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The hepato-enteric immune axis in health and disease

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Introduction

The gut not only digests, absorbs and processes nutrients but also acts as a barrier to pathogens entering from the intestinal lumen. The gastrointestinal immune system is shaped by interactions with commensal bacteria, and changes in the gut microbiota can lead to systemic effects on immunity and inflammation, including a predisposition to non-alcoholic fatty liver disease.[1] Mucosal immune responses occur in the intestinal mucosa and underlying lamina propria. Transport of nutrients and translocation of pathogens into the portal venous circulation mean, however, that appropriate immune responses in the liver are required to deal with antigens that evade the enteric immune system. Immune responses in the gut, and to a lesser degree in the liver, have been well-described but the explicit mechanisms that facilitate cross-talk between both organs are not fully understood.[2] Improving our understanding of the so called 'hepato-enteric' immune axis might provide insights into the pathogenesis of diseases that affect both sites.

Gut-specific cellular trafficking

The intestinal mucosal barrier reduces bacterial translocation and the innate immune system provides a rapid and potent response to pathogens that cross this barrier. Antigens in the gut lumen as well as those that penetrate the mucosal barrier are sampled and processed by specialised antigen presenting cells (APCs) called dendritic cells (DCs). They are then carried through the lymphatic system to draining mesenteric lymph nodes (MLNs), where they activate the differentiation of naive lymphocytes into antigen-specific effector T-cells or regulatory T-cells (T_{reg}).

A system of 'tissue-specific' lymphocyte trafficking has evolved to target lymphocytes to areas of infection or injury. This trafficking system is controlled by combinations of chemokines and adhesion molecules (addressins) that are expressed in target tissues. These act as a molecular 'postal code' to attract specific subsets of lymphocytes that express the appropriate counter-receptors.[3] The gut postal code is characterised by the expression of mucosal addressin cell adhesion molecule-1 (MAdCAM-1) on mucosal endothelium; and of the chemokine CCL25, which is expressed by small-bowel epithelium. These molecules interact with the lymphocyte receptors α4β7-integrin and the chemokine receptor CCR9, respectively. MAdCAM-1 is widely expressed in mucosal vessels and in the intestinal lamina propria, whereas CCL25 is largely restricted to the thymus and small bowel. The 'imprinting' of a 'gut-homing' phenotype on mucosal lymphocytes, as characterised by the cellular expression of CCR9 and α4β7, is orchestrated by a subset of $CD103^+$ gut DCs in a process that is dependent on retinoic acid (Fig 1a) and occurs during lymphocyte activation within Peyer's patches and mesenteric lymph nodes. α4β7 is also involved in the recruitment of activated T-cells to the colon. CCL25 is absent from normal murine colonic epithelium although it has been reported in the colon in active colitis.[4] During exacerbations of inflammatory bowel disease (IBD), MAdCAM-1 expression is upregulated and promotes the sustained recruitment of circulating lymphocytes that express α4β7. It also promotes the establishment of chronic bowel inflammation. Antibody inhibition of α4β7 reduces inflammation in animal models of IBD, and clinical trials are underway using either α4β7 or CCR9 antagonists to treat IBD.

The liver: a balance between immune tolerance and immune response

Despite immune surveillance by the mucosal immune system, some pathogens penetrate intestinal defence mechanisms and enter the liver through the portal circulation, leading to the evolution of a second line of immune protection in the liver; however, the liver is constantly exposed to harmless food antigens and so it has also evolved tolerogenic mechanisms to prevent it being constantly inflamed by immune activation.[5] A vigorous intrahepatic immune response depends on the activation of T-cells by fully activated DCs within secondary lymphoid tissues; conversely, direct activation within the liver by hepatic-resident antigen-presenting cells usually results in tolerance.[6] The fact that local presentation of antigens in the liver usually results in tolerance might be explained by a combination of factors, including the finding that hepatic endothelial cells are unique tolerogenic APCs. The relative insensitivity of these cells and Kupffer cells to lipopolysaccharides is a vital property that prevents the liver from being in a constant state of immune activation in response to gut-derived bacterial products in the portal circulation.[5]

Immunosuppressive T_{reg} and immune tolerance

Peripheral T_{reg}, which suppress the activation of effector T-cells, are generated when naive T-cells are activated by immature DCs, or in the presence of cytokines IL10 and TGF-β. Intrahepatic T_{reg} in the human liver use the chemokine receptor CXCR3 to respond to IFNγ-dependent chemokines (i.e. CXCL9 and CXCL10) that are produced within the inflamed liver. They use CCR10 to

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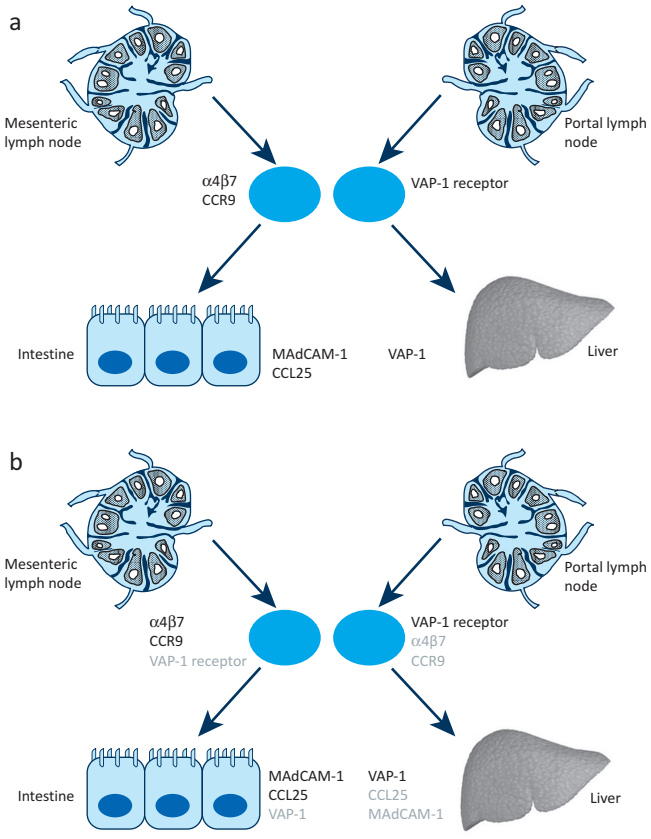

Fig 1. Enterohepatic homing of lymphocytes. (a) Under normal physiological conditions, gut-derived antigens are presented to naive lymphocytes in the draining mesenteric lymph nodes. Lymphocytes are activated by gut dendritic cells that imprint them with a 'gut-homing' phenotype in a process dependent on retinoic acid. Gut-tropic lymphocytes are characterised by the expression of the chemokine receptor CCR9 and the integrin α4β7. These receptors direct the migration of the activated lymphocytes back to gut tissue, where their respective ligands CCL25 and MAdCAM-1 are expressed. Similarly, lymphocytes that are primed against hepatic antigens gain expression of adhesion molecules that allow them to traffic to the liver by interacting with molecules such as VAP-1, which is expressed on hepatic endothelium. (b) In primary sclerosing cholangitis (PSC) and inflammatory bowel disease (IBD), the system of 'selective' lymphocyte homing becomes altered. Expression of the gut-specific adhesion molecules (CCL25 and MAdCAM-1) is no longer restricted to the intestine and becomes detectable in the liver. VAP-1 expression on mucosal vessels also increases in IBD. The end result is that lymphocytes that have been generated to recognise gut antigens in the setting of IBD are now misdirected to the liver, where they contribute to inflammation and biliary destruction.

localise to CCL28 secreted by biliary epithelial cells (BEC), resulting in their accumulation around bile ducts.[7] CCL28 is also expressed by intestinal epithelium and thus similar signals might localise T_{reg} in the gut and in the liver. Interactions between T_{reg}, pathogens, APCs and other liver cells regulate immune activation in the liver and the transition from tolerance to inflammation.

Diseases affecting the gut and liver

In light of the close integration of the mucosal and hepatic immune systems and their shared exposure to antigens, it is not surprising that the liver can be affected in immune-mediated diseases that primarily affect the gut.

Coeliac disease

Asymptomatic individuals are increasingly diagnosed with coeliac disease on the basis of serological tests (using anti-tissue transglutaminase antibody or anti-endomysial antibody). Abnormal liver enzymes are detected in up to 60% of patients with coeliac disease, and 10% of patients with unexplained elevated transaminases have detectable anti-endomysial antibodies. The exact reasons for liver dysfunction in coeliac disease are unclear. Most commonly, liver biopsy shows a non-specific lymphocytic infiltrate that resolves when the patient adopts a gluten free-diet. Autoimmune hepatitis (AIH), primary sclerosing cholangitis (PSC) and primary biliary cirrhosis (PBC) can also be detected in patients with coeliac disease (up to 7%) and could be part of an inherent predisposition to the development of autoimmunity. Unfortunately, treatment with a gluten-free diet does not improve the outcome of autoimmune liver diseases, although it might alleviate non-specific symptoms such as fatigue and lethargy. Non-alcoholic fatty liver disease is also associated with coeliac disease, possibly as a result of changes in the gut microbiota and increased gut permeability.[1]

Inflammatory bowel disease

An association between hepatopancreatobiliary disease and IBD has been recognised since the 19th century. Several mechanisms could be involved, including hepatic toxicity from certain IBD-related medications and aetiopathogenic mechanisms that are shared with IBD (Table 1). Liver disease, in the form of AIH or PSC, develops in 2.4–7.5% of patients with IBD. Moreover, the majority of northern European patients (70–85%) who have PSC will suffer from IBD at some point in their lifetime. The strongest association is with ulcerative colitis (UC; 90%); Crohn's disease predominates in the remaining 5–10%. The distribution of intestinal inflammation in IBD with PSC is unique (Table 2), typically being pan-colonic (87%) albeit with rectal sparing (51–65%). Hence, this inflammation can be missed on rigid sigmoidoscopy. Furthermore, the inflammation tends to be most severe on the right side of the colon (52%) and is frequently associated with backwash ileitis (51%). Although the colitis of IBD with PSC is usually mild and follows a relatively quiescent course, the risk of colonic neoplasia is substantially increased (30% lifetime risk) compared to that observed in IBD alone, being greatest in the proximal colon.[8] The pattern of hepatobiliary disease in PSC does not parallel that of colonic inflammation, and IBD can develop for the first time after patients have undergone liver transplantation for PSC. Furthermore, patients can develop PSC for the first time many years after total colectomy for colitis. Interestingly, when performed in the pre or peri-liver transplant period, colectomy might protect against the development of recurrent PSC in the liver allograft, whereas this is not the case for colectomy performed post-transplantation.[9]

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Table 1. Hepatopancreatobiliary manifestations of inflammatory bowel disease.

IBD medication related	• Drug-induced hepatitis (relating to methotrexate, cyclosporine, thiopurines or infliximab) • Reactivation of hepatitis B (relating to infliximab) • Pancreatitis (relating to mesalazine or thiopurines)
Associated with the pathophysiology or severity of IBD	• Choledocholithiasis • Portal vein thrombosis • Hepatic artery thrombosis
Possible shared aetiopathogenesis with IBD (autoimmune disorders)	• PSC • Autoimmune hepatitis–PSC overlap • Small-duct PSC (pericholangitis) • IgG4-cholangitis/pancreatitis
Miscellaneous recognised associations with IBD	• Fatty liver • Hepatic abscess • Amyloidosis • Hepatic granulomas

Genetic links between PSC and IBD. The prevalence of PSC among first-degree relatives of patients who suffer from this disease is 100-times greater than that in the total population, and the risk of developing PSC and/or UC is also significantly increased in this group compared to controls. Given the strong association between PSC and IBD, it would not be surprising to find that they share some common genetic basis. Recently, genome-wide association studies established human leukocyte antigen (HLA) as the most important risk locus in PSC,[10] the strongest associations being for HLA-B*08 and -DRB*03. HLA class-II alleles conferring susceptibility to, or protection from, PSC were found to be associated with PSC patients regardless of their IBD status and were not associated with UC. Furthermore, HLA alleles that are associated with UC are not linked to PSC. Many other IBD susceptibility loci tested to date fail to show a common genetic link to PSC. The lack of a more common genetic basis supports the paradigm that IBD with PSC is a unique phenotype.

Bacteria and cellular immunity. The translocation of bacteria or bacterial components across the 'leaky' inflamed colonic mucosa can occur in patients with colitis, and these microbial products might subsequently enter the liver via the portal-venous system. Cytokines released from Kupffer cells in the liver might then attract macrophages, lymphocytes, activated neutrophils and fibroblasts, resulting in an inflammatory reaction centred on the portal fields. The ensuing concentric fibrosis would cause cholangiocyte atrophy secondary to ischaemia and would lead to progressive cholestasis, ongoing fibrosis and secondary biliary cirrhosis.[11] Experimental evidence to support the 'leaky-gut' hypothesis suggests that bacterial overgrowth in the small intestine and the infusion of bacterial antigens into the portal circulation of mice can indeed lead to hepatic inflammation with at least some characteristic features mimicking human PSC. It is therefore tempting to hypothesise that in genetically susceptible individuals bacterial antigens function as molecular mimics to trigger the immune response

Table 2: IBD in PSC — a unique intestinal clinical phenotype.

	IBD alone		IBD with PSC
	Ulcerative colitis	**Crohn's disease**	
Male:female skew	No	No	Male predominance
Distribution of inflammation	Starts at the rectum and progresses proximally in a continuous manner	Most common distribution is ileocaecal disease, but any part of the gastrointestinal tract can be affected	Typically pancolonic with more severe disease in the proximal colon 23% have right-sided disease only
Severity of inflammation	Variable 25% develop at least one episode of acute, severe colitis in 12.7 years	Variable depending upon disease location: 12% likely to be relapse-free over 10 years 50% will require IBD-related surgery during their lifetime	Usually quiescent and asymptomatic intestinal disease
Risk of colonic neoplasia	Up to 5% over 20 years	Up to 5% (with colonic disease) over 20 years	Up to 30% over 20 years
Backwash ileitis	12–17%	N/A	51%
Rectal sparing	Uncommon; 5%	Common	Common; 51–65%

IBD = inflammatory bowel disease; PSC = primary sclerosing cholangitis.

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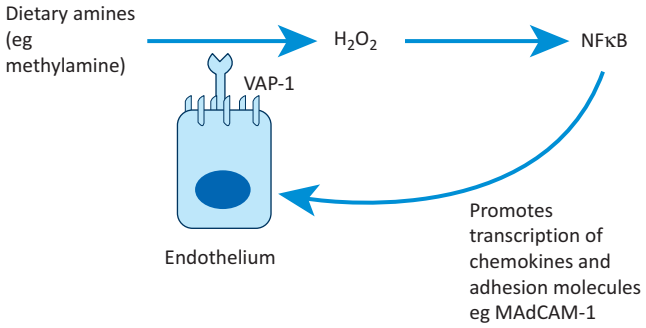

Fig 2. Vascular adhesion protein-1 (VAP-1), an adhesion molecule with amine oxidase enzymatic activity. The deamination of dietary methylamine in the presence of a proinflammatory stimulus leads to the transcriptional upregulation of chemokines and other adhesion molecules such as MAdCAM-1.

that initiates PSC. Human studies, however, have provided little evidence for increased portal vein bacteraemia in PSC patients. Support against this concept might also be drawn from studies that have found antibiotics inefficacious in treating PSC patients in the absence of bacterial ascending cholangitis.

Cellular immunity and aberrant lymphocyte homing. The portal infiltrate in PSC consists predominantly of T-lymphocytes. The final common pathway in IBD and its hepatic complications is that of a destructive inflammatory infiltrate, and evidence implicates mucosal-derived lymphocytes in the pathophysiology of extra-intestinal disease. Recent insights into the molecular basis of lymphocyte homing have suggested novel mechanisms to explain how extra-intestinal complications can occur many years after inflammation in the gut has resolved. In adults, although the liver and gut have distinct endothelial phenotypes, there might be overlapping expression of certain adhesion molecules and chemokines. Under normal conditions, vascular adhesion protein-1 (VAP-1) expression on hepatic endothelial cells (HEC) is far stronger than that observed on mucosal vessels. However, gut expression of VAP-1 is greatly increased in IBD, suggesting that liver-derived lymphocytes (LDL) that express the VAP-1 receptor might be able to enter inflamed bowel. Similarly, the expression of MAdCAM-1 and the chemokine CCL25 are normally restricted to the gut; but in PSC, MAdCAM-1 is also found on portal vein endothelium and CCL25 on periportal sinusoidal endothelium. Over 90% of lymphocytes in the small bowel express CCR9 and α4β7; in PSC, 20% of liver-infiltrating lymphocytes (LILs) are also $CCR9^+\alpha4\beta7^+$, most likely being recruited to the liver by aberrant expression of CCL25 and MAdCAM-1.[12] This would allow immune surveillance across both sites in a process by which long-living, mucosal-derived memory cells are recruited rapidly to the liver if some trigger leads to upregulation of hepatic MAdCAM-1 (Fig 1b). If these memory cells were to become reactivated, by cross-reactive antigens in the liver or by gut antigens entering via the portal circulation, this could initiate an inflammatory response and result in chronic hepatic inflammation.

The exact factors leading to aberrant expression of CCL25 and MAdCAM-1 in the PSC liver are incompletely understood, although recent work has demonstrated that the imprinting and plasticity of a gut-homing phenotype on human T-cells (expressing CCR9 and α4β7) requires primary activation or reactivation by DCs resident in the gut. The inability of liver DCs to imprint gut tropism supports the notion that $\alpha4\beta7^+CCR9^+$ T-cells that infiltrate the liver in PSC are primed in the gut.[13]

VAP-1, as well as behaving as an adhesion molecule, possesses amine oxidase activity (Fig 2). Recent evidence has shown that deamination of methylamine (a compound found in various foodstuffs, wine and cigarette smoke) by VAP-1 is able to induce the expression of functional MAdCAM-1 on endothelial cells *in vitro* and *in vivo*.[14] Increased levels of dietary methylamine resulting from enhanced absorption via an inflamed gut may thus act as a substrate for VAP-1, thereby increasing MAdCAM-1 expression in the hepatic endothelium. This could promote the uncontrolled recruitment of mucosal effector cells and result in tissue damage that is characteristic of both IBD and PSC.

Infections of the gut and liver

Most T-cells that infiltrate the liver are 'primed' cells, including those with specificity for persistent viruses, suggesting that the trafficking of memory T-cells through the liver contributes to immune surveillance. The ability of mucosal memory lymphocytes to enter and respond to antigens in the liver might, however, be restricted by the tolerogenic cytokine milieu, and could result in the death of many enteric lymphocytes that enter the liver. The cytokine IL10 is a key mediator of liver tolerance and it is implicated in the resolution of gut inflammation and in the regulation of immune responses to gut parasites, some of which also infect the liver. For example, oral infection with the nematode *Trichinella spiralis* results in a severe hepatitis in IL10 knockout animals as a consequence of a potent $CD4^+$ T-cell-mediated response to parasites migrating via the portal vein into the liver.[15] An ongoing intestinal immune response is necessary for the development of hepatitis, which is driven by gut-derived $CD4^+$T-cells that are recruited to the liver. In wild-type animals, IL10 prevents the development of hepatitis in response to parasites entering the liver, allowing the worm to survive outside the gut, but if IL10 is absent, a vigorous immune response ensues.[15] This infection provides a very clear example of how local IL10 can completely suppress immune responses to pathogens that enter the liver from the gut.

Conclusions

Knowledge of the mechanisms that underpin co-ordinated immune responses between the gut and liver will allow us to further elucidate the pathophysiology of how intestinal diseases are associated with specific hepatic manifestations. Although data supporting a direct role for intestinal microbiota is contentious, the translocation of gut bacteria or bacterial products

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might be episodic, could be hard to detect and might contribute to hepatobiliary disease progression rather than initiation. Future studies that endeavour to dissect the liver and gut microbiome might provide further clues about the underlying the pathophysiology behind linked intestinal and hepatic diseases. The important role played by the homing of mucosal T-cells expressing α4β7 in response to aberrantly expressed gut addressins suggests that blocking these pathways might prevent the recruitment of effector cells into the liver. Therapeutic inhibitors of both CCR9 and α4β7 are currently in development for the treatment of IBD, and it will be interesting to see whether these reagents provide benefit in treating associated hepatobiliary disease.

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