Lymphocyte homing in the pathogenesis of extra-intestinal manifestations of inflammatory bowel disease

Bertus Eksteen, Alice E Miles, Allister J Grant and David H Adams

ABSTRACT – Inflammatory bowel disease is associated with extra-intestinal manifestations which occur either at the same time as flares of bowel inflammation (skin and eye disease) or run a course that is independent to inflammation in the bowel (liver and some joint syndromes). It has been suggested that the skin and eye complications occur as a consequence of the recruitment of activated effector cells released from the gut into the circulation to extra-intestinal site where they cause acute damage. However, this does not explain how patients can develop primary sclerosing cholangitis many years after having their colon removed for colitis. We propose that long-lived populations of memory lymphocytes arise as a consequence of bowel inflammation and that these cells express homing receptors that direct their subsequent migration not only to the gut but also to the liver. These long-lived cells may recirculate to the liver for many years and, in the absence of a local activating stimulus, will not cause damage. However, if they are subsequently activated in the liver this will lead to the development of inflammation and tissue damage which promotes the recruitment of more mucosal lymphocytes resulting in persistent inflammation and disease. The recent findings that MAdCAM-1 and CCL25, previously thought to be restricted to the gut, are up-regulated in the liver during inflammatory liver diseases that complicate IBD support the concept that common mechanisms control lymphocyte recruitment to the inflamed liver and gut.

KEY WORDS: chemokines, endothelium, inflammatory bowel disease, lymphocyte homing, primary sclerosing cholangitis

Inflammatory bowel disease (IBD) is often associated with inflammation at extra-intestinal sites, including the joints, eyes, skin and liver. Non-specific complications of IBD such as pyrexia, lethargy and anorexia may be attributable to systemic circulating pro-inflammatory stimuli including tumour necrosis factor α, but this does not explain the development of specific inflammatory diseases at extra-intestinal sites. Genetic and environmental factors have been linked to IBD, but the initial triggers involved in gut inflammation and the mechanisms involved in establishing disease at extra-intestinal sites remain poorly understood.

Both IBD and its extra-intestinal complications are characterised by an influx of destructive inflammatory cells into tissue. Following some initial trigger, highly activated immune cells, particularly lymphocytes, accumulate at these sites, with the subsequent production of pro-inflammatory cytokines and persistent tissue destruction. Infiltrating lymphocytes in the extra-intestinal disorders of IBD are originally activated in the gut and then aberrantly recruited to extra-intestinal tissues.[1] Elucidating the mechanisms for lymphocyte homing to these tissues is therefore of great importance in advancing our understanding of the pathology of IBD.

Extra-intestinal disorders associated with IBD can broadly be classified into two groups:

- those that occur in parallel with active gut inflammation such as pyoderma gangrenosum, erythema nodosum and anterior uveitis, and
- those that appear to be independent of the course of IBD, such as primary sclerosing cholangitis (PSC) and ankylosing spondylitis.

The association between PSC and IBD is of particular interest as patients undergoing a total colectomy for fulminant colitis can develop *de novo* PSC many years later; similarly, colonic inflammation can occur for the first time after patients have undergone liver transplantation for PSC.[2] The mechanisms of lymphocyte homing to both the normal and inflamed gut are well characterised, but less is known about the processes leading to the development of inflammation at extra-intestinal sites such as the liver.

This review will present an overview of recent advances in this area, focusing in particular on the link between IBD and inflammatory liver disease.

Lymphocyte recirculation and tissue-specific homing

Lymphocytes are the predominant leukocyte at sites of chronic inflammation; they are unique in their

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ability to recognise specific antigen and to provide long-lived immunological memory so that rapid immune responses can be activated on re-encounter of the antigen. T lymphocytes develop in the thymus where autoreactive cells are removed, emerging into the circulation as naïve T cells programmed to respond to specific antigenic epitopes. Naïve T cells circulate continuously between the blood and secondary lymphoid organs (eg lymph nodes, spleen, Peyer's patches). It is in the microenvironment of lymphoid tissue that naïve T cells are primed by dendritic cells which present specific antigen epitopes to the naïve T cell on major histocompatibility complex antigens. The interactions between dendritic cells and lymphocytes are intimate and complex; they induce not only antigen recognition but also knowledge of the tissue and environment in which dendritic cells encounter the antigen,[3] allowing effector and memory T cell populations to home preferentially to the tissue in which they were originally activated (Fig 1). This has recently been elegantly demonstrated by a study in which naïve T cells were primed with antigen by dendritic cells isolated from different tissues and then re-infused and tracked *in vivo*. Only dendritic cells isolated from gut tissue were able to educate T lymphocytes to traffick selectively to the gut *in vivo*.[4]

Lymphocyte recruitment from the circulation to target tissue is a highly regulated process depending on sequential interactions with a series of adhesion molecules (Fig 2).[5] The dynamics of flow within blood vessels means that erythrocytes flow in the centre of the stream, promoting the margination of leukocytes to the vessel wall and endothelial surface. Initial transient interactions between lymphocyte and endothelium (mediated largely by selectins and their glycoprotein ligands) induce rolling or 'tethering'. This allows the lymphocyte to sample the endothelial environment for cytokines (called chemokines) immobilised by glycosaminoglycans on the endothelial glycocalyx. When chemokines bind to specific G protein-coupled receptors on the lymphocyte, this triggers cytoskeletal rearrangement and activation of lymphocyte integrins. Integrin activation leads to high affinity binding to ligands belonging to the immunoglobulin superfamily (eg intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), mucosal addressin cell adhesion molecule-1 (MAdCAM-1)), with subsequent arrest on endothelium followed by transendothelial migration into tissue in response to appropriate chemotactic signals.

The patterns of lymphocyte recirculation and homing are determined by the tissue-specific expression of endothelial adhesion molecules and chemokines, leading to recruitment of specific lymphocyte subsets expressing the appropriate receptors. Some adhesive interactions, such as those mediated by the endothelial adhesion molecules ICAM-1 and VCAM-1 and their integrin ligands $\alpha_L\beta_2$ (lymphocyte function-associated antigen-1 (LFA-1)) and $\alpha_4\beta_1$ (very late antigen (VLA-4)), are involved in lymphocyte recruitment to many tissues, especially in the context of inflammation. However, other molecules show a more restricted expression and act as 'addressins'

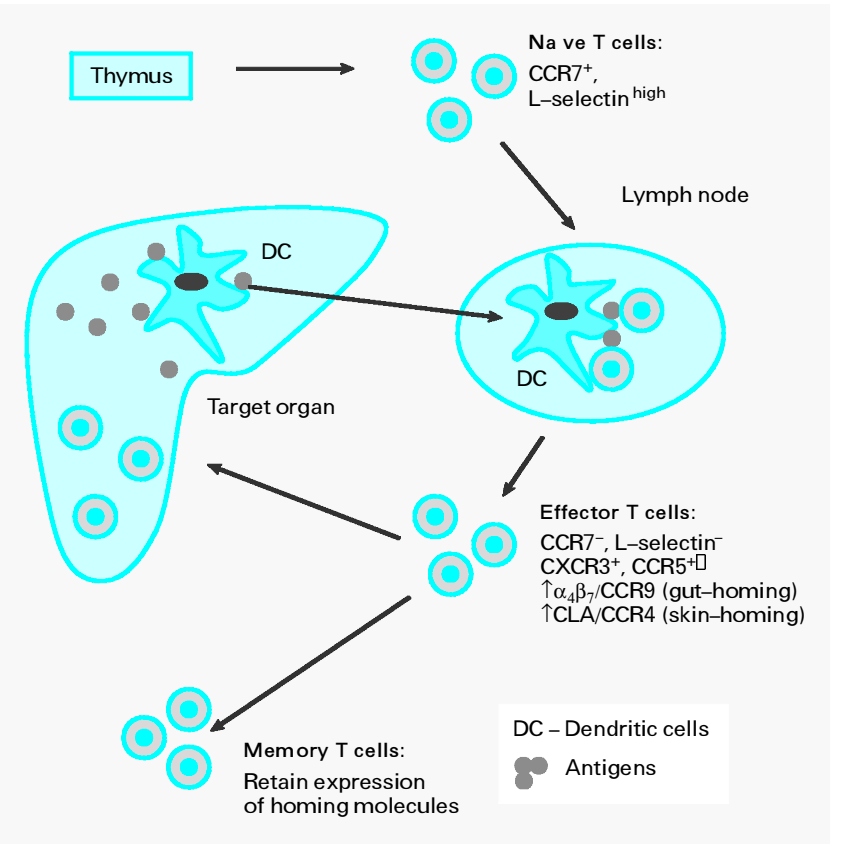

Fig 1. Lymphocyte development. Naïve lymphocytes released from the thymus circulate continuously between blood and secondary lymphoid organs. Entry to secondary lymphoid tissue via high endothelial venules depends on expression of L-selection and CCR7 which allows the naïve T cell to interact with peripheral node addressin (PNAd) and the chemokine CCL21, respectively, both of which are expressed in secondary lymphoid tissues. When primed by antigen on antigen presenting cells (such as dendritic cells) naïve lymphocytes undergo differentiation with downregulation of CCR7 and L-selectin, and upregulation of pro-inflammatory chemokine receptors such as CXCR3 and CCR5 and specific adhesion molecules that direct the lymphocyte to its target tissue. After the antigen has been eliminated some of these effector cells persist in the circulation as long-living memory cells; they retain the expression of these specific adhesion molecules which allows them to recirculate preferentially back to the tissue in which they were originally activated (CLA = cutaneous lymphocyte antigen).

directing the recruitment of particular lymphocyte subsets to specific tissues.

On release from the thymus, naïve lymphocytes have a well described phenotype, expressing high levels of L-selectin and the chemokine receptor CCR7. This allows them to home to lymphoid tissue via interactions with the L-selectin ligand peripheral node addressin (PNAd) and the chemokine CCL21 (secondary lymphoid-tissue chemokine (SLC)), both of which are generally restricted to endothelium in secondary lymphoid tissues.[6] Once primed, lymphocytes downregulate expression of both L-selectin and CCR7 excluding them from lymphoid tissue, and increase expression of other adhesion molecules which allows them selectively to home to the target tissue in search of antigen. For example, skin homing lymphocytes express high levels of cutaneous lymphocyte antigen (CLA) which binds E-selectin on dermal vessels, and CCR4 which responds to the chemokine CCL17 in the skin. Lymphocytes which home to the gut, however, express low levels of CLA but high levels of $\alpha_4\beta_7$ integrin, allowing them to bind the endothelial ligand MAdCAM-1 which is largely restricted to mucosal vessels.[3] Once inflammation has subsided, some of these cells persist in the circulation as long-lasting memory cells retaining the ability both to home back to the original site of activation and to mount an immune response if the antigen is again encountered (Fig 1).

Lymphocyte homing to the gut

Antigen presentation and priming of naïve lymphocytes in the gut is facilitated not only by dendritic cells in mesenteric lymph nodes (MLN) but also in highly specialised mucosal-associated lymphoid tissues (MALT) such as Peyer's patches. Naïve lymphocytes recruited to these tissues are primed exclusively to gut-derived antigens and acquire at least two crucial molecules that allow specific gut-homing: $\alpha_4\beta_7$ integrin and the chemokine receptor CCR9. MAdCAM-1, the ligand for $\alpha_4\beta_7$, is expressed widely in mucosal vessels and related lymphoid tissue and is the predominant adhesion molecule in the intestinal lamina propria. MAdCAM-1/$\alpha_4\beta_7$ interactions not only mediate strong adhesion of lymphocytes to endothelium but can also support rolling interactions in the absence of selectin-dependent adhesion.[7]

IBD is associated with destructive infiltration of inflammatory cells into the bowel with subsequent cytokine release and tissue damage. In both ulcerative colitis and Crohn's disease this inflammatory infiltrate includes lymphocytes and neutrophils. However, Crohn's disease is mediated by type 1 helper (Th1) cells and is associated with non-caseating granulomata, whereas in ulcerative colitis inflammation is restricted to the colon and characterised by crypt abscesses. It is thought that upregulation of MAdCAM-1 during gut inflammation leads to the sustained recruitment of circulating $\alpha_4\beta_7$+ lymphocytes and the establishment of chronic

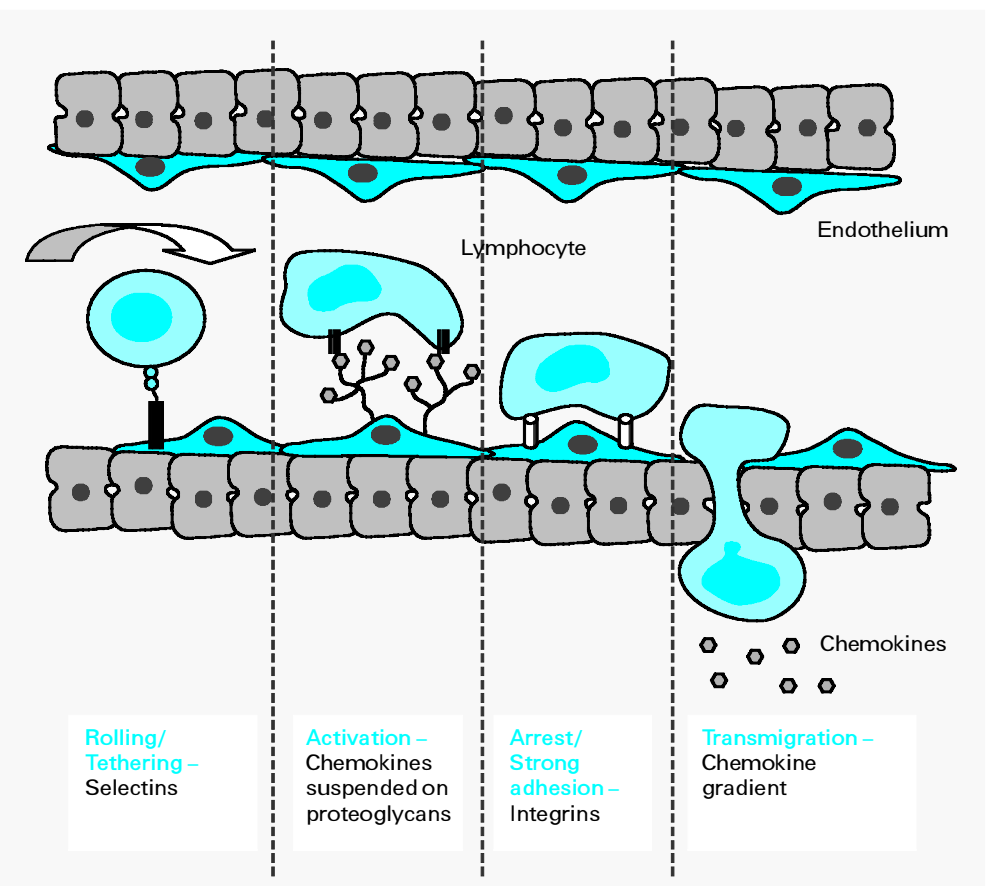

Fig 2. Lymphocyte recruitment to tissue. Initial transient interactions between selectins and their glycoprotein ligands induce rolling of lymphocytes along the endothelium, exposing them to chemokines immobilised on endothelial proteoglycans. Chemokines bind to specific lymphocyte receptors, causing activation of lymphocyte integrins and triggering firm adhesion or arrest of the lymphocyte. Finally, transendothelial migration or 'extravasation' into tissue occurs in response to chemotactic gradients.

bowel inflammation. Evidence for this includes the ability of antibodies to $\alpha_4\beta_7$ to reduce inflammation in animal models and in patients with colitis.[8,9]

Homing to the small bowel is enhanced by the restricted expression of CCL25 (thymus-expressed chemokine (TECK)) by crypt and glandular epithelium, and subsequent presentation on small bowel venular endothelium.[7] Ligation of CCR9 by the chemokine ligand CCL25 triggers conformational changes in the $\alpha_4\beta_7$ integrins and firm adhesion to MAdCAM-1. There is over 90% expression of $\alpha_4\beta_7$ on lymphocytes in the small bowel, which mirrors the expression of the chemokine receptor CCR9. The importance of both molecules is emphasised by the fact that both CCR9 -/- and β_7-integrin -/- mice have deranged mucosal lymphocyte compartments and a reduction in total lymphocyte numbers in the gut.[7]

Intriguingly, lymphocytes that home to the colon have limited expression of CCR9 (<20%) and no detectable CCL25 production on the colonic epithelium,[10] but the chemokine receptor CCR10 is expressed on colonic homing lymphocytes of both T and B cell lineages. CCL28 (mucosal-expressed chemokine (MEC)), the ligand for CCR10, is detectable on colonic mucosal vessels and epithelial cells during both physiological and inflammatory states.[11] Adhesion molecule expression in the colon is less well characterised than in the small bowel: MAdCAM-1 expression is detectable but much reduced compared with the small bowel. It is thus likely that other adhesion molecules such as ICAM-1 and VCAM-1 will also contribute to colonic lymphocyte adhesion. Evidence in support comes from studies of experimental murine colitis models in which VCAM-1 mediated adhesion blockade significantly ameliorated inflammation whereas MAdCAM-1 blockade was much less effective.[12]

Small bowel and colonic homing lymphocytes also express chemokine receptors that do not generally dictate tissue-specific homing but rather define an activated pro-inflammatory phenotype. CXCR3, CCR5 and CXCR4 are detectable on gut-homing lymphocytes, with their expression dramatically enhanced during states of inflammation. The first two are associated with Th1 type immune responses in virtually all organ systems including the liver, joint and skin. The ligands for CXCR3, CXCL10 (interferon-inducible protein of 10Kd (IP-10)), CXCL9 (monokine induced by interferon γ (MIG)) and CXCL11 (interferon-inducible T-cell alpha chemoattractant (I-TAC)) and the ligands for CCR5, CCL5 (regulated on activation, normal T-cell expressed and secreted (RANTES)), CCL3 (macrophage inflammatory protein-1α (MIP-1α)) and CCL3 (MIP-1β) are expressed in both ulcerative colitis and Crohn's disease.[13] CXCR4 is widely expressed on leukocytes and is thought to have an important role in retaining lymphocytes in inflamed tissue.[14]

Lymphocyte homing to the liver

The unique dual blood supply of the liver has led to a complex system of homing molecules that differs both between normal conditions and disease states and also between anatomical areas within the liver lobule. The hepatic portal tracts consist of a branch of the hepatic artery, a portal vein and a bile duct. Lymphocyte infiltrates are seen in the portal tracts during normal conditions and probably provide immune surveillance to both systemic and portal derived antigens. Chronic inflammation restricted to the portal tracts is often termed 'chronic persistent hepatitis'. This may be associated with bile duct damage, but does not usually lead to hepatocyte loss and progressive fibrosis in the absence of interface hepatitis – chronic inflammation extending beyond the portal areas leading to hepatocyte destruction, fibrosis and ultimately cirrhosis.

Both sinusoidal and portal endothelium express the conventional cellular adhesion molecules ICAM-2, ICAM-1 and VCAM-1 under normal conditions, with marked increases in the latter two during inflammation.[15] Co-operation between the selectins ICAM-1 and VCAM-1 is critical in mediating lymphocyte recruitment via the portal tracts. However, the sinusoidal bed provides a unique low-shear environment which does not require capture mediated by selectins. A novel endothelial adhesion molecule, vascular adhesion protein-1 (VAP-1) mediates adhesion and transmigration of lymphocytes on sinusoidal endothelium. VAP-1, a homodimeric transmembrane protein, is constitutively expressed on both vascular and sinusoidal endothelium in the liver, but in the absence of inflammation is largely absent from endothelium at other non-lymphoid sites. It has been shown to support T cell adhesion to both normal and inflamed hepatic endothelium *in vitro*, although the lymphocyte receptor is not known.[16] VAP-1 shows monoamine oxidase activity; a soluble form of this enzyme can be detected in the systemic circulation, with raised levels in the serum of patients with inflammatory liver disease but not of those with other inflammatory conditions such as rheumatoid arthritis. It is thought that VAP-1 may function as an addressin, allowing the selective recruitment of lymphocyte subpopulations to the liver.

Recruitment of lymphocytes to the liver also requires specific chemokine signals. Liver homing effector lymphocytes (similar

Key Points

Although most patients with primary sclerosing cholangitis will have a history of inflammatory bowel diseaase (IBD) the liver disease usually runs a course that is independent of the IBD

The ability to home to a particular tissue is imprinted on lymphocytes by tissue-specific dendritic cells in regional lymph nodes. This homing depends on the lymphocyte expressing receptors that allow it to recognise tissue-specific combinations of signals on the endothelium of target organs

In hepatic complications of IBD, the liver endothelium expresses molecules that are normally restricted to the gut resulting in the inappropriate recruitment of mucosally activated lymphocytes to the liver. Because the mucosal lymphocytes are long-lived memory cells, liver inflammation can occur many years after bowel inflammation has become quiescent

to gut homing lymphocytes) express the pro-inflammatory chemokine receptors CXCR3, CCR5 and CXCR4; the first two are closely associated with most forms of chronic hepatitis and determine portal versus parenchymal inflammation. The ligands for CCR5, CCL5, CCL3 and CCL4 are expressed in the portal tracts and provide an important initial signal to drive recruitment to the liver. There is evidence that retention of lymphocytes in the portal tracts, important for both antigen clearance and limiting parenchymal damage, is mediated by the CXCR4 ligand CXCL12 (stromal-cell-derived factor-1 (SDF-1)). In some instances, pathogens escape portal immune regulation and infiltrate the parenchyma, inducing expression of the interferon-dependent CXCR3 ligands by periportal hepatocytes, Kupffer cells and sinusoidal endothelium. This promotes the recruitment of CXCR3 high effector lymphocytes via the sinusoids. Interface hepatitis ensues which, if it persists, eventually leads to cirrhosis.[17]

Aberrant homing of mucosal lymphocytes in extra-intestinal disease

Lymphocyte infiltration is a feature of the extra-intestinal complications of IBD. The concept that these lymphocytes are originally activated in the gut and then aberrantly recruited to extra-intestinal tissues first arose from the observation that mucosal immunoblasts from IBD patients can bind to peripheral lymph nodes and to synovium.[18] Extra-intestinal inflammation during active episodes of gut inflammation is thought to develop as a result of an increase in circulating activated mucosal lymphocytes with the ability to enter any tissue in which low levels of inflammatory ligands such as ICAM-1 and VCAM-1 are expressed. Such inflammation would follow a parallel course to active episodes of IBD and resolve once gut inflammation has subsided and the supply of effector cells diminished.[2]

However certain extra-intestinal complications such as ankylosing spondylitis, autoimmune hepatitis and PSC often progress independently from bowel inflammation. As stated earlier, patients can develop PSC for the first time many years after total colectomy for colitis, and colonic inflammation can occur for the first time after patients have undergone liver transplantation for PSC.[2] The mechanisms of these secondary episodes of inflammation are poorly understood, but any proposed model explaining the link between IBD and PSC must account for the time delay often observed between onset of inflammation in the liver and gut.

Hepatic complications of inflammatory bowel disease: evidence of enterohepatic lymphocyte recirculation

PSC will develop in 2.4–7.5% of patients with underlying IBD, while 70–85% patients with PSC will suffer from IBD at some point in their lives. Of these, most (90%) will develop ulcerative colitis, with Crohn's colitis predominating in the remainder. As liver disease can occur in the absence of a diseased colon, the link cannot simply be explained by release from the diseased gut of recently activated effector cells or inflammatory factors. Long-living memory T lymphocytes originally activated in the gut may also recirculate through the liver and be able subsequently to trigger inflammation under the right conditions. Such an inflammatory response, if perpetuated, could lead to chronic hepatitis and cirrhosis. The teleological explanation for such an 'enterohepatic' lymphocyte recirculation would be to provide a mechanism for immune surveillance across both liver and gut, allowing the immune system to respond to gut antigens entering via the portal circulation.

Evidence suggests that there may be enterohepatic lymphocyte recirculation. During development, the gut is populated by lymphocyte precursors derived from the developing liver, suggesting a common origin for lymphocytes at these two sites. Furthermore, although the liver and gut have distinct endothelial phenotypes in adults, there is overlapping expression of many molecules including the two potential addressins VAP-1 and MAdCAM-1. Under normal conditions VAP-1 expression on liver endothelium is far stronger than the patchy expression seen on mucosal vessels. Gut expression is greatly increased in IBD, suggesting that liver derived lymphocytes expressing the VAP-1 receptor may be able to enter the inflamed gut.[19] Similarly, endothelial expression of MAdCAM-1, previously thought to be restricted to the gastrointestinal tract, has recently been demonstrated on hepatic portal endothelium in inflammatory liver disease associated with IBD, including PSC. This hepatic endothelial MAdCAM-1 was shown to support $\alpha_4\beta_7$ mediated lymphocyte adhesion *in vitro*,[1] suggesting that it may have a functional role in lymphocyte recruitment.

Thus, we propose that mucosal derived memory lymphocyte cells recirculate between both liver and gut using MAdCAM-1, VAP-1 or both (Fig 3). In the normal situation, this would allow immune surveillance across both sites, but long-living mucosal derived memory cells could be recruited rapidly to the liver if some trigger leads to hepatic inflammation and upregulation of hepatic MAdCAM-1. If these cells were to become activated, for example by cross-reactive antigens in the liver or by gut antigens that have entered via the portal circulation, this could result in an inflammatory response which, if sustained, would lead to chronic inflammation.

MAdCAM-1 expression alone is probably not sufficient to explain the chronic lymphocyte infiltrate observed in PSC and several other factors are likely to be involved in this process. A feature of chronic inflammatory liver diseases such as PSC is the formation of secondary lymphoid tissue, portal associated lymphoid tissue (PALT), in the portal tracts during chronic inflammation. PALT provides an environment for cross-talk between memory cells and dendritic cells, as well as the expansion of effector cells, thus playing a fundamental part in sustaining chronic inflammation. Lymphoid neogenesis in chronic inflammatory conditions occurs in response to cytokine release and provides a mechanism for continuous lymphocyte recruitment to tissue. There is evidence that lymphoid neogenesis may be associated with overexpression of the chemokine CCL21.[20]

CCL21 activates $\alpha_4\beta_7$ dependent adhesion to MAdCAM-1 and plays an important role in recruiting naïve lymphocytes to

the Peyer's patches of the gut.[7] CCL21, previously thought to be restricted to secondary lymphoid tissue, is upregulated on vascular endothelium in PALT in PSC, and intrahepatic PSC lymphocytes migrate to CCL21 *in vitro*. Furthermore, MAdCAM-1 is detectable in vessels and $\alpha_4\beta_7$ on lymphocytes in PALT. It is therefore possible that CCL21 in PSC plays a role in recruiting $\alpha_4\beta_7$+ lymphocytes to the liver by activating firm adhesion to MAdCAM-1.

Further evidence for a link between lymphocyte recirculation to gut and liver comes from our recent unpublished findings that suggest a role for the gut associated chemokine CCL25 in lymphocyte recruitment to the liver in PSC. CCL25 expression has been detected in PSC liver associated with high numbers of CCR9+ intrahepatic lymphocytes. Moreover, the CCR9+ cells all co-express $\alpha_4\beta_7$ integrin, suggesting that they are derived from the mucosa. That both gut-homing and liver-homing lymphocyte populations share expression of several other chemokine receptors such as CCR5, CXCR3 and CXCR4 also suggests that common mechanisms may be involved in lymphocyte recruitment to both sites (Fig 4).

Summary

Selective lymphocyte recruitment to target tissues is crucial in maintaining immune surveillance by allowing long-lived memory cells actively to patrol sites where potential harmful antigens might be detected and subsequently to mount a targeted effective response. Patterns of lymphocyte homing are controlled by the expression of adhesion molecules and chemokines which recruit particular lymphocyte subsets expressing the appropriate receptors. Aberrant expression of such adhesion molecules is likely to lead to inappropriate tissue inflammation and manifestations of autoimmunity. Some of these adhesion molecules show widespread expression and are involved in lymphocyte recruitment to several tissues, while others are more specific, acting as 'addressins' for lymphocyte recruitment to particular organs. For example, lymphocytes primed to an antigen in the gut express $\alpha_4\beta_7$ and CCR9, allowing them to be directed to mucosal tissue to which the $\alpha_4\beta_7$ ligand MAdCAM-1 and the CCR9 ligand CCL25 are largely confined. Long-living memory cells retain expression of these molecules allowing them to recirculate preferentially to the gut.

IBD disease and its extra-intestinal complications are associated with destructive infiltration into tissue of lymphocytes which were probably originally activated in the gut then aberrantly recruited to other tissues. The primary triggers involved in these processes are unknown, but alterations in adhesion molecule expression provide a strong clue as to their pathogenesis. The inflammatory response following recognition of antigen in the gut involves effector cell expansion and release of pro-inflammatory cytokines, resulting in upregulation of endothelial molecules such as VCAM-1 and ICAM-1 and inflammatory chemokines such as CXCR3 and CCR5 ligands. Thus, during gut inflammation there is a loss of specificity, with expression of non-gut-specific inflammatory molecules in favour of an overwhelming response to clear the antigen. Extra-intestinal complications during active episodes of gut inflammation could therefore be explained by high numbers of circulating gut-activated effector lymphocytes expressing high levels of adhesion molecules such as $\alpha_L\beta_2$ (LFA-1) and $\alpha_4\beta_1$(VLA-4), allowing them to be recruited to other sites where endothelial ligands such as ICAM-1 and VCAM-1 are constitutively expressed or where mild inflammation is present. Clinically, such individuals will present with skin and joint

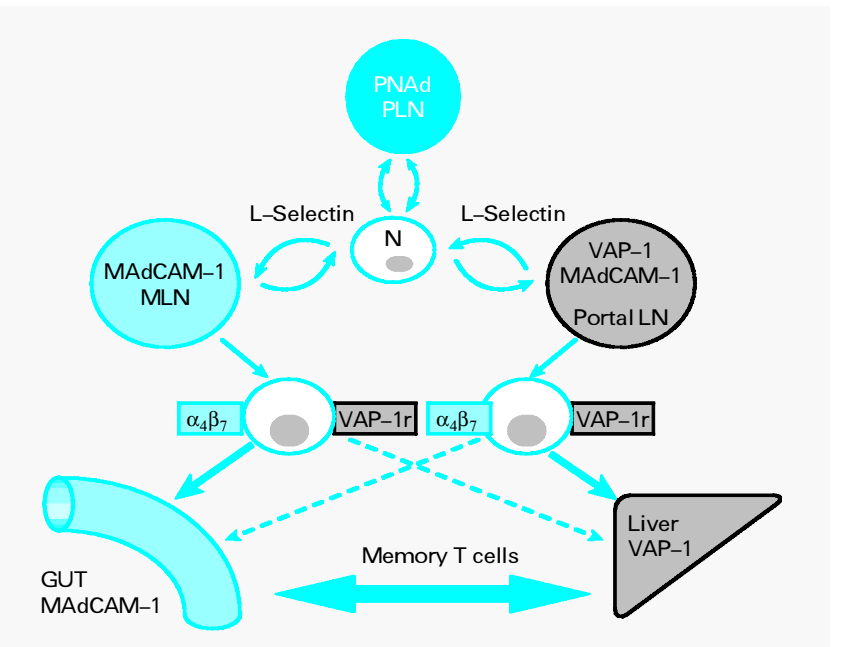

Fig 3. Naïve lymphocytes (N) use peripheral node addressin (PNAd) to enter peripheral lymph nodes (PLN) and MAdCAM-1 to enter mesenteric lymph nodes (MLN) and portal lymph nodes (Portal LN). Lymphocytes (L) activated in MLN will gain $\alpha_4\beta_7$ expression and selectively circulate to the gut via interactions with MAdCAM-1. Conversely, lymphocytes activated in Portal LN will express the vascular adhesion protein-1 (VAP-1) receptor and circulate to the liver where VAP-1 is expressed. However, during inflammation VAP-1 can be upregulated in the gut which could lead to the aberrant recruitment of liver-derived lymphocytes. Similarly, MAdCAM-1 can be expressed in the liver during inflammation and recruit gut homing lymphocytes (broken arrows). A number of lymphocytes will become long-lived memory cells with the ability to traffick to both sites, independent of their original activation site (MLN or Portal LN).

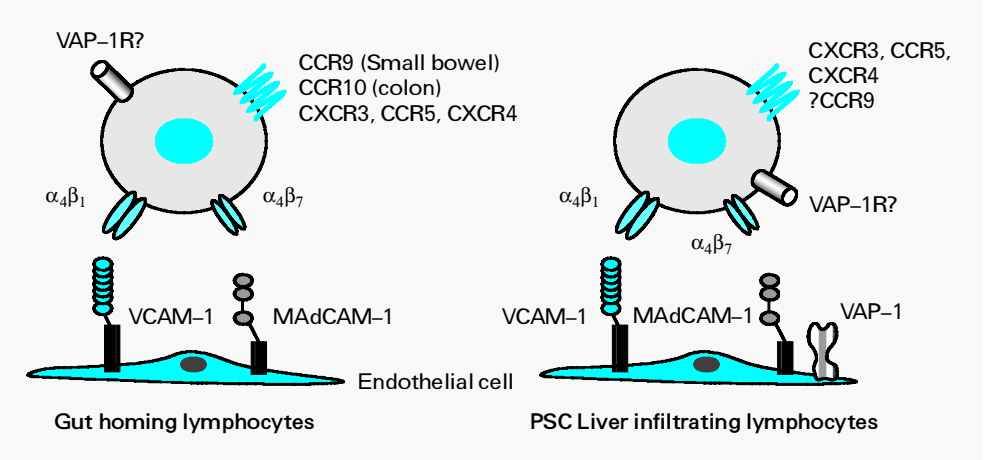

Fig 4. Lymphocyte phenotypes. Relevant adhesion molecules that define or are shared by gut and primary sclerosing cholangitis (PSC) liver-homing lymphocytes (MAdCAM = mucosal addressin cell adhesion molecule; VCAM = vascular cell adhesion molecule; VAP = vascular adhesion protein).

inflammation at the same time as their gut disease; inflammation rapidly resolves once gut inflammation subsides, as is the case with pyoderma gangrenosum, erythema nodosum and acute arthritis associated with IBD.

However, these mechanisms cannot explain the hepatic extra-intestinal manifestations of IBD which progress independently from gut inflammation. Many pro-inflammatory adhesion molecules are shared by the liver and the gut such as CXCR3 ligands, CCR5 ligands, ICAM-1 and VCAM-1, but it is rare for patients to have active disease at both sites. We suggest that extra-intestinal complications such as PSC are mediated by long-lived memory lymphocytes derived from the mucosa and able to recirculate between gut and liver due to the overlapping expression of 'tissue-specific' adhesion molecules. This could allow for a specific immune response to a gut antigen to be triggered in the liver or alternatively an immune response directed to a liver antigen in the gut, independent of inflammation at the original site where the antigen was first encountered. Under the right conditions this could be perpetuated, leading to chronic inflammation.

The concept that common mechanisms control lymphocyte recruitment to the inflamed liver and gut is supported by the recent findings that MAdCAM-1 and CCL25, previously thought to be restricted to the gut, are upregulated in the liver during inflammatory liver diseases including PSC, and that VAP-1, constitutively expressed in the liver, is upregulated in the gut in IBD.

Much more work is needed to fully understand the pathogenesis of IBD and its extra-intestinal complications, but knowledge of these processes has greatly improved in recent years and multiple potential therapeutic targets are emerging. Anti-integrin therapies have been successful in animal models of colitis and are now emerging as therapeutic agents to treat Crohn's disease by targeting α_4 or $\alpha_4\beta_7$ integrins.[9] As our understanding of the molecular mechanisms underlying lymphocyte recruitment in extra-intestinal disorders of IBD improves, similar approaches may become directly applicable to 'difficult to treat diseases' such as PSC.

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