Homing of Immune Cells: Role in Homeostasis and Intestinal Inflammation

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Abstract: Rather like a satellite navigation system directing a vehicle to a particular destination defined by post-code, immune cells have homing molecules or "immune post-codes" enabling them to be recruited to specific organs, such as the intestine or skin. An efficient system would be designed such that the site of entry of an antigen influences the homing of effector T cells back to the appropriate organ. For example, to mount an immune response against an intestinal pathogen, T cells with a propensity to home to the gut to clear the infection would be induced. In health, there is such a sophisticated and finely tuned system in operation, enabling an appropriate balance of immune activity in different anatomical compartments. In disease states such as inflammatory bowel disease (IBD), which is characterized by intestinal inflammation and often an inflammatory process involving other organs such as skin, joints, liver, and eye, there is accumulating evidence that there is malfunction of this immune cell trafficking system. The clinical importance of dysregulated immune cell trafficking in IBD is reflected in recently proven efficacious therapies that target trafficking pathways such as natalizumab, an α4 integrin antibody, and Traficet-EN, a chemokine receptor-9 (CCR9) antagonist. Here we review the mechanisms involved in the homing of immune cells to different tissues, in particular the intestine, and focus on alterations in immune cell homing pathways in IBD. Unraveling the mechanisms underlying the immune post-code system would assist in achieving the goal of tissue-specific immunotherapy.

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TROPISM OF T LYMPHOCYTES TO THE INTESTINE

Lymphocytes continuously migrate around the body, optimizing their chance of encountering an antigen. Every day nearly a trillion lymphocytes leave the circulation and enter the tissues, before reentering the blood. Such a massive migration needs to be tightly controlled. Lymphocyte subsets express unique patterns of homing molecules and the various types of vascular endothelium in different tissues express specific ligands, enabling migrating lymphocytes to be guided to their target tissue via site-specific pathways. A particular combination of homing factors specifically targets antigen-experienced immune cells to different tissues expressing the respective ligands.

Naïve T cells express homing receptors that allow them to migrate to lymphoid organs like lymph nodes, but they are usually excluded from peripheral tissues. Once T cells have become activated by antigen, they change their pattern of homing receptors and can migrate to peripheral sites. There is a specialized stepwise cascade of events that occurs allowing circulating lymphocytes to gain access to the tissues. After initial rolling and tethering with transient interactions between lymphocytes and endothelial cells, lymphocytes are activated and then increase their adhesiveness, allowing them to attach firmly to endothelial cells before migrating across the endothelium.[1] Lymphocyte migration into tissue depends on regulation at each of these steps. In particular, lymphocyte migration is dependent on the ability of lymphocytes to interact with the endothelium of postcapillary venules through a multistep process governed by three prominent families of proteins: selectins, integrins, and chemoattractants. This process involves the capturing or "tethering" of lymphocytes by the endothelial cells after which lymphocytes "roll" over the endothelium and are "activated," usually mediated by several chemoattractants presented on the endothelium. This mechanism subsequently would allow endothelial cells of the postcapillary venules to "arrest" and "stick" lymphocytes on their surface to guide them for extravasation into the target tissue.[2,3]

In the intestine lymphocytes activated in mesenteric lymph nodes have a propensity to home back to the intestine. The molecules on lymphocytes which enable homing to the intestine include the integrin $\alpha4\beta7$, which is attracted

to its ligand mucosal addressin cell adhesion molecule-1 (MAdCAM-1), on high endothelial venules in the intestine, and the chemokine receptor CCR9 attracted to its ligand CCL-25 (TECK).[4,5]

Several lines of evidence support a role for the integrin $\alpha4\beta7$ and its ligand MAdCAM-1 in mediating homing of lymphocytes to the gut, since MAdCAM-1 is expressed on intestinal lamina propria venules.[6] First, $\beta7$-deficient mice have severely impaired formation of their gut-associated lymphoid tissue throughout the gastrointestinal tract, with decreased T and B cells present,[7] and $\beta7$-deficient cells are impaired from entering the intestine.[8] Absence of $\beta7$ causes a defect in the transition from lymphocyte rolling to firm adhesion in the high endothelial venules in the intestine. Second, antibodies to $\alpha4$ and $\beta7$ integrins reduce homing of lymphocytes to the murine intestine and inhibit binding of lymphocytes to MAdCAM-1.[9] In addition, natalizumab (an antibody against the $\alpha4$ integrin) has been successfully used for the treatment of Crohn's disease (CD) by inhibiting the migration of effector T cells into the gut.[10] Third, reactivity to the intestinal pathogen, rotavirus, is particularly seen in the "gut homing" $\alpha4\beta7+$ subset of circulating memory T cells and, in adoptive transfer models, this subset of cells clears rotavirus infection, implying that priming to an intestinal antigen occurs in the gut and circulating memory T cells return to the gut to deal with the antigen.[11,12] In contrast, antigens introduced by other routes, for example, the skin, do not cause an enriched response in the "gut homing" subset of memory T cells, but preferentially prime effector T cells with "skin homing" receptors.[13]

Similar lines of evidence support a role for CCR9 and its ligand CCL-25/TECK in the homing of lymphocytes to the intestine. CCR9 deficiency or CCL25/TECK blockade reduces the number of antigen-specific lymphocytes in the small intestine.[14–17] CCR9 is expressed at high levels on lamina propria mononuclear cells in the small intestine[5] and its ligand CCL25/TECK is expressed in both lamina propria venules and small intestine enterocytes.[18,19]

TROPHISM TO DIFFERENT ANATOMICAL COMPARTMENTS OF THE INTESTINE

The pathways involved in recruitment of lymphocytes to different anatomical parts of the intestinal tract are different. For example, differences exist in expression of homing molecules and their ligands in the small intestine compared with the large intestine. In the small intestine both $\alpha4\beta7$/MAdCAM-1 and CCR9/CCL25/TECK pathways are involved in migration of lymphocytes. Ligation of CCR9 by CCL25/TECK results in conformational changes in $\alpha4\beta7$ integrins and allows firm adhesion of $\alpha4\beta7$ integrins to MAdCAM-1. In contrast, in the colon expression of the chemokine receptor CCR9 and its ligand CCL25/TECK is limited. Only a small subset of cells in the colon expresses CCR9 and the level of expression is lower than in the small intestine.[18,19] Furthermore, the ligand for CCR9, CCL25/TECK is not expressed in the colon.[19] Functionally, while blockade of CCL25/TECK or CCR9 desensitization prevents homing of T cells to the small intestine, there is no effect on migration of cells into the colon, and in vitro CCL25/TECK is selectively chemotactic for small bowel but not colonic lamina propria mononuclear cells.[19] This implies a restricted and limited role for CCR9 and its ligand CCL25/TECK in the large intestine.

Further underlying anatomical compartmentalization, there are differences in pathways involved in recruitment of lymphocytes in different parts of the small intestine. There is a gradient of expression of CCL25/TECK on epithelial cells, with the highest expression in the proximal small intestine, lower expression in the ileum, and no detectable expression in the colon.[20] In the proximal small intestine (duodenum and jejunum) homing of T cells is more dependent on CCL25/TECK-CCR9 interaction compared with homing into the distal ileum.[21] There is no evidence of differential expression of MAdCAM-1 along the axis of the small intestine but MAdCAM-1 is detected in small intestine and expression is greater than expression in colonic tissue.

The molecules that control the immune post-code system not only have a role in targeting the tissue of migration, but also on controlling the type of cell that migrates there. CCL27/CTACK is highly expressed by colonic epithelial cells[23] (in addition to skin keratinocytes in the epidermis[22]) but its chemokine receptor CCR10 has not been identified on intestinal T cells, but has been identified on Ig-A secreting B cells migrating to the colonic lamina propria.[24] Therefore, B and T cells use differential homing markers.[2,3] These different mechanisms of different leukocyte population recruitment may permit functional specialization of immune responses in different segments of the gastrointestinal tract.

TROPISM OF LYMPHOCYTES TO OTHER ORGANS

Specific homing pathways exist for other organs.[3] T-cell stimulation by skin antigens induces effector T cells expressing skin homing receptors such as P- and E-selectin and the chemokine receptors CCR4, CCR8, and/or CCR10.[25,26] However, recent evidence suggests that CCR10 may be a "general" mucosal homing receptor,[27] which might be more related to retaining T cells in the skin after they have reached it, instead of governing their migration.[2]

These effector T cells trafficking to the skin are devoid of the gut homing molecules $\alpha4\beta7$ and CCR9. Conversely, gut tropic cells do not have skin-homing molecules. Compartmentalization has also been observed for the mucosal surface in the lungs, joints, and the central

nervous system, although less is known about the pathways involved.

ROLE OF DENDRITIC CELLS IN THE HOMING OF LYMPHOCYTES

In the intestine, lamina propria dendritic cells (LPDCs) make tight junctions with epithelial cells and sample luminal antigens by extending their dendrites into the gut lumen. In this way, DC are in touch with constituents of the intestinal microbiota and potential pathogens. In addition to constitutive trafficking of DC to the draining lymph node in the presence of danger signals such as pathogens or cytokines, DC upregulate molecules such as CCR7 and move to the draining lymph node. Here DC present antigens to naïve T cells and are unique among antigen-presenting cells in their ability to do this. Antigen-bearing DC are therefore perfectly placed to "inform" T cells of the tissue and environment in which the DC encountered the antigen.

Work from our laboratory elegantly demonstrated in mice that DC from mesenteric lymph nodes, but not from peripheral lymph nodes, induced the gut homing receptor $\alpha4\beta7$ on T cells, thus enabling T cells to home back to the intestine and access the anatomical site most likely to contain their cognate antigen.[28] Others have shown that intestinal DC from Peyer's patches, mesenteric lymph nodes, and the lamina propria induce $\alpha4\beta7$ and CCR9 expression on T cells.[16,29,30] It was demonstrated in vivo that DC from intestinal tissue, but not DC from other tissues, were able to educate T lymphocytes to traffic selectively to the gut. In contrast, activation of the same T cells by DC from lymph nodes draining the skin did not express these gut-homing molecules but instead expressed skin-homing molecules (P- and E-selectin).[29] These studies show that in addition to the central role of DC in regulating immunogenic versus tolerogenic immune responses, they are crucial in the generation of tissue trophic effector T lymphocytes.

The mechanisms by which DC imprint homing molecules are becoming clearer, and appear to involve fat-soluble vitamins, like vitamin A and vitamin D, which have well-established roles in regulation of immunity. While retinoic acid (RA), which can be generated by intestinal DC through the metabolism of vitamin A, appears to induce expression of intestinal homing markers on T cells,[31] vitamin D promotes development of DC that stimulate T cells to express skin-homing markers.[22] In addition to endogenous production of RA, vitamin A is derived from the diet. Liver, butter, eggs, cheese, and orange fruit and vegetables are rich sources of vitamin A. Vitamin A from foods of animal origin is absorbed in the intestine as retinol, before being converted to retinal or RA, whereas vitamin A from fruit and vegetables is often in the form of beta-carotene, which is converted into retinol.

Although other soluble factors are likely to contribute to the homeostatic function of gut DC and oral tolerance, vitamin A appears to elicit a central role. RA is core in determining the tissue-specific properties of murine intestinal DC and the induction of the oral tolerance; the upregulation of gut-homing receptors on lymphocytes, generation of tolerogenic Treg cells, and the induction of B-cell class switching to IgA-producing cells are all dependent on RA.[32–35] Iwata et al[31] showed that exposure of T cells to RA led to induction of $\alpha4\beta7$ and CCR9 on responding T cells (and a concomitant suppression of the skin-homing molecules E- and P-selectin) and impressed upon T cells the ability to migrate to the intestine, thereby contributing to compartmentalization of the immune response. These effects were reproduced by a synthetic agonist of RA and were partially inhibited with the retinaldehyde dehydrogenase (RALDH) inhibitor Citral and a pan-RAR antagonist. The physiological importance of RA in achieving intestinal tropism was demonstrated in vitamin A-deficient mice, which contained reduced numbers of effector T cells in the gut lamina propria, whereas the numbers of T cells in other anatomical compartments, including the lung or liver, was unaffected. Intestinal DC, but not DC from other sites, expressed the prerequisite enzymes, RALDHs,[36] for conversion of vitamin A to RA and inhibitors of these enzymes prevented induction of $\alpha4\beta7$ on T cells. The exact subpopulation of DC that produced RA is not clear, but it has been reported in mice that CD103(αE)+ DC in the intestine are specially equipped for producing RA and promote expression of CCR9 on T cells.[30] Thus, intestinal CD103+ DC, but not DC at other anatomical sites or intestinal CD103− DC, express key enzymes involved in the oxidative metabolism of retinol and can thereby generate immunoregulatory all-trans RA from dietary precursors.

The ability to induce CCR9 on T cells is not a property of all $CD103^+$ DCs (which are present in many mucosal tissues and lymph nodes) but seems to be a selective property of mesenteric lymph node and small intestinal LP $CD103^+$ DC.[37] Mesenteric lymph nodes and small intestinal LP $CD103^+$ DC efficiently induce enhanced RA receptor signaling in T cells and generate CCR9+$\alpha4\beta7$+ gut tropic T cells in vitro, indicating that they selectively have this ability in the small intestinal mucosa. The nature of these imprinting signals is currently unknown.

However, as mentioned earlier, RA may not be the only factor that regulates expression of gut-homing molecules; other factors are likely to contribute. While RA induced expression of both CCR9 and $\alpha4\beta7$, expression of these two homing molecules is not always linked, as is the case with T cells infiltrating the colonic mucosa, which are $\alpha4\beta7$ high but CCR9−, implying that other factors are involved.

Whereas dietary RA upregulates gut-homing molecules and simultaneously inhibits induction of skin-homing

molecules, another fat-soluble vitamin, vitamin D, which is naturally generated in the skin, appears to have the opposite effect on induction of tissue-homing molecules. 1,25(OH)(2)D(3), the active form of vitamin D3, signals T cells to express CC chemokine receptor 10, which enables them to migrate to the skin-specific chemokine CCL27, while suppressing the gut-homing receptors $\alpha4\beta7$ and CCR9.[22]

There is evidence for compartmentalization of DC themselves, since DC express homing molecules. Fresh circulating DC express both gut ($\beta7$) and skin (CLA) homing molecules. On the contrary, tissue-specific DC have lost their multipotential homing phenotype by the time they elicit specific tissue-homing properties in stimulated T-cells (Mann et al, unpubl. data). It is likely that DC acquire more specific homing characteristics following encounters with antigen or other stimuli such as cytokines or microbial products. This distribution may underlie the "knock-on" effect of DC on the T cells that they stimulate. Others have shown that CCR9 is a homing receptor for a subset of DC, plasmacytoid DC, enabling migration to the small intestine.[38] CCR9 expression on plasmacytoid DC appears to define a physiologically important tolerogenic DC subset well positioned in lymphoid tissues to participate in homeostatic immune regulation.[39] A key question is how DC themselves are educated to acquire gut-homing potential. One possibility could be that many of the unique properties of gut DC appear to be a result of conditioning by the local microenvironment. Supporting this concept, the capacity to induce a homing profile on responding T cells upon antigen presentation by DC seems to be derived from the tissue microenvironment in which the antigen presentation takes places, as discussed below.[40] Toll-like receptor (TLR) signals, commensal bacteria, diet, or other environmental factors may be involved.

Recent evidence suggests that in addition to DC, stromal cells from the mesenteric lymph node but not peripheral lymph node also express RA-producing enzymes and support induction of the intestinal homing molecule CCR9 on activated T cells in vitro.[41]

PLASTICITY IN THE SYSTEM

The degree to which homing phenotypes are stably imprinted versus being dynamic, dependent on the environment, is a subject of debate. However, there is evidence to suggest that immune cells' migratory behavior displays plasticity. It has been shown that when DC are injected into the abdominal peritoneum, irrespective of whether they are derived from mesenteric lymph nodes or peripheral lymph nodes, they upregulate the gut-homing molecule $\alpha4\beta7$ on T cells.[42] Such work indicates a dominant role for the local tissue environment in shaping the mucosal imprinting capacity of DC. Such flexibility is well recognized for other functional aspects of DC. For example, it is seen in their ability to polarize Th1, Th2, or T-regulatory ability, depending on their exposure to particular cytokine and microbial signals.[43]

Plasticity of expression of homing molecules is demonstrated in the context of inflammation. The chemokine receptors CXCR3, CCR5, and CXCR4 are present on gut-homing lymphocytes particularly in states of inflammation. CXCR4 is widely expressed on leukocytes and is thought to have a role in retaining lymphocytes in inflamed tissue.

Therefore, after being conditioned by the local tissue microenvironment, DC would acquire on the one hand the local homing profile, and on the other the capacity to imprint that profile on responding cells in the draining lymph nodes. Thus, DC would be able to induce tissue-specific homing receptors and therefore pass the information about the tissue of origin in which T cells can find the antigen.[44]

GUT TISSUE MICROENVIRONMENT EDUCATES DC INTO HOMEOSTATIC DC

The differences in the homing profile of DC from different tissues and their differential functional properties may indicate the preexistence of functionally distinct homing-specific DC subsets; however, the multi-homing phenotype of blood DC supports the fact that the tissue microenvironment educates DC to acquire specific homing and differential functional properties. Supporting this possibility are mouse studies showing that tissue-specific DC transferred into a different tissue fail to induce their corresponding T-cell homing phenotype in the "foreign" microenvironment, but induced the homing profile of the new tissue.[42]

Conditioning DC in the supernatant (SN) from intestinal epithelial cell line monolayers results in the generation of tolerogenic DC with a release of IL-10 and IL-6, but not IL-12, directing T-cell polarization toward a noninflammatory Th_2 profile.[45] Conditioned DC had lower surface expression of MHC class II, CD80, and CD86 compared with their unconditioned counterparts, and were less stimulatory for allogeneic T cells. Conditioned DC reduced production of IL-12p70 but increased production of homeostatic cytokines IL-10 and TGF-β.[40] Bone marrow-derived murine DC conditioned in the presence of SN from intestinal epithelial cell line monolayers upregulated expression of CD103 and acquired the capacity to induce Treg cells with a gut-homing phenotype.[46] SN from freshly isolated human intestinal epithelial cells condition DC to become tolerogenic and drive the development of adaptive FoxP3 Tregs. Moreover, if monocytes were conditioned through the whole differentiation into monocyte derived DC, they upregulated CD103 expression.[47] Therefore, it seems plausible that once DC leave the blood and enter the gut

environment, in the steady state, they would be conditioned into tolerogenic gut DC responsible for maintaining gut homeostasis and oral tolerance.

Thus, gut DC (either from the MLN or the LP) have a central role in gut homeostasis and in mechanisms of oral tolerance.[35,48,49] Gut DC have a homeostatic phenotype characterized not only by expression of gut-homing markers, like CD103 and CCR9,[37,39,50] but also by reduced levels of costimulatory molecules and TLRs compared with their blood counterparts,[87] and gut DC have a higher production of IL-10 with a higher CCR7 expression. Therefore, in the absence of danger signals, gut DC would migrate to the lymph nodes with that homeostatic profile, where they contribute to the oral tolerance against gut nutrients and/or antigens from the commensal microflora.[51]

Supporting these roles of the tissue conditioning on DC, new evidence has emerged showing the gut lamina propria to have at least two different DC subsets, with differential homing profiles and immunological properties. They may control the immune system and help to modulate the response against autoantigens, either from the diet or the commensal microflora (homeostasis or oral tolerance) or trigger an immune response when required against harmful antigens. Thus, DC in the gut can be subdivided into tolerogenic gut-homing (CD103+, CCR7+, and CX_3CR1-) and proinflammatory (CD103−, CCR7−, CD11b+, CX_3CR1+, and CD14+) DC.[52–54] It seems plausible, therefore, that in the steady state condition newly arrived DC would be influenced by the gut tissue microenvironment to acquire a gut-homing phenotype with homeostatic properties that would make them responsible for the oral tolerance. On the contrary, upon the presence of danger signals, there would be new recruitment of monocytes and an "in situ" differentiation into DC,[52–55] which, in this inflammatory context, would trigger an immune response. Therefore, the different origins of gut DC may explain how the intestinal immune system has the capacity to destroy harmful pathogens (by intervention of newly recruited nongut-homing DC) while tolerating beneficial bacteria and nutrients (mediated by the conditioning of blood DC in the steady state condition into tolerogenic gut-homing DC).[56]

REGULATORY T CELLS DISPLAY TISSUE TROPISM

T cells with regulatory properties (Treg) play a central role in maintenance of immunological homeostasis and tolerance in the gut. Tregs are not a homogenous group of cells, but are subdivided into naïve-like subsets that preferentially recirculate through lymphoid tissue and effector/memory subsets that migrate into peripheral sites. Similar to other T cells, Tregs have homing receptors allowing migration into specific tissues. In particular, the gut-homing molecule $\alpha4\beta7$ is induced on Treg when exposed to DC from mesenteric lymph nodes but not from peripheral lymph nodes, and RA appears to be central to this induction of $\alpha4\beta7$ and homing to the intestine.[34,57] As with conventional T cells, the DC subset, CD103+ DC, is equipped for converting antigen-specific T cells into Treg cells.[58] The CD103+ DC subset displays an enhanced ability to generate Treg compared with their CD103− counterparts in vitro, and this ability is blocked with an RAR antagonist.[59,60] One difference between mouse and human Tregs is that RA alone induces expression of FoxP3 (a transcription factor that characterizes Tregs) in human cells but requires additional TGF-β to do the same in mouse T cells. RA also allows induction of FoxP3 in the presence of high levels of costimulation, which normally prevents FoxP3 induction, suggesting that RA can permit development of Tregs even in the presence of inflammation.[61] As part of intestinal immune homeostasis, production of RA by intestinal DC may help to target regulatory cells to intestinal tissue, limiting the activity of gut-homing effector populations. However, one recent study found that CD103+ DC induced Th17 T-cell differentiation in vitro that was also inhibited by addition of an RAR antagonist to the culture medium.[62] Addition of high concentrations of RA inhibited Th17 cell differentiation, whereas addition of low doses of RA promoted Th17 cell differentiation. Thus, DC-derived RA can act as a cofactor for the generation of FoxP3+ regulatory cells and, dependent upon the prevailing cytokine milieu and levels of RA signaling, influence the balance between the generation of regulatory cells and effector, Th17, cells. Thus, many similar factors influence the activation of "conventional" T cells and regulatory T cells. Further work to determine the role of RA signaling in vivo in the generation of this spectrum of regulatory versus effector gut homing T-cell populations is needed.

B CELLS DISPLAY TISSUE TROPISM

IgA is the most abundant immunoglobulin isotype produced in the body (around 3 g/day) and ≈80% of all IgA-antibody-secreting cells are located in the intestinal mucosa and play an important role in combating intestinal infections. Migration of B cells and antibody-secreting cells into the gut is necessary to enable protection against intestinal infections. A similar mechanism of tissue tropism is noted for B cells as for T cells. Gut-homing receptors, $\alpha4\beta7$ and CCR9, are upregulated on B cells by intestinal DC and by RA. Additionally, gut-associated DC induce B cells to become IgA-antibody-secreting cells by a mechanism that is, at least in part, dependent on RA, in combination with IL-6 or IL-5 or other as yet unknown factors.[32] Thus, intestinal DC and RA induce imprinting of gut-homing molecules and effector activity. Another chemokine receptor, CCR10, has been proposed as a general mucosal homing receptor.[23,63,64]

Most antibody-secreting cells express CCR10[64] and one of its ligands (CCL28/MEC) is expressed by most mucosal epithelia.[23] However, although CCR10 is apparently necessary for the homing to the colon by B cells, its role in migration to the small bowel remains controversial.

ROLE OF LYMPHOCYTE MIGRATION IN IBD

In IBDs there is an abnormal infiltrate of destructive inflammatory cells into the intestinal mucosa. It is thought that this abnormal immune response is driven by the microbial flora in genetically predisposed individuals, but mechanisms involved in establishing disease in the intestine, and indeed at different parts of the intestine, and at extraintestinal sites are poorly characterized. Both tissue-specific homing of lymphocyte populations and an imbalance of regulatory and effector responses are likely to contribute to the dysregulated immune response of IBD.

Animal Models

Animal models of IBD inform us of the role of immune cell trafficking pathways in these diseases. Upregulation of MAdCAM-1 expression in intestinal lamina propria has been demonstrated in some experimental models of colitis such as dextran-sulfate induced intestinal inflammation.[65,66] Anti-MAdCAM-1 treatment causes a reduction of lymphocyte recruitment to the gut mucosa and a reduction in intestinal inflammation in several experimental models of colitis.[67–71] In the cotton-top tamarin, a primate which develops spontaneous and chronic intestinal inflammation that clinically and histologically resembles ulcerative colitis (UC) in humans, administration of anti-$\alpha 4$ and anti-$\alpha 4\beta 7$ monoclonal antibodies ameliorates intestinal inflammation and is associated with reduced density of mucosal $\alpha 4\beta 7+$ lymphocytes.[72–74] In some models, blocking several integrins is required for effective treatment and resolution of inflammation. For example in models of CD-like ileitis (SAMP1/YitFc and CD4+ T cell transfer models), pathogenic T cells use not only the $\alpha 4\beta 7$/MAdCAM-1 pathway to recirculate to the chronically inflamed small intestine, but also engage other recruitment pathways such as $\alpha 4\beta 1$ and L-selectin.[75] In two murine chronic ileitis models (SAMP/yitFc and TNFdeltaARE mice), blockade of CCR9-CCL25 activity either genetically or with neutralizing antibodies had limited or no effect on disease outcome. Furthermore, in the SAMP/yitFc model administration of anti-$\alpha 4\beta 7$ antibody alone had no effect on disease outcome, whereas TNF-delta ARE mice bred on a $\beta 7^{-/-}$ background failed to develop disease. These findings highlight a potential functional redundancy in the mechanisms of T-cell recruitment to the inflamed intestinal mucosa and suggest that combinatorial therapies targeting multiple homing receptors may provide added benefit for treating intestinal inflammation.

Molecular mechanisms involved in how lymphocytes "stick" and "unstick" to their ligands are becoming clearer. Consequently, abnormalities of these mechanisms may be involved in immune cell trafficking malfunction and disorders of intestinal inflammation. For example, the ability of T cells to induce colitis in the T-cell transfer colitis model was reduced when lymphocyte migration into the gut was paralyzed by impaired unsticking and/or excessive sticking of lymphocytes, caused by a mutation in $\beta 7$ integrin.[76]

Human Tissue

Organ-specific homing pathways have also been assessed in human IBDs. Whereas in healthy mucosa MAdCAM-1 is constitutively expressed on intestinal lamina propria high endothelial venules, its expression dramatically increases in IBD. In actively inflamed tissue of patients with UC or CD, the proportion of venular endothelium within lamina propria that expresses MAdCAM-1 is increased compared with noninflamed tissue.[77–79] In inflamed tissue from CD patients, more extensive expression of MAdCAM-1 was noted than in inflamed tissue from UC patients and was found in deeper layers of intestinal tissue.[78] Additionally, inflamed colonic lamina propria of patients with CD and UC showed increased density of $\alpha 4\beta 7+$ cells compared with controls.[77]

Data from our group has shown altered numbers and cytokine production of $\beta 7+$ circulating T lymphocytes in patients with active UC and CD.[80,81] In particular, there is a redistribution of $\beta 7+$ T cells from the circulating pool to the mucosa. Given that this $\beta 7+$ cell population is comprised of cells primed in the intestine with the capacity to home back to the intestine, the functional properties of these cells may reflect their function in the intestinal mucosa. In UC, patients with a relapse of their disease demonstrated an increase in both Th1 (TNF-α) and Th2 (IL-4) cytokines by intestinal homing $\beta 7+$ memory T cells.[81]

Regarding the CCR9/CCL25 pathway, in CD affecting the small intestine, but not the colon, increased CCR9+ lymphocytes were found in peripheral blood. CCL25 expression was altered in inflamed small intestine from CD patients, but was not detected in normal or inflamed colon of CD patients. In CD patients, CCR9+ T cells from small intestine lamina propria had a predominant Th1 and Th17 cytokine profile.[82] In small bowel CD, CD103+ DC were present in the mesenteric lymph node and these DC induced CCR9 on responding T cells.[37]

There is some evidence that extraintestinal manifestations of IBD are related to aberrant homing of immune cells. Mucosal immunoblasts from IBD patients bind to peripheral LN and to synovium. Some extraintestinal inflammation occurs during episodes of gut inflammation and it has been postulated that skin and eye complications occur as a consequence of recruitment of immune cells primed in

the gut to the extraintestinal site from the circulation. Other extraintestinal manifestations, for example, primary sclerosing cholangitis (PSC), occur at a time that does not correlate with inflammation in the gut, and indeed patients who have had a colectomy for colitis can develop inflammation in the biliary tree. It has been suggested that long-lived populations of memory lymphocytes that arise as a consequence of bowel inflammation migrate not only to the gut but also to the liver; MAdCAM-1 and CCL25, previously thought to be restricted to the gut, are upregulated in the liver in PSC,[83] providing a mechanism for infiltration of immune cells into the liver.

The functional importance of migration of effector T cells (controlled by intestinal DC) in intestinal inflammation has been shown by the efficacy of monoclonal antibodies against α4 integrin in clinical trials. Natalizumab, a recombinant humanized monoclonal antibody against α4 integrin, is efficacious in inducing remission in moderate to severe CD and demonstrates impressive efficacy in maintaining remission of disease by blocking gut-homing.[10,84] Also, natalizumab has been successfully used to treat multiple sclerosis, by blocking the brain-homing VLA-4 ($\alpha4\beta1$).[85] However, natalizumab is associated with side effects. A few patients with CD and multiple sclerosis treated with natalizumab have developed progressive multifocal leukoencephalopathy, caused by reactivation of a clinically latent JC polyomavirus. Natalizumab inhibits the shared α4 integrin moiety of both $\alpha4\beta7$ and $\alpha4\beta1$, which imparts a broader tissue effect on immune function. It may be that inhibition of lymphocyte trafficking and impaired immune surveillance lead to reactivation of infections in particular when inhibition of lymphocyte trafficking is not tissue-specific, but has overlap with other organs. A second humanized monoclonal antibody, MLN-02 (LDP-02), developed against $\alpha4\beta7$, has also shown efficacy in UC and CD and does not appear to have the associated risks of more widespread immunosuppression.[86]

FUTURE/CONCLUSIONS

Selective expression of chemokines by differentiated epithelia and expression of tissue-specific homing molecules on immune cells may represent an important mechanism for specialization and compartmentalization of immune responses. Targeting such pathways provides a mechanism for modulating generation of gut tropic immune cells and entry of these cells into the intestinal mucosa. Strategies that selectively affect organ-specific homing molecules may limit systemic immunosuppressive effects, while targeting the end-organ inflammation. Unraveling the immunological post-code system could inform future therapeutic strategies in IBD.

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