

## Concluding remarks

With the current pace of technological advance, one can safely predict that our understanding of liver disorders and their treatment will continue to improve. Advances in ultrasound, computed tomography, and magnetic resonance imaging, of which space has not allowed consideration, are already enabling the detection of small hepatocellular cancers at the stage when local ablation techniques can give long-term survival. The use of stem cells to enhance regeneration and remodelling of the liver may be but a pious hope but there is already evidence from Professor John Iredale that the breakdown of fibrosis is a feasible proposition.

Enabling clinicians to work alongside scientists with multi-disciplinary skills in dedicated centres will continue to be the most effective way of enhancing knowledge and expertise in the specialty, a view from which I have never deviated.

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## CURRENT KEY DEVELOPMENTS

### Mucosal lymphocytes in the pathogenesis of the hepatic complications of inflammatory bowel disease

David H Adams FRCP

Professor of Hepatology, Liver Research Group, Medical Research Council Centre for Immune Regulation, University of Birmingham Medical School

Email: d.h.adams@bham.ac.uk

Inflammatory bowel disease (IBD) is associated with extra-intestinal manifestations which occur either at the same time as bowel inflammation (joint, skin and eye) or run an independent course (autoimmune hepatitis and primary sclerosing cholangitis (PSC)). It has been suggested that eye, skin and joint manifestations are driven by the trapping of gut-derived effector cells in capillaries in these sites; however, this cannot explain the liver diseases that develop when bowel inflammation is quiescent or even after colectomy.<sup>1,2</sup> This led us to propose that long-lived memory lymphocytes that arise as a consequence of bowel inflammation express homing receptors that direct their subsequent migration not only to the gut but also the liver.<sup>3</sup> Such cells could recirculate between the liver and gut without causing damage for many years but if they subsequently encounter an antigen in the liver this could result in their activation and the promotion of tissue damage and disease. This could explain how a patient can develop liver disease many years after their IBD has become quiescent. In order to prove the hypothesis we needed to:

- demonstrate that lymphocytes in the liver of patients with PSC were originally activated in the gut
- provide a mechanism to explain how these cells are recruited to the liver
- show that they are critical for disease pathogenesis.

Over the last nine years we have answered the first two questions and thus the hypothesis is still valid.<sup>4</sup>

When lymphocytes are activated by dendritic cells (DC) in gut-associated lymphoid tissues (GALT) they are not only programmed to respond to antigen but are also imprinted with a homing phenotype which directs their subsequent trafficking back to the gut.<sup>5</sup> After antigen has been cleared, a population of long-lived memory cells remain that retain gut tropism and thereby provide immune surveillance against the same pathogen entering the gut in the future. The molecular basis of this tissue-specific homing has recently been elucidated. Lymphocytes are recruited into tissues from the blood by sequential interactions with adhesion molecules and chemotactic cytokines called chemokines presented on the endothelium lining the vessels in the target tissue. Adhesion molecules allow lymphocytes with an appropriate receptor to recognise and bind the endothelium and chemokines can then direct migration through the endothelium

into tissue.<sup>5,6</sup> A cell will only be recruited if it expresses receptors that allow it to respond to the particular molecules presented on the target endothelium. Endothelium in the gut expresses a unique adhesion molecule called mucosal addressin cell adhesion molecule-1 (MADCAM1) that is absent from other vascular beds and a unique chemokine CCL25 which is restricted to the small bowel. Activation of naive lymphocytes by antigen-bearing DCs in GALT imprints the responding lymphocytes with the receptors for these gut-specific molecules: the integrin  $\alpha 4\beta 7$  and the chemokine receptor CCR9 respectively.<sup>7,8</sup> We have shown that this imprinting is dependent on DCs from the gut, and DCs from other tissues, including the liver, cannot do this<sup>8</sup> so  $\alpha 4\beta 7$  and CCR9 are only found together on lymphocytes activated in the gut. We have shown that 20% of the thymus (T) cells infiltrating the liver in PSC are  $\alpha 4\beta 7$ +CCR9+ and thus of gut origin whereas<sup>9</sup> these cells are found at very low frequencies in other liver diseases.<sup>9,10</sup> Furthermore, these cells are memory/effector T cells that secrete interferon  $\gamma$  suggesting that activation by antigen *in vivo* would rapidly expand an effector population capable of promoting liver inflammation.<sup>9</sup>

The functional relevance of  $\alpha 4\beta 7$  and CCR9 expression is supported by observations that both MADCAM1 and CCL25, which are absent from normal liver<sup>11</sup> are present on hepatic endothelium in liver diseases associated with IBD<sup>10</sup> and that  $\alpha 4\beta 7$ +CCR9+ lymphocytes from PSC livers bind MADCAM1 on liver tissues and respond to CCL25 in adhesion and migration assays.<sup>9,10</sup> Finally, another adhesion molecule vascular adhesion protein-1 (VAP-1), which we have shown to be involved in lymphocyte recruitment to the human liver where it is constitutively expressed,<sup>12,13</sup> is increased on mucosal vessels in IBD.<sup>14</sup> Thus we have demonstrated that T cells originally activated in the gut infiltrate the liver in PSC in response to aberrant expression of homing molecules usually restricted to the gut. However, the signals responsible for inducing expression of MADCAM1 and CCL25 in the liver in IBD are unknown and are currently a major focus of research in our group.

In summary, we propose that some mucosal lymphocytes can bind liver endothelium, possibly via VAP-1, allowing them to recirculate between the liver and gut to provide immune surveillance across both sites.<sup>4</sup> However, in PSC hepatic inflammation leads to the up-regulation of hepatic MADCAM1 and CCL25 and increased recruitment of mucosal T cells. If these cells are activated by cross-reactive liver antigens or gut antigens that have entered through the portal circulation, this leads to their local expansion and the establishment of chronic inflammation.<sup>3</sup> If we are correct and PSC is caused by lymphocytes activated in the gut then blocking  $\alpha 4\beta 7$ /MADCAM1 or CCR9/CCL25 may prevent them getting into the liver to cause disease. Because the same signals are involved in gut inflammation in IBD, new treatments currently being developed for IBD may also be effective in PSC.

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## Recent developments in targeting liver fibrosis

**John Iredale** DM FRCP(Lond) FRCP(Ed) FMedSci  
Professor of Medicine, University of Edinburgh

Email: john.iredale@ed.ac.uk

## Introduction

Liver fibrosis and cirrhosis present a continuous disease spectrum characterised by an increase in total liver collagen and other matrix proteins which disrupt the architecture of the liver and impair its function. Fibrosis in the liver is mediated by myofibroblasts which in turn are derived from hepatic stellate cells, resident myofibroblasts and bone-marrow-derived stem cells. Previously considered to be at best irreversible and at worst relentlessly progressive, recent research involving tissue culture, animal and human models, has indicated that hepatic fibrosis is dynamic and has the potential to resolve with diminution of scarring.<sup>1</sup> The identification of the key regulating mediators of inflammation and fibrosis in the liver has spurred the interest of

investigators in academia and industry who are actively involved in the design of specific and targeted therapies.

### New developments in the field of liver fibrosis

In injured areas, incoming inflammatory cells release cytokines which cause resident hepatic stellate cells to become activated to myofibroblasts.<sup>2</sup> Additionally, local myofibroblasts may be recruited and there is increasing evidence that myofibroblasts derived from bone marrow stem cells also play a major role in the development of fibrosis.<sup>3,4</sup> Key mediators involved in this process include pro- and anti-inflammatory cytokines and the major profibrotic cytokine, transforming growth factor beta-1.<sup>5</sup> Activated hepatic stellate cells/myofibroblasts proliferate and secrete the collagens and other matrix proteins which characterise fibrosis. Stellate cells and other cells involved in the fibrotic process, including macrophages and Kupffer cells also secrete a repertoire of matrix degrading metalloproteinase enzymes.<sup>6–8</sup> These enzymes have the potential to degrade the collagen and excess matrix and while their activity decreases with progressive fibrosis, recent research indicates that this occurs as a result of enzymatic inhibition. This inhibition is mediated by powerful metalloproteinase inhibitors (the tissue inhibitor of metalloproteinases (TIMPs) 1 and 2).<sup>9–11</sup> These data emphasise the potential dynamic nature of scarring within the liver and indicate that there is a potential for matrix degradation even in advanced cirrhosis but it is held in check by the concurrently secreted TIMPs. There is significant interest in un-harnessing the matrix degrading capacity of the fibrotic liver to facilitate matrix degradation and a return to normal or near normal architecture or to upregulate the matrix degrading capacity of an injured liver.<sup>11–13</sup>

Reassuringly, human and animal models indicate that this process of matrix degradation occurs *in vivo* even in comparatively advanced cirrhosis.<sup>13</sup> Studies using pathological specimens and paired biopsies from trials of antiviral regimens in chronic hepatitis have shown that matrix degradation occurs even in advanced human cirrhosis.<sup>14</sup> In parallel, rodent models, in which spontaneous recovery from liver fibrosis and cirrhosis occurs, have allowed the frequent sampling that is necessary to identify the critical features of the process. These studies have demonstrated that the expression of TIMPs 1 and 2 decrease rapidly while matrix degrading metalloproteinases, possibly derived from inflammatory macrophages, continue to be expressed resulting in increased collagenase activity and consequent matrix degradation within the liver.<sup>8</sup>

Together with matrix degradation, apoptosis of the stellate cells occurs. In very advanced cirrhosis there is evidence for cross-linking of the matrix that prevents its effective degradation and promotes survival of the activated stellate cell/myofibroblasts. Even in this context, however, there may be significant remodelling which may be sufficient to enhance hepatic activity to a level that is compatible with survival of the patient, providing other complications of fibrosis, such as portal hypertension, are effectively treated. Studies in the area of regulating TIMPs and matrix metalloproteinases (MMPs) are

limited to experimental animal models but they auger well for attenuation of liver fibrosis by manipulating the TIMP–MMP balance or enhancing stellate cell apoptosis.<sup>15</sup>

Stem cell therapy offers the opportunity to repopulate the liver with effective functioning hepatocytes. Sadly, experimental evidence from rat and human models suggests that in the context of ongoing inflammation in the liver, stem cells are consistently recruited to inflammatory cell and myofibroblast lineages.<sup>3,4</sup> This suggests that some modification to stem cells will be necessary to cause them to preferentially develop into fully functioning hepatocytes. Nevertheless, the data highlight the opportunity to design therapies to impede the recruitment of stem cells to myofibroblasts – a potential mechanism to regulate the development of the hepatic scar. Alternatively, stem cells destined to become inflammatory cells could be modified and used as a vehicle to deliver antifibrotic therapies. This approach represents a logical extension of so-called ‘macrophage targeting’ which has been successfully deployed in animal models of renal disease.<sup>15</sup>

### Conclusion

Antifibrotic therapies are an emerging reality. The platform on which these strategies are designed is the result of the burgeoning evidence base for the reversibility of liver fibrosis and the identification of the key mediators of fibrosis and fibrosis reversal. There is real hope that specific and targeted therapies applicable to this serious disease will be developed in the near future.

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