyer’s patches</td>
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<tr>
<td>β7</td>
<td>Hypocellular Peyer’s patches and reduced lymphocyte number in lamina propria</td>
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<tr>
<td>αE</td>
<td>Reduced number of lymphocytes in lamina propria and epithelium</td>
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<tr>
<td>LFA-1/ICAM-1</td>
<td>Aberrant lymphocyte pattern in epithelium, lamina propria, and Peyer’s patches</td>
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<tr>
<td>P-selectin</td>
<td>Altered T-cell populations in gut epithelium</td>
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<tr>
<td>VAP-1</td>
<td>Reduced lymphocyte homing to Peyer’s patches</td>
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<tr>
<td>CCR9</td>
<td>Reduced number of intraepithelial T cells, reduced number of IgA+ plasma cells in lamina propria</td>
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<tr>
<td>CCR7</td>
<td>Impaired homing of lymphocytes (especially T cells) to Peyer’s patches</td>
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<tr>
<td>CCL19/CCL21</td>
<td>Impaired homing of lymphocytes (especially T cells) to Peyer’s patches</td>
</tr>
<tr>
<td>CXCR4</td>
<td>Aberrant B-cell follicles formed ectopically in intestinal lamina propria around Peyer’s patches</td>
</tr>
<tr>
<td>CXCR5/CXCL13</td>
<td>Developmental abnormalities and reduced number of Peyer’s patches</td>
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</table>
CCR9-deficient mice, IgA+ plasma cells are substantially reduced in number in the lamina propria of the small intestine. In adoptive transfer experiments, CCR9-deficient IgA+ plasma cells show impaired migration into the small intestine compared with wildtype controls. Furthermore, CCR9-deficient mice fail to mount a normal IgA response to an orally administered antigen, although the architecture and cell-type composition of Peyer’s patches and MLNs are unaffected (50).

Another epithelial chemokine present in small intestine is CCL28. It attracts IgA blasts, which bear its receptor, CCR10 (51). CCL28 is also present in large intestine, whereas CCL25 is essentially absent from the colon. Consistent with this, only less than 20% of lymphocytes homing to the colon are CCR9+ (52). On the other hand, CCL28 is present in epithelia in other organs considered to belong to the common mucosal system, whereas CCL25 is absent from there as well. There has been quite a lot of discussion whether homing mechanisms to small and large intestine differ from each other. Although many molecules involved in homing to the small intestine and colon seem to be shared between these two locations, differential expression of CCL25 and CCL28 may suggest that the small intestine has certain unique characteristics within the common mucosal immune system. Interestingly, CCL28 has a dual role in mucosal immunity. It is a chemokine with broad-spectrum anti-microbial activity against Candida albicans and various Gram-positive and Gram-negative bacteria. It is secreted to low-salt body fluids as saliva and therefore can play an important role as a first-line defense mechanism (53).

Other molecules involved

Besides the molecules responsible for the organ-specific homing and localization of lymphocytes to mucosal tissues, there are many other molecules without any clear tissue tropism that are needed to allow a lymphocyte to enter a tissue anywhere in the body. The most important ones of these molecules are described briefly below, emphasizing their contribution to lymphocyte homing to mucosal sites (Fig. 4, Table 1).

Leukocyte function-associated antigen-1 (LFA-1) (CD11a/CD18) and its Ig superfamily ligand intercellular adhesion molecule-1 (ICAM-1) (CD54) mediate firm attachment and transmigration of lymphocytes to lymphoid organs, including mucosa-associated lymphoid tissues (17). Development of the mucosal immune system is affected in animals even with partial loss of expression of ICAM-1 or CD18. Normal expression of CD18 integrins and ICAM-1 is required for the development of the CD8(αβ) TCR(αβ) compartments in lamina propria and epithelium and for the generation of normal Peyer’s patch lymphocyte populations (54, 55).

Inflammation-inducible selectins, P-selectin (CD62P) and E-selectin (CD62E), together with their counter-receptors P-selectin glycoprotein ligand-1 (PSGL-1) (CD162) and E-selectin ligand-1 (ESL-1) mediate leukocyte tethering and rolling on vasculature throughout the body (23). However, experimental evidence supports the idea that P-selectin also contributes to the physiological lymphocyte migration to the intestine, because mice lacking P-selectin have slight aberrations in their intestinal T-cell numbers. P-selectin-deficient mice have 20–30% higher proportions of αβ+ T cells and a concomitant decrease in γδ+ T cells in the intraepithelial compartment. Despite these changes within the mucosal immune system of mutant mice, their resistance against oral infection with Listeria monocytogenes was apparently unimpaired (55).

The principal hyaluronan receptor CD44 mediates lymphocyte binding to mucosal HEV (56). Recent studies have shown that CD44 on activated T cells can initiate contact and mediate rolling on hyaluronan on endothelial cells. Moreover, CD44 can form a bimolecular complex with α4β1 integrin (CD49d/CD29) in this rolling interaction allowing subsequent firm adhesion to vascular cellular adhesion molecule-1 (VCAM-1) (CD106), which is the counter-receptor of α4β1 (57). The role of CD44 in leukocyte trafficking in normal conditions seems to be dispensable, because no obvious mucosal phenotype has been reported in mice deficient in CD44 when unchallenged.

Vascular adhesion protein-1 (VAP-1) is a molecule possessing both adhesive and enzymatic properties. In most organs, it is stored within intracellular vesicles, from which it is translocated to the endothelial cell surface on inflammatory stimuli (58). Recent studies with VAP-1-deficient mice suggest that it also plays a role in physiological lymphocyte trafficking to Peyer’s patches and is involved in rolling, adhesion, and transmigration steps during lymphocyte extravasation (59).

Chemokines CCL21 and CCL19 are presented on HEVs in Peyer’s patches, where they attract and activate CCR7+ lymphocytes (51). Interestingly, the expression of these chemokines is segmented, concentrating to interfollicular areas, where they seem to preferentially guide the homing of T cells. In contrast, B cells leave the blood within follicles or in close vicinity to them (60). Contribution of CCR7 and its ligands, CCL21 and CCL19, is evident in mutant plt/plt mice, which lack CCL21 and CCL19, as well as in CCR7-knockout animals. These mice show severely impaired T-cell homing to Peyer’s patches, while homing of B cells is less affected (60–62).

The major chemoattractants for B cells are CXCL12 that is displayed broadly on HEVs and CXCL13 that is found selectively on Peyer’s patch follicular HEVs in the gut (63).
In addition to cell entry, these chemokines are also important in the organization of germinal centers, as CXCL13 directs the traffic of CXCR5⁺ lymphocytes to the light zone and CXCL12 guides CXCR4⁺ cells to the dark zone (64). This activity was demonstrated in knockout mice, in which CXCR4-deficient cells were excluded from the dark zone when transferred to wildtype mice and deficiency in CXCL13 was associated with aberrant light-zone localization of lymphocytes (64). Moreover, CXCR4 deficiency leads to the formation of aberrant B-cell follicles ectopically in intestinal lamina propria around Peyer’s patches (65) and mice lacking CXCR5 show reduced number of Peyer’s patches and B cells derived from CXCR5-deficient mice remain trapped in the T-cell–rich zones (66–68). Thus, CXCR4 and CXCR5 seem to be involved in controlling lymphocyte migration in more than one checkpoint.

Several molecules have been implicated to be important in transmigration of leukocytes through endothelial cell junctions in vessels with tight junctions (11, 29, 30). In this aspect, the mucosal vessels may function similarly to vessels in other lymphoid organs. Transmigration proceeds by sequential homotypic interactions between lymphocyte and endothelial CD31, CD99, JAM-A, and JAM-C. In addition, endothelial JAM-A can use LFA-1, JAM-C may utilize Mac-1 (CD11b/CD18), and JAM-B uses α4β1 as their leukocyte counter-receptors (reviewed in 11). The well-accepted dogma in the field has been that leukocytes transmigrate via endothelial cell junctions. However, contradictory opinions have been presented throughout the years (reviewed in 21), and recent reports unambiguously show that lymphocytes can also transmigrate through the endothelial cell body (69, 70). Diapedesis seems to take place by a novel ‘cup-like’ transmigratory structure. This endothelial structure is composed of ICAM-1- and VCAM-1-enriched vertical microvilli-like projections that surround transmigrating leukocytes. The transmigratory cup may be essential in guiding the transmigrating cell to the right migratory path through the cell (69).

**Imprinting of the organ specificity**

Although the existence of organ-specific homing routes has been known more than 40 years and certain molecules responsible for tissue-specific trafficking for more than 20 years, the regulatory elements behind the imprinting of the homing properties have remained a mystery until very recently. During the last 2–3 years, the importance of the local microenvironment and a critical role of DCs in imprinting of the migratory phenotype to T cells have been convincingly demonstrated by several groups (71–74). The experimental set-ups have utilized the known dichotomy between skin- and gut-homing lymphocytes (see above). The results demonstrate that the imprinting takes place in local lymph nodes relatively quickly after immunization, because within 2 days of systemic immunization, CD4⁺ T cells activated in lymph nodes draining the skin upregulate P-selectin ligands and downregulate α4β7, while those responding to the antigen in intestinal lymph nodes start to express high levels of α4β7 and to respond effectively to CCL25 (71). Further studies have shown that DCs are major players in this phenotypic transformation, because isolated DCs either from Peyer’s patches (PP-DCs) or from MLNs (MLN-DCs) are much more efficient in upregulating the expression of α4β7 and CCR9 on the surface of CD8⁺ T cells than DCs isolated from PLNs (PLN-DCs) or spleen. Contrary to mucosal DCs, PLN-DCs upregulate the selectin ligands, especially E-selectin on T cells (73–75). Consistent with the activated phenotype, the CD8⁺ T cells activated with PP-DCs home significantly better to MLNs than those co-cultured with PLN-DCs, which preferentially home to PLNs and inflamed skin (73–75).

As there are several different subsets of DCs in different organs, an interesting question is whether any specific subtype is responsible for imprinting of the tissue specificity. So far, the analyses indicate that all PP-DC subtypes induce higher levels of α4β7 than the corresponding PLN-DC subsets, but the CD11b⁺ DC fraction is the most effective inducer of α4β7 (76). Among MLN-DC subpopulations, both CD8α⁺ and CD8α⁻ DCs stimulated T cells to express CCR9 and α4β7 and to downregulate L-selectin (74).

The factors, mediators, and triggers responsible for differential imprinting have been searched for among the surface molecules and T-helper 1 (Th1)/Th2 cytokines. Compared with PLN-DCs, PP-DCs express higher levels of many surface molecules, such as αβ, costimulatory molecules, B7-H1/PDL1, and B7-DC/PDL2, but blocking studies with monoclonal antibodies have excluded the involvement of these molecules in imprinting. Contributions of MAdCAM-1, OX-40L, Qa2, CCL25, interleukin-10 receptor (IL-10R), pertussis toxin, and transforming growth factor-β have been ruled out. Moreover, although Th1 and Th2 cytokines can influence the expression of homing-associated molecules on T cells, neither IL-4 nor IL-12 seems to be responsible for imprinting (76). The activation of T cells, however, seems to be a prerequisite for the induction of gut-homing phenotype (76).

A recent report demonstrates the first substance contributing to the imprinting of gut-homing phenotype. Incubation of T cells with a vitamin A metabolite, retinoic acid, enhances the
expression of α4β7 and CCR9 on T cells on activation and imprints them with the gut tropism. In line with this, vitamin A deficiency causes a reduction in α4β7+ memory and effector cells in lymphoid organs and depletion of T cells from the intestinal lamina propria (77).

As dynamic responsiveness is one of the characteristics of a well-working immune defense, a fundamental question is whether imprinting is irreversible. Recent studies demonstrate that imprinting is indeed reversible, and the T cells can be re-educated by different types of DCs to display new homing properties. This ability guarantees effective relocation of memory T cells in the case that their action is needed at a site different from the original site of imprinting (76).

The imprinting studies have concentrated on T cells, but in vivo animal and human studies suggest that comparable education may take place also in B cell–DC interactions within follicles. In human vaccination studies with Salmonella, the oral vaccination route produces significantly more α4β7+ and less L-selectin+ antibody-secreting cells (ASCs) than the parenteral route of immunization (78). Interestingly, re-immunization via the oral route induces L-selectin onto α4β7-expressing cells making them ‘dual specific’ (78). Based on these findings, the B cells producing specific antibody against Salmonella obtain further imprinting after the re-immunization via unknown mechanisms. Most likely, the ‘improved’ phenotype after re-immunization allows the ASC to distribute more effectively throughout the body than after the primary activation.

It may also be possible to educate DCs to imprint the desired homing properties to T cells. Recent studies show that the route of injection determines which type of homing properties the DCs are able to imprint. Bone marrow-derived DCs injected intracutaneously imprint skin-homing potential to T cells, whereas the same cells given via the intraperitoneal route induce T cells with the gut-homing phenotype (75).

Mucosal homing in inflammation

On inflammation, multiple changes occur in the mucosal vasculature. Concomitantly with the altered hemodynamics and permeability, the local microvascular endothelial cells become activated (79). This is manifested as the appearance or induction of many endothelial adhesion molecules, including E-selectin, P-selectin, MAdCAM-1, ICAM-1, VAP-1, and VCAM-1 (31, 80). Upregulation of each of these molecules has typical kinetics. For instance, luminal P-selectin is seen within seconds after exposure to certain pro-inflammatory stimuli, whereas upregulation of ICAM-1 typically starts after several hours. In inflammatory bowel diseases, PNAd carbohydrates appear in vessels of lamina propria and increased numbers of L-selectin+ cells enter the gut (81). In addition to adhesion molecules, the chemokine pattern also changes in inflammation, along with chemokine receptor profiles on lymphocytes (82, reviewed in 83). For example, in small bowel Crohn’s disease expression of CCR9 by lamina propria lymphocytes is reduced, and CCL25 disappears from the endothelial cells and has a patchy expression profile in intestinal crypts (52). Moreover, there is a 30-fold increase in CCR2+ lamina propria lymphocytes in this disease (84).

The changes in adhesion and activation molecules can partially explain the typical appearance of different leukocyte subsets in inflamed gut (31). Soon after the inflammatory stimulus, granulocytes start to enter the affected tissue. Later on, markedly increased flux of lymphocytes and monocytes is observed. During a chronic inflammatory stimulus, the endothelial changes are more aggravated, and massive influx of inflammatory cells ensues. The aberrant expression of adhesion and activation molecules in inflammation can explain why the dichotomy between the peripheral and mucosal recirculation routes is compromised in inflammation (81). The signals that eventually cause the resolution of the inflammatory phenotype remain poorly characterized. However, there is evidence that this process is dependent on the downregulation of pro-inflammatory signals and that it may involve active anti-inflammatory signals (85).

Recirculation routes and extra-intestinal manifestations of mucosal inflammation

Lymphocyte migration pathways may provide an explanation for the common association of mucosal inflammations and infections with secondary inflammatory lesions in anatomically distinct organs and tissues. Inflammatory bowel diseases (IBDs) are often complicated by reactive arthritis, uveitis, and skin and liver inflammation (86). For instance, about two-thirds of spondylarthropathy patients show histological signs of gut inflammation. It has been shown that mucosal immunoblasts have the unexpected property of binding to inflamed synovial vessels (but not inflamed PLN vessels) (56, 87). This finding implies that these cells have a potential to migrate either back to the mucosa or aberrantly to the synovium. If the triggering antigen is present in the synovium, and it has activated the synovial microvasculature, the preactivated gut-originated cells would then be able to immigrate into the synovial tissue and trigger the inflammatory circle. For instance, on contact with endothelial cells, monocytes fed with bacteria can trigger

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P-selectin expression (88). P-selectin appears to play a very central role in allowing endothelial adherence of gut-originated mononuclear cells (89, 90), which can take the triggering antigen with them into the synovial tissue. The vasculature of the inflamed synovium then starts to proliferate and express multiple endothelial adhesion molecules, which can mediate binding of mucosal immunoblasts isolated from IBD patients.

The connection between gut and liver inflammation is also intriguing (91). IBD can be complicated with primary sclerosing cholangitis (PSC), even years after the colon has been removed. Moreover, colonic inflammation can develop for the first time after a liver transplantation has been performed for PSC. These findings have led to the idea of enterohepatic recirculation (91). According to this hypothesis, long-living memory cells that have been originally activated in the gut can recirculate to liver. Under normal conditions, this circulation would allow the hepatic immune system to respond to gut-derived antigens that enter the liver via the portal tract. These cells then harbor the potential of triggering inflammation under suitable activation conditions. Evidence that supports this concept is rapidly accumulating. First, there are certain endothelial molecules that show overlapping expression patterns in the liver and gut. VAP-1, for instance, is brightly expressed in human liver and in inflamed gut (92, 93). Even more uniquely, MadCAM-1, which was long thought to be mucosa specific, is induced in the portal endothelium of liver in the inflammation associated with IBD (94). These vascular adhesion molecules have been shown to be functional at both anatomical sites in supporting lymphocyte adhesion, and hepatic lymphocytes express α4β7 receptor for MadCAM-1 (94, 95). In the absence of endothelial selectins in liver, VAP-1 appears to be able to account for lymphocyte rolling on the sinusoidal endothelium (95). Moreover, CCL21, which has been suggested to be selectively expressed only in secondary lymphoid tissues, has been found on the vascular endothelium in portal-associated lymphoid tissue in liver (91). The gut-associated CCL25 is expressed in PSC liver, and it activates α4β7 on CCR9+ gut-homing lymphocytes to bind MadCAM-1 on the hepatic endothelium (96). The aberrant expression of homing-associated molecules may explain, in part, the molecular basis of enterohepatic recirculation.

**Mucosal inflammation and anti-homing receptor therapy**

The finding that α4β7–MadCAM-1 pathway is central for mucosal homing has raised enthusiasm in targeting these molecules as a novel form of precision anti-inflammatory therapy. Several animal models have indeed shown promising results. In a cotton-top tamarin model, an anti-α4 antibody markedly improved the clinical and histological scores in the colitis that resembles human IBD (97). As α4 can pair with either β1 or β7, it has been rational to target specifically the mucosal homing receptor α4β7 with antibodies that only recognize this heterodimer. Anti-α4β7 therapy markedly improved colitis within 3 days in the treated animals (98). α4β7 and anti-MadCAM-1 monoclonal antibodies have also been effective in reducing intestinal inflammation in a model of adoptive lymphocyte transfer in severe combined immunodeficiency (scid) mice (99).

A humanized anti-α4-integrin antibody (natalizumab) has been tested in several clinical studies for treatment of Crohn’s disease. In a large phase III trial, two intravenous boluses of anti-α4 monoclonal antibody at 1-month intervals resulted in significantly improved remission and clinical response (100). As predictable in chronic inflammation, the beneficial responses were unfortunately quite short lived after the cessation of the injections. Anti-α4 treatment caused lymphocytosis, suggesting that it targets lymphocyte homing. However, at this stage, the contribution of α4 to matrix adhesion and lymphocyte proliferation cannot be excluded. The treatment has appeared to be safe in a large group of patients, but two recent and very serious adverse effects in patients receiving natalizumab in combination with interferon-β for the treatment of multiple sclerosis necessitates close re-examination of this issue (http://www.fda.gov/cder/drug/advisory/natalizumab.htm).

Humanized anti-α4β7 monoclonal antibody has been analyzed in phase II studies in ulcerative colitis and found to be useful in alleviating the inflammation (101, 102). However, in Crohn’s disease, there was no apparent benefit of this antibody (103).

Neutralizing anti-ICAM-1 antibodies and ICAM-1 anti-sense oligonucleotides have also been successfully used in animal models of IBD (104–106). In humans, the initial enthusiasm generated by successful small trials of treating Crohn’s disease with ICAM-1 anti-sense oligonucleotides (107) has been fading away after two larger studies that could not demonstrate marked effects (108, 109).

Importantly, however, the α4 data in Crohn’s disease and multiple sclerosis show for the first time that targeting of lymphocyte homing receptors with monoclonal antibodies can yield clinically useful responses. Therefore, these findings are expected to boost similar studies with other adhesion molecules that are relatively specific to a particular homing route.
Lymphocyte homing to the gut (111). These cells then drain to the subcapsular situation may be even more complex depending on in vivo and in vitro have naive B- and T-cell phenotype, and they express low levels of α4β7, whereas the memory cells are generally α4β7high and mostly L-selectin− (111). These cells then drain to the subcapsular sinus of the MLN, and they can immigrate into the lymphoid stroma through the wall of afferent lymphatic cords. After further maturation, the lymphocytes again leave the organized lymphoid tissue by entering lymphatic sinusoids that coalesce into larger sinuses and eventually exit the lymph node as the efferent lymphatic vessel at the hilus of the node. When released into the systemic circulation, the memory and effector cells (about 10% of all cells in mesenteric lymph) are expected to recirculate into distant intestinal effector sites (111). Some molecules involved in lymphocyte migration into the lymphatics have been elucidated recently. CCL21 is expressed in lymphatic endothelial cells (112), and it may be involved in attracting cells into this type of vasculature. This hypothesis is supported by the findings that in CCL21-deficient animals, the exit of DCs from peripheral organs (skin) is compromised (61). Furthermore, certain adhesion molecules such as macrophage mannose receptor and common lymphatic endothelial and vascular endothelial receptor-1 (CLEVER-1) can support lymphocyte binding to lymphatic endothelium in vitro assays (113, 114). Sphingosine-1-phosphate receptor type 1, is also crucial for lymphocyte exit from the lymphatic tissue into the lymphatic vasculature. In the absence of this molecule, lymphocytes can extravasate relatively normally. However, they are retained in the lymphatic tissue in abnormally high numbers, and the draining lymphatic sinuses appear empty (115, 116). Further elucidation of these mechanisms will allow dissection of the exit arm of lymphocyte recirculation, which is exactly as important for the normal immune homeostasis as the entry step.

Vaccination and mucosal homing
Migratory pathways of immune cells have great impact on the distribution of immune responses after vaccination. Analysis of the homing of plasma cells after topical application of antigen has been helpful to delineate the tissue-selective homing (51). In general, IgA ASCs isolated from gut-associated lymphoid tissue can populate many mucosal effector sites, including the intestine, respiratory and urogenital tracts, as well as mammary and salivary glands (8, 32, 35, 78). IgA-secreting plasma cell precursors isolated from the respiratory system or nasopharyngeal area, in contrast, show only low level of migration to the intestine. IgG ASC precursors, even if isolated from gut-associated lymph nodes, also home poorly to mucosal tissues and preferentially migrate to other lymphoid tissues.

Most IgA ASCs induced in intestinal lymphoid tissues express high levels of α4β7 (117). Both IgA and IgG ASCs triggered via systemic sites express low levels of α4β7 and high levels of L-selectin, and they can express α4β1 (118, 119). Thus, at least differential interactions between α4β7 and α4β1 with MadCAM-1 (mainly expressed in intestinal mucosa, and further upregulated by mucosal vaccination) and VCAM-1 (expressed in non-intestinal mucosa) may partially contribute to the different homing selectivity. However, the in vivo situation may be even more complex depending on the re-encounter of the antigen. When orally immunized volunteers were re-immunized either orally or parenterally, most of the ASCs remained highly α4β7+, but almost all subjects started to express L-selectin as well (78). These data suggest that re-stimulation of orally induced ASCs may allow them to home to either mucosal or systemic sites.

Tissue-selective function of chemokines is probably equally important in determining the homing routes of ASCs. Thus, the expression of CCR9, CCR10, and CXCR4 on IgA ASCs induced in small intestine allows these cells to interact with the corresponding ligands expressed in the intestine and other mucosal sites (120, 121). In contrast, the cells induced by a respiratory antigen lack CCR9 expression that hampers their homing to gut (120, 122). IgG ASCs express CXCR3 and CXCR4 (and lack CCR9) and are therefore targeted to non-mucosal sites, such as areas of peripheral inflammation and bone marrow (123–125). This knowledge together with recent results regarding imprinting may prove clinically relevant when designing most optimal immunization routes.

Concluding remarks
The molecular mechanisms of lymphocyte homing to gut have been successfully unraveled during the last decades. The signals involved in attraction, adhesion, and, most recently, imprinting are beginning to be understood. This knowledge has also been utilized in the development of
more efficient anti-inflammatory regimens and vaccines. Nevertheless, we are still facing substantial challenges. The mucosa-selective homing of various lymphocytes compellingly documented in early in vivo studies remains partly unexplained in molecular terms. The function of each adhesion and activation molecule appears to become more and more tissue non-selective as it is dissected in greater detail. Therefore, it appears that the tissue specificity of mucosal homing may indeed be understandable only through appreciation of the combinatorial use of different molecules during the multistep adhesion cascade. Unfortunately, this complexity makes it inherently more difficult to target leukocyte traffic into the intestine. Also, the homing of various leukocyte types into the mucosa is not well studied. Most of the imprinting studies, for instance, have been performed with CD8⁺ T cells. Therefore, it remains to be seen whether the homing results can be generalized to other cell populations (e.g. CD4⁺ T cells, B cells, and IELs) and to cells at different activation stages. Moreover, there is an urgent need to understand the lymphatic exit step of the leukocyte traffic in gut that is absolutely essential for the regulation of immune cell homeostasis in this immunologically highly active organ. Finally, the effect of superimposed gut inflammation (altered normal microbiota, allergens, infections, IBD, vaccination, etc.) on leukocyte trafficking into the gut and other mucosal tissues may still dramatically complicate our view on the cellular traffic into and out of the gut. Nevertheless, the complexity of leukocyte traffic into gut will at the same time guarantee that it will remain in the spotlight for new discoveries and applications in the field of leukocyte homing.

References


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