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THE ROLE OF CHOLANGIOCYTES IN THE DEVELOPMENT OF CHRONIC INFLAMMATORY LIVER DISEASE

David H. Adams and Simon C. Afford

The Liver Research Laboratories, MRC Centre for Immune Regulation, The University of Birmingham Institute of Clinical Science, The Queen Elizabeth Hospital, Edgbaston, Birmingham B15 2TH, United Kingdom

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1. ABSTRACT

Cholangiocytes constitute the biliary epithelium, an important barrier to infection entering the liver via the gastrointestinal tract. These cells have developed mechanisms to respond to infection by recruiting and interacting with effector leukocytes to clear bacterial or viral pathogens. Cholangiocytes are also targets of immune mediated damage in several liver diseases and under these circumstances protective mechanisms, for example the secretion of chemokines and expression of adhesion molecules, become harmful and promote the inappropriate recruitment and retention of effector cells within portal tracts. In chronic inflammatory biliary diseases such as primary biliary cirrhosis and primary sclerosing cholangitis, infiltrating leukocytes destroy bile ducts by killing cholangiocytes via complex molecular mechanisms involving Fas-dependent apoptosis and autocrine and paracrine interactions with other members of the TNF superfamily including CD40. A better understanding of these processes may lead to novel therapeutic approaches aimed at switching off the chronic inflammatory response and protecting bile ducts from apoptosis.

2. INTRODUCTION

The main functions of the liver include the removal of toxins from the blood, the uptake and processing of nutrients delivered via the portal vein from the gut, the production of proteins and the secretion of bile. In addition the liver plays an important role in immune surveillance by clearing infectious agents and regulating immune responses to antigens delivered via the portal vein from the gut(1). The bulk of the liver mass is formed by hepatocytes arranged in hexagonal lobules encapsulated by connective tissue septa. Within each lobule hepatocytes are arranged in contiguous polarised double rows or sinusoids. On the luminal surface the hepatocytes are separated from the blood by the space of Disse, and a unique fenestrated endothelium which allows for exchange of soluble blood borne components. The sinusoids also contain two types of resident haematopoetic cells, tissue macrophages (Kupffer cells) and natural killer lymphocytes (Pit Cells) which provide a first line innate immune response against microbial agents and tumour cells(2). A specialised fibroblast, the hepatic stellate cell or Ito cell resides within the Space of Disse and can be activated in response to inflammation or tissue damage(3).

On the abluminal surface, the hepatocytes are in contact via tight junctions and canaliculi between pairs of hepatocytes form the terminal branches of the biliary tree into which hepatocytes secrete bile. The canaliculi drain into intrahepatic bile ducts, true ductular structures formed by a distinct epithelial cell, the cholangiocyte. These intrahepatic bile ducts drain into larger segmental ducts and eventually form an interconnecting branching system which transports bile from the liver into the gut. The intrahepatic bile ducts are found in portal tracts between the liver lobules. Together with branches of the hepatic artery and the portal veins the intrahepatic bile ducts form the portal triad.

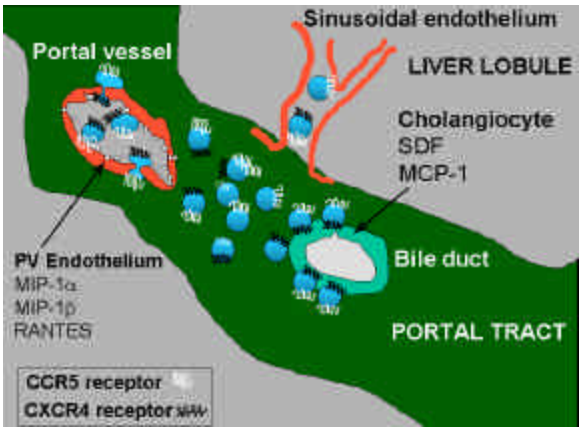

Figure 1. Leukocyte recruitment to the portal tract. Leukocytes are recruited via portal vessels in response to appropriate adhesion molecules and promigratory signals delivered by chemokines including the CCR5 ligands RANTES, MIP-1α? and MIP-1β on portal endothelium. The infiltrating cells are localised to the biliary epithelium in bile ducts by chemokines expressed and secreted by cholangiocytes. Non-inflamed cholangiocytes express SDF the chemokine ligand for CXCR4 strongly, and when inflamed secrete several other chemokines including IL-8, RANTES and MCP-1 allowing them to localise effector cells to the epithelial barrier.

Blood entering the liver from the portal vein and hepatic artery mixes in the sinusoids before returning to the systemic circulation via the central or terminal hepatic veins at the centre of each lobule (4). The liver is therefore exposed to exogenous antigens from two distinct sources. Firstly antigens which evade the mucosal immune system are delivered to the liver via the portal vein in association with nutrients and harmless food antigens. This need to discriminate and respond accordingly to potentially harmful antigens and benign essential food antigens is responsible for the liver's ability to generate immune tolerance against antigens delivered via the portal vein (1). The other site of exposure is at the level of the biliary epithelium. The biliary system is continuous with the GI tract and thus exposed to microbial agents in the gut lumen. It is thus critical that mechanisms are present to prevent ascending infection via the biliary system.

Protection against infection is afforded by several mechanisms. Firstly, the continuous bile flow discourages ascending infection by luminal bacteria. This is demonstrated by the high incidence of severe bacterial infection and ascending cholangitis in patients with biliary tract obstruction (5). If microbial agents do gain access to the biliary epithelium, several mechanisms have evolved to limit the infection. The portal tracts contain elements required to generate both innate and cognate immune responses against invading pathogens. The immediate response is afforded by resident intraepithelial type lymphocytes associated with the bile ducts(6). These bile duct associated T cells display cytolytic activity, and can lyse intestinal epithelial cell lines, suggesting that they share characteristics with intestinal intraepithelial lymphocytes and are able to act as a first line defence to antigens entering via the biliary tract.

In addition cholangiocytes respond to inflammatory damage or exposure to bacterial and viral products by secreting cytokines and chemokines that are capable of stimulating an inflammatory response. For example when stimulated by proinflammatory cytokines, cholangiocytes secrete several chemokines at concentrations which are capable of attracting leukocytes in *in vitro* migration assays suggesting that they contribute significantly to local chemokine levels (7). These epithelial-derived chemokines can be transcytosed to the portal endothelium where they promote the recruitment of leukocytes by stimulating transendothelial migration into the portal tracts in addition to attracting recruited cells to the epithelial surface (8) **(**Figure 1**)**. The type of chemokines secreted by cholangiocytes and thus the nature of the leukocytes recruited depends on the activating stimulus. Interleukin-1 (IL), bacterial lipopolysaccharides (LPS) or tumor necrosis factor alpha (TNFα) result in secretion of large amounts of IL-8, ENA-78 (epithelial cell derived neutrophil activating protein) and GRO, which predominantly recruit neutrophils. Activation of these signals by bacterial infection would trigger a rapid and appropriate neutrophil response. In contrast, BEC treated with interferon gamma (IFNγ) show reduced IL-8 secretion associated with a marked upregulation of monocyte chemoattractant protein 1 (MCP-1) and three other chemokines involved in lymphocyte recruitment, monokine induced by IFNγ (Mig), interferon inducible T cell alpha chemoattractant (ITAC) and interferon gamma inducible protein 10 (IP10) (7,9). The ability of IFNγ to stimulate selectively the production of chemokines that act on monocytes and lymphocytes in preference to those that act on neutrophils has been reported for other human epithelial cells including hepatocytes(10,11). These observations suggest that the epithelium itself plays a role in switching the cellular response from one in which neutrophils predominate to a more chronic inflammatory response characterised by mononuclear cell recruitment in response to MCP-1, Mig and IP-10.

This ability of cholangiocytes to express and secrete chemokines will be important for local immune defence within the liver. The biliary epithelium is an important point of contact and potential pathogenic micro-organisms in the bile or ascending from the intestine via the biliary tract need to provoke a rapid neutrophil response. Cholangiocyte derived chemokines, secreted in response to bacterial products such as LPS or cytokines such as IL-1 and TNFα that are released early in the course of bacterial infection, could be key factors in the initial recruitment and activation of leukocytes to combat invading pathogens.

The biliary epithelium can be infected by several pathogens including viruses and cryptosporidiosis. Infectious agents are rarely associated with direct bile duct damage but they may be contributing factors to bile duct loss. Hepatitis C virus (HCV) infection can be associated with a lymphocytic inflammation of intrahepatic bile ducts and AIDS cholangiopathy is characterised by a sclerosing

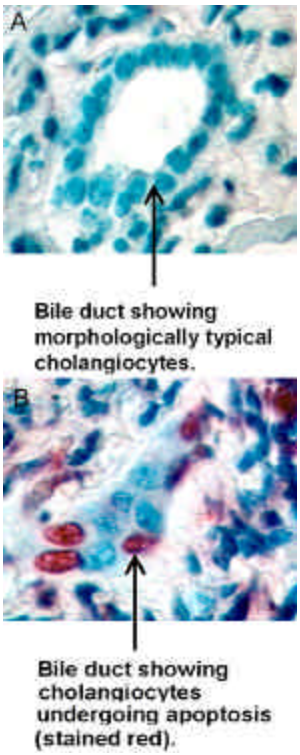

Figure 2. In normal non inflamed liver, bile ducts show little evidence of cholangiocyte apoptosis (Panel A). However during sustained inflammation of the portal tract, in cases of PBC or chronic allograft rejection for example, bile ducts surrounded by an inflammatory infiltrate of CD3 +ve T cells and CD14/CD68+ve monocytic cells frequently contain cholangiocytes undergoing apoptosis which are revealed by in situ DNA end labelling methods and nuclear morphology (Panel B).

inflammatory cholangitis (12,13). In some immunocompromised individuals, protozoal infections have also been reported to cause a sclerosing cholangitis like syndrome with accompanying bile duct loss (14). If bile ducts are directly infected then the immune system needs to be able to selectively kill infected cholangiocytes. The mechanisms involved in these processes are poorly understood although there is evidence that when they are deficient, as is the case in HIV infection or X-linked hyper IgM syndrome susceptibility to biliary infections increases (14-16).

3.1. Inflammatory Diseases of the Biliary Tract – The Vanishing Bile Duct Syndromes

Although the liver experiences regular inflammatory insults, its capacity to mount a regenerative response together with its relative immune privilege, ensures that the inflammation is controlled and normal liver architecture and function is restored in most cases. However, in a number of circumstances inflammation in the liver fails to resolve and permanent progressive damage occurs leading ultimately to fibrosis, and cirrhosis (3). In biliary cirrhosis, the cholangiocytes are the initial targets for immune or inflammatory attack and once significant cholangiocyte destruction occurs, the integrity of the biliary system becomes compromised to the extent where bile transport is obstructed, and a vicious irreversible cycle of inflammation and tissue damage ensues (17-20).

Cholangiocytes are important cellular targets for immune-mediated damage in a number of liver diseases characterised by a progressive destruction of intrahepatic bile ducts and collectively referred to as the vanishing bile duct syndromes (VBDs). Vanishing bile duct syndromes are defined histologically by the loss of bile ducts from more than 50% of identifiable portal tracts(21). Whilst it is often the small interlobular ducts that are affected, larger ducts may also be involved in some conditions. The diseases associated with bile duct loss include developmental diseases such as intra and extrahepatic biliary atresia, where there is disordered development of the biliary tree during embryogenesis usually accompanied by a vasculopathy. The commonest group in adults are the immune–mediated diseases which include autoimmune cholangitis, primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), graft versus host disease (GVHD) and liver allograft rejection. In all of these diseases, the progressive loss of bile ducts is associated with leukocytic infiltration of the portal tracts and lymphocyte-mediated apoptosis of cholangiocytes (22,23) (Figure 2).

The reasons why bile ducts are infiltrated in these diseases are poorly understood. Under certain circumstances cholangiocytes can express auto and alloantigens and co-stimulatory molecules that would render them susceptible to cell-mediated damage and their ability to contribute to the active recruitment of leukocytes suggest they are active participants in inflammatory biliary disease(24-27). Thus inflammatory bile duct diseases may be the consequence of an aberrant inflammatory response, which is either triggered inappropriately or which fails to resolve. In this review we shall concentrate on the role of cholangiocyte apoptosis and proliferation in regulating this switch to chronic inflammatory damage.

3.2.What makes cholangiocytes targets for inflammatory attack?

The liver responds to injury by undergoing a proliferative response leading to restoration of normal liver architecture and function. Anti-proliferative mechanisms have evolved to limit this response and to prevent the development of a malignant phenotype in response to regeneration (28). As a consequence both cholangiocytes and hepatocytes are highly sensitive to apoptotic death (29). In addition, epithelial cell apoptosis in the liver can be triggered by infiltrating effector leukocytes. This response is beneficial when it allows the immune system to rapidly clear infected epithelial cells, but when it is triggered inappropriately or allowed to continue after clearance of the offending pathogen, it can result in irreversible inflammatory bile duct damage and loss (30).

3.3. The role of tumor necrosis factor receptor (TNFr) superfamily members in cholangiocyte apoptosis

Human and animal studies conducted over the last decade have established that hepatocytes are

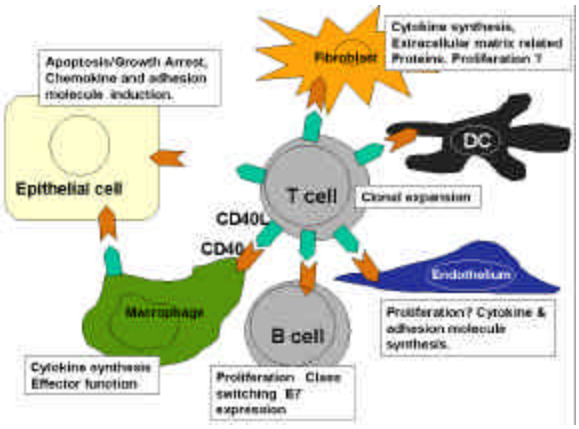

Figure 3. Activation of CD40 during Liver Inflammation may have complex consequences. CD40 is expressed by a wide range of liver cell types during inflammation (epithelium, macrophages, endothelium, fibroblasts, B cells and dendritic cells) suggesting that it is involved in the promotion of a wide range of cell specific proinflammatory responses. CD40L expression is confined (in human liver tissue) to activated T cells and macrophages. Another important source of CD40L is platelets which may present functional ligand to hepatic endothelium. Synthesis of CD40L by endothelial cells has been reported *in vitro*, although the significance of this remains to be demonstrated *in vivo*.

intrinsically sensitive to apoptotic death mediated via activation of members of the TNFr superfamily particularly Fas (CD95) (31-34). Fas is widely expressed on hepatocytes and upregulated on both hepatocytes and cholangiocytes during inflammation (35). Direct activation of Fas has been shown in a number of systems to invoke massive hepatocyte damage and fulminant liver failure (36). More recent evidence suggests that cholangiocytes are also highly susceptible to Fas-mediated attack(30). Given the marked ability of Fas engagement to kill cells by apoptosis, it is not surprising that regulatory mechanisms have developed in which Fas activation is controlled by other molecules, acting in a paracrine or autocrine fashion. In these models Fas mediated apoptosis can be amplified by activation of other TNFr family members thereby providing a mechanism for controlling Fas-mediated responses(37). One of the first examples of this mechanism was described in hepatocytes and involves the activation of another closely related member of the TNFr family, CD40(35). We have now generated data to support a similar role for CD40 in cholangiocyte apoptosis in PBC and chronic graft rejection (30).

In both hepatocytes and cholangiocytes, activation of CD40 on the cell surface triggers autocrine Fas ligand (CD95L) upregulation leading to autocrine and paracrine activation of Fas. The requirement for Fas in this model is demonstrated by the ability to block the effects of CD40 activation by neutralising either Fas or FasL. Under basal conditions hepatocytes and cholangiocytes express little CD40 *in vivo*, but expression is upregulated in response to inflammation, and *in vitro* CD40 expression increased on cholangiocytes in response to cytokines including TNF(30). *In vitro*, soluble CD40 ligand (CD40L, CD154, gp39) or crosslinking antibodies can both activate CD40 mediated Fas dependent killing although the role of soluble TNFs including CD40L *in vivo* is not yet known. In inflammatory liver diseases CD40L expression is increased at sites of active bile duct inflammation where cholangiocyte apoptosis occurs, and is restricted to macrophages and infiltrating effector lymphocytes. Thus activation of this amplification mechanism will only occur in the presence of an inflammatory response characterised by a chronic inflammatory infiltrate. Since the reports of cooperative interactions between CD40 and Fas, similar results have been reported for other TNFr family members(37). For example the cytotoxic effects of TNFR2, CD30 and CD40 have been shown in other systems to be mediated via autocrine or paracrine activation of TNFR1. Our unpublished data suggest this mechanism may also apply to cholangiocytes.

A number of studies provide compelling circumstantial support for the importance of CD40 mediated Fas dependent cell death in the liver. Studies in mice and humans suggest that genetic perturbations of the CD40/CD40L system have a number of liver specific consequences. CD40L deficient mice demonstrate an inability to clear certain biliary infections resulting in persistent cholangitis(16). Humans with the X linked hyper IgM syndrome are also deficient in functional CD40L and these patients display a similar phenotype characterised by a failure to clear cryptosporidial infections of the biliary tract leading to persistent chronic infection and a cholangiopathy with features similar to sclerosing cholangitis. The lack of CD40L also results in a cholangiocytic dysplasia predisposing these patients to the development of cholangiocarcinoma (14).

The consequences of CD40 activation in the inflammatory environment are likely to be more far reaching than simply the destruction of cholangiocytes and hepatocytes via Fas mediated apoptosis. That the CD40/CD40L system plays a pivotal role in modulating many facets of the inflammatory response is well illustrated by recent studies demonstrating that overexpression of CD40 ligand in murine epidermis leads to a massive inflammatory response in the skin(38). CD40 can be expressed by the majority of cell types in the liver, including cholangiocytes, hepatocytes, endothelial cells, infiltrating leukocytes and fibroblasts, whereas expression of CD40L is restricted to platelets, a subpopulation of activated T lymphocytes and macrophages (30,39,40). Signals mediated via CD40 modulate a wide range of biological functions including stimulating adhesion molecule expression, chemokine secretion and extracellular matrix deposition as well as the provision of costimulatory signals which promote T cell/B cell interactions and proliferation (Figure 3).

3.4. Other mechanisms of cholangiocytes apoptosis

Within the context of the inflammatory response, there are a number of ways in which cholangiocytes can be eliminated via apoptotic mechanisms, in addition to direct

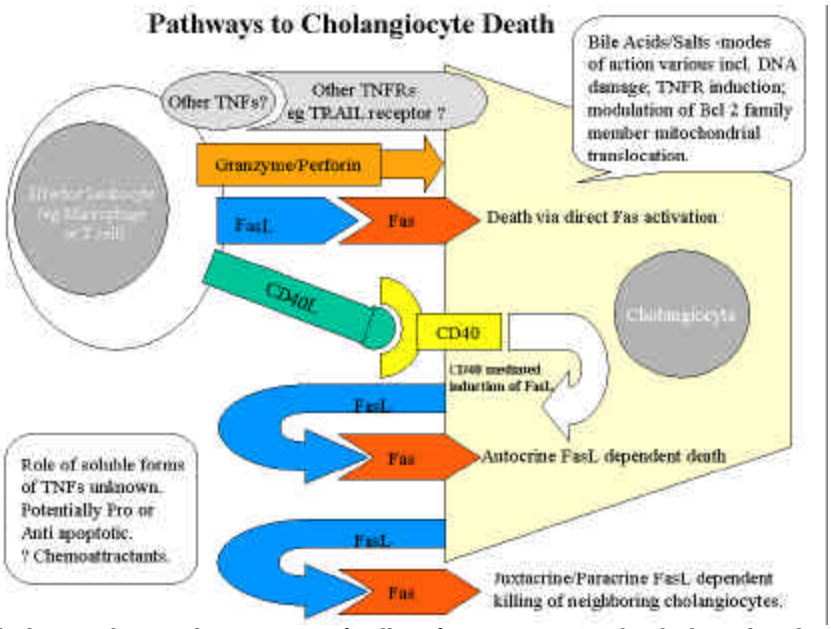

Figure 4. Activated leukocytes have a diverse array of cell surface weaponry with which to directly induce cholangiocyte apoptosis including TNF, Fas ligand, possibly other TNF family members, and the granzyme/perforin system. Autocrine or paracrine Fas-mediated cholangiocyte apoptosis can also be induced via CD40L expressed on activated macrophages or T cells. Which pathway or combination of pathways is utilised will depend upon the inflammatory microinvironment. Soluble forms of TNF family members including FasL and CD40L may exist, although their function in tissues remains unknown. During biliary obstruction and cholestatic liver disease, components of bile can act in a number of ways to exacerbate cholangiocyte death via diverse mechanisms which can involve direct DNA damage, induction of TNFRs or modulation of survival factor translocation to the mitochondrial membrane.

activation of cell surface Fas. 1) TNF? , which is produced by mononuclear cells, including Kupffer cells and endothelial cells in the inflamed liver can kill cholangiocytes directly (31) as well as by activating the Fas-dependent mechanisms described above. 2) Cytolytic T lymphocytes express perforin and potent apoptosis inducing proteolytic enzymes called granzymes which they inject into target cells resulting in caspase activation and apoptosis. There is circumstantial evidence that cholangiocytes may be killed by this pathway alongside Fas-mediated mechanisms in chronic inflammatory liver diseases(41,42). 3) Although some bile acids or salts such as ursodeoxycholic acid (UDCA) are cytoprotective(43) others, particularly tauroursodeoxycholate (TDC) and glycochenodeoxycholate (GCDC) can induce apoptosis directly. Their capacity to do so generally correlates with their degree of hydrophobicity and their modes of action seem to be various(44). Thus in severe cholestatic liver diseases this mechanism may contribute and amplify bile duct loss occurring as a consequence of immune mediated attack(45).

The hierarchical importance of these mechanisms *in vivo* is not clear. It seems likely that any or all of them could contribute to cholangiocyte apoptosis, but that different contributions will come from the specific pathways depending on the underlying disease\pathogenesis (Figure 4).

3.5. The molecular basis of leukocyte/cholangiocyte interactions

Cholangiocytes can express many of the key molecules which promote interactions with effector leukocytes including the cell adhesion molecules intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) and MHC antigens(46,47). However, under most circumstances they do not express CD80 or CD86, ligands for the T cell receptor CD28 signals through which are required for optimal costimulation (26,27,48). In response to appropriate inflammatory signals they will also secrete a number of chemokines including IL8, MCP 1, Mig, IP10 and ITAC, which are important for effector cell recruitment. Thus cholangiocytes are able to actively recruit and retain effector leukocytes allowing these cells to interact via a number of pathways, including CD40. There is evidence that under certain conditions murine cholangiocytes can act as antigen presenting cells when they express MHC II antigens and costimulatory molecules including CD40 and the CD28 ligands CD80 and CD86(24-26) although it is not clear whether this is functionally important in man (27,48). The consequences of such interactions *in vivo* are not clear. Evidence from other systems suggests that when epithelial cells act as non-professional antigen presenting cells, they are unable to provide full costimulation for naïve T cells and in fact induce non-responsiveness in such cells(49,50). However, the majority of cells that come into contact with activated cholangiocytes will be primed T cells, and cholangiocytes

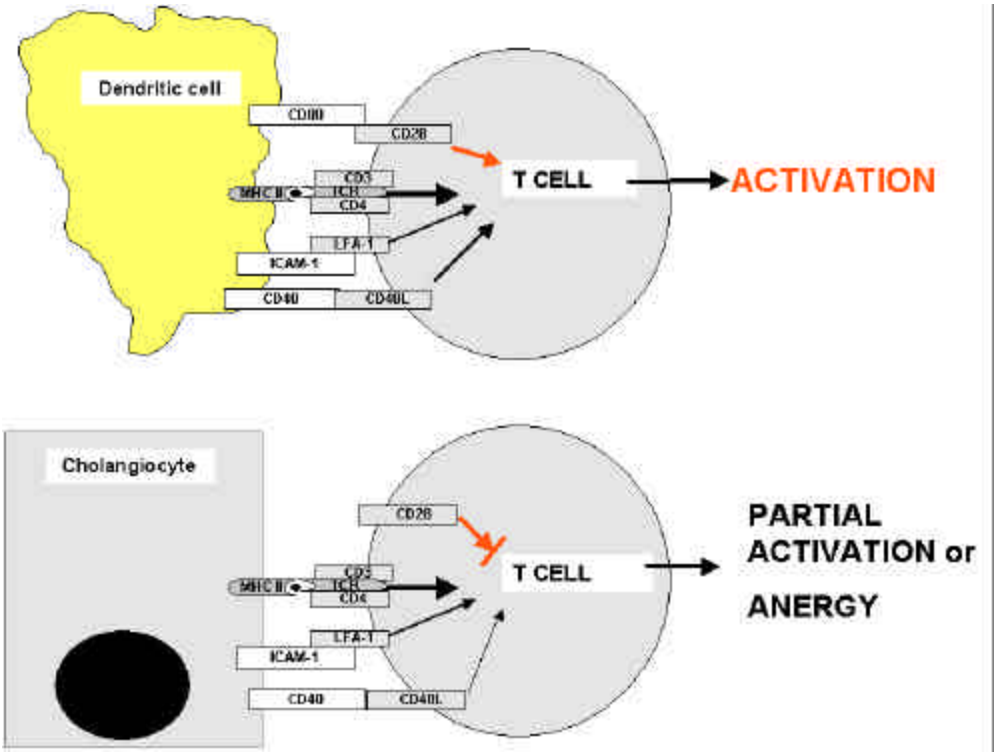

Figure 5. Resting cholangiocytes express MHC I but low levels of MHC II and molecules capable of providing co-stimulation to infiltrating T cells. When activated cholangiocytes express higher levels of ICAM-1, facilitating interactions with T cells, and upregulate MHC II and CD40, which can act as a co-stimulatory signal. However they lack the CD28 ligands CD80 or CD86 suggesting that they will still not be able to fully activate T cells. Indeed their ability to provide a signal through the T cell receptor in the absence of costimulation through CD28 suggests that they could induce anergy. This mechanism applies to other epithelial cells and may be an important mechanism for down-regulating autoimmune responses in peripheral tissues.

may be able to provide signals which promote the local proliferation and survival of such cells (Figure 5).

3.6. Lymphocyte recruitment in chronic biliary disease – Resolution or Progression to Chronic Inflammation?

The chronic inflammatory biliary diseases are characterised by a number of common histopathological features including the progressive loss of intralobular bile ducts with evidence of increased apoptosis in surviving ducts (Figure 1), and the development of interface hepatitis and bridging fibrosis. These processes are associated with activation of myofibroblasts or stellate cells which are responsible for the development of fibrosis and cirrhosis(23,51). The majority of end-stage liver failure is the consequence of chronic inflammation which fails to resolve and in most chronic inflammatory biliary diseases, this process cannot be switched off using current anti-inflammatory or immunosuppressive therapy. In the absence of effective therapies, new approaches to the treatment of chronic inflammatory liver disease are required. For such approaches to be effective and have few long term adverse effects, it is likely that treatment will be required early in the disease process before the vicious cycle which sustains chronic inflammation becomes established. This approach would require identifying at risk patients before they are symptomatic which is not currently practical for the majority of biliary diseases. Failing that, preventative intervention therapy needs to be able to break the cycle which sustains chronic inflammation before irreversible damage is done. Such approaches depend on understanding the signals that drive chronic inflammation.

Experimental and clinical evidence implicates TNFα and the lymphotoxins in these processes. Ectopic expression of lymphotoxin transgenes in tissue leads to chronic inflammation and the establishment of tertiary lymphoid tissue which can act as routes for the continued recruitment of lymphocytes (52-54). Such tertiary lymphoid tissue or neolymphogenesis is a feature of chronic biliary diseases including PBC, PSC and hepatitis C(55,56) when infiltrates organise into lymphoid follicles containing B and T lymphocytes, dendritic cells (DCs) and new $CD34^+$ $MAdCAM\text{-}1^+$ vessels with the morphology of high endothelial venules(56,57). This process of lymphoid neogenesis, or the development of new lymphoid tissue in inflammatory sites when normal lymph node development is complete, has been reported in several chronic immune-mediated diseases including rheumatoid arthritis(58,59). Because these inflammatory lymphoid follicles provide a microenvironment for the recruitment and retention of lymphocytes at sites of chronic inflammation, understanding the signals involved in lymphocyte

recruitment to these sites will provide insights into the pathogenesis of chronic inflammation and may suggest new therapeutic targets(60).

In addition to lymphoid follicles, the fibrous septa which are present in cirrhotic livers are also rich in newly formed blood vessels. These have been referred to as "fibrovascular membranes" (61) and are an important component of evolving fibrosis because they provide a potential pathway for lymphocyte recruitment. Fibrovascular membranes may also be important for producing the lesion of interface hepatitis, which is seen at the periphery of portal tracts in many biliary diseases, including PBC and PSC.

The chemokine stromal cell derived lymphocyte chemoattactant (SLC, CCL21) is expressed predominantly in lymphoid tissue where it acts to recruit lymphocytes and dendritic cells (DCs) bearing its receptor chemokine receptor 7 (CCR7, 62-64). That SLC plays a critical role in the development and organisation of lymph nodes(65) is shown by the failure of lymph node development in mice that are deficient in SLC(66). Moreover, recent animal studies report that SLC expression is sufficient for lymphoid neogenesis because tissue-specific expression of an SLC transgene(65) or induction by lymphotoxin(52) results in ectopic lymphoid neogenesis.

We have recently reported the induction of SLC (CCL21), which was previously considered to be a constitutive chemokine, on stromal tissues surrounding portal vessels and in lymphoid aggregates in chronic inflammatory biliary disease(55). SLC was also expressed on the endothelium of CD34+ neovessels at the periphery of fibrous septa and consistent with a role for SLC, significant numbers of both $CD45RA^+$ and RA^- $CCR7^+$ T cells were detected within the liver of patients with PSC and PBC. Thus increased expression of SLC in portal tracts may be important for sustaining the recruitment and retention of lymphocytes in chronic biliary diseases leading to bile duct damage and destruction. The endothelial adhesion molecule MAdCAM-1 is critical for lymphocyte homing to mucosa associated lymphoid tissues(67,68) including inflamed mucosal tissue in inflammatory bowel disease(67). MAdCAM-1 has recently been detected *de novo* on portal vessels and in lymphoid aggregates in inflammatory liver diseases including PSC associated with portal lymphoid follicles(56, 69) suggesting that the recruitment of mucosal lymphocytes to the liver may be critical in the pathogenesis of these diseases.

3.7. What drives persistent inflammation and bile duct damage in VBDS?

In the end-stages of chronic allograft rejection, the inflammatory infiltrate may be reduced compared with early disease but despite this tissue damage, including the loss of bile ducts, continues relentlessly. This may be a consequence of aberrant activation of Fas-mediated apoptosis by the mechanisms outlined above. Our data suggest that CD40 amplification of Fas-mediated apoptosis will also continue to be important because a high proportion of the persisting inflammatory cells, either macrophages or T lymphocytes are strongly positive for CD40L in the setting of high CD40 expression.

This persistent CD40L expression in chronic inflammation contrasts with *in vitro* studies of T lymphocytes where CD40L expression in response to activation is usually transient in nature. Little is known about the regulation of CD40 ligand expression and the factors which may maintain its expression *in vivo.* It is likely that chronic inflammation alters the microenvironment in favour of persistent CD40L expression(70) and this process may involve the activation of specific signal transduction pathways and transcription factors. These are likely to include nuclear factor kappa B (NFκB) dependent mechanisms but may also involve other as yet poorly understood mechanisms including activation of the novel AT hook transcription factor AKNA 1 which has a profound influence on CD40/CD40L expression in T lymphocytes *in vitro*(71).

This persistent CD40L expression may be a critical factor in driving and sustaining chronic inflammation. In addition to providing the signal for effector cell amplification of target cell apoptosis, activation of CD40 on other cell types may help to shape the chronic inflammatory microenvironment. For example, activation of CD40 on fibroblasts triggers the release of inflammatory mediators including chemokines, extracellular matrix proteins, and metalloproteinase inhibitors and thus promotes fibrogenesis and activation of CD40 on endothelial cells leads to endothelial cell (EC) proliferation, chemokine secretion and a proinflammatory phenotype which will promote the continuing recruitment of leukocytes thereby helping to sustain the chronic inflammatory response(72). Finally CD40/CD40L interactions are also likely to have direct effects on the infiltrating CD40L bearing effector cells. Intracellular signalling via CD40L in T lymphocytes leads to JNK, p38 and lck activation, which are likely to have profound effects of cell survival and activation although the downstream functional consequences of such pathways in the context of tissue leukocytes are not known(73).

3.8. Potential for Therapeutic Intervention

If the lymphoid follicles and chronic inflammatory response in VBDS are driven by TNF, lymphotoxin or CD40L targeting these cytokines may switch off the persistent inflammation. Anti-TNF therapy is successful in rheumatoid arthritis and Crohn's disease, two chronic inflammatory diseases which respond poorly to conventional immunosuppression and probably works by remodelling lymphoid tissues rather than blocking acute inflammation(74,75). It is therefore possible that anti-cytokine therapies may be effective in chronic biliary diseases, if given early enough before bile duct loss is too advanced.

CD40 may be a particularly attractive target. CD40 blockade down-regulates antigen-specific responses in models of transplantation by its effects on lymphocyte costimulation(76,77). However, it may also act to regulate chronic inflammation as discussed above and in the context

of VBDS by preventing cholangiocyte apoptosis. The fact that patients who lack CD40L have a failure of cholangiocyte apoptosis provides further evidence of the biological importance and thus evolutionary conservation of this function (14). Thus CD40 blockade may be effective in chronic liver disease. If sustained production of CD40L by tissue macrophages and effector lymphocytes is the pivotal mechanism that promotes CD40 activation, chronic inflammation and bile duct apoptosis as we suspect, then therapies could be aimed specifically at these populations of cells as well as at blocking CD40.

4. PERSPECTIVE

Cholangiocytes are important targets of immune mediated diseases and they contribute actively to local inflammation by secreting cytokines and chemokines capable of recruiting and localising effector cells within portal tracts. In chronic inflammatory diseases of the bile ducts, this results in the establishment of tertiary lymphoid structures and continuing leukocyte recruitment and retention within the liver. The mechanisms of bile duct loss in these diseases involve Fas-dependent apoptosis and this may be amplified by complex autocrine and paracrine interactions involving members of the TNF superfamily, particularly CD40 and its ligand. A better understanding of these processes may lead to novel therapeutic approaches aimed at switching off the chronic inflammatory response and protecting bile ducts from apoptosis.

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Send correspondence to: Dr Simon C. Afford, The Liver Research Laboratories, MRC Centre for Immune Regulation,The University of Birmingham Institute of Clinical Science, The Queen Elizabeth Hospital, Edgbaston, Birmingham B15 2TH UK.Tel: 44-0121-472 1311 Ext. 3805, Fax: 44-0121-627-2497, E-mail: S.C.AFFORD@bham.ac.uk