

Regulatory T cells and autoimmune hepatitis: What happens in the liver stays in the liver

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Autoimmune hepatitis (AIH) is a classical autoimmune liver disease that has characteristic features of autoimmunity. It is more commonly seen in women, strongly associated with variants in the major histocompatibility complex region and usually responsive to immunosuppression. Although the aetiology is still unknown the development of the disease involves a loss of immunological self-tolerance to liver antigens resulting in a T cell mediated destruction of hepatocytes [1]. The majority of AIH patients require lifelong immunosuppression to maintain remission and the treatment for AIH still relies on corticosteroids and azathioprine, which were first introduced more than 25 years ago [2]. In recent years it has been suggested that one of the factors that predisposes to autoimmune disease is an imbalance between effector and regulatory arms of the immune system [3,4]. Evidence of this comes not only from clinical studies but also from animal studies in which defects in immune regulation and a lack of functional regulatory T cells (Tregs) is associated with the development of autoimmunity including autoimmune hepatitis. However, it is less clear whether the defect in patients with AIH is due to a failure of regulatory cell recruitment or function, or whether regulatory cells are simply overwhelmed by dominant effector cell responses. The article by Taubert and colleagues in this edition of the *Journal of Hepatology* sheds more light on this debate. They conducted a correlative study using immunohistochemistry and flow cytometry to quantify the relative proportions of effector cells, regulatory T cells and B cells in the liver and blood of patients with type1 AIH. Consistent with other studies they reported that intrahepatic Tregs are enriched in untreated type1 AIH but that their numbers fall in response to therapy. The authors suggest that this effect of therapy might explain the high relapse rates seen after discontinuation of immunosuppression in AIH and speculate that therapies that

increase or preserve intrahepatic immunoregulation might be better suited for the long-term control of AIH.

Lymphocytes comprise around 25% of intrahepatic immune cells and include effector CD4 T cells, cytotoxic T cells, B cells, invariant iNKT, and MAIT cells [5]. Regulatory networks particularly intrahepatic CD4⁺CD25^{high}CD127^{low}FOXP3⁺ regulatory T cells control the effector responses. The intrahepatic frequency of these cells varies from 1% to 5% of the lymphocyte population in most chronic liver diseases [6] and is associated with a reversal of the CD4/CD8 ratio in the liver compared with blood [7]. In autoimmune hepatitis, CD4 T cells dominate in the early stage of the disease, particularly Th1 cells [8]. Subsequently, T helper cells recruit cytotoxic CD8 T cells leading to higher CD8 T cell frequencies and possibly more aggressive cytotoxic T cell mediated destruction of hepatocytes as the disease progresses as described by Taubert and colleagues [9].

Staining for the FOXP3 transcription factor, the master regulator of Treg differentiation and function has been widely used to quantify CD4 Tregs in tissues [10]. However, FOXP3 is also expressed transiently during T cell activation and in some other lymphocyte subsets, potentially reducing the reliability of single colour immunohistochemistry to quantify Tregs in hepatic inflammation [6,11]. The current report partially overcomes this by using double immunohistochemistry. However, in the inflamed environment some Tregs may display lineage plasticity and express both FOXP3 and the Th1 transcription factor TBet [12] and high local levels of TGF- β and retinoic acid may generate induced Tregs [13,14]. A more detailed phenotypic analysis of freshly isolated intra-hepatic Tregs with multi-colour flow cytometry would answer these questions particularly if this includes the TSDR methylation status, which correlates closely with stability and function of thymic derived Tregs [15].

AIH flares are characterized by high frequencies of both Tregs and effector T cells (Teffs) in the inflamed liver [8,9] (Fig. 1). Now Taubert and colleagues show that patients reaching biochemical remission have higher intrahepatic Treg/Teff and Treg/B cell ratios compared to those who fail to respond. This suggests that Tregs are important in gaining and maintaining remission. The finding that intrahepatic Tregs decrease during therapy is novel and particularly interesting and could explain the high relapse rates after discontinuation of immunosuppression. The authors'

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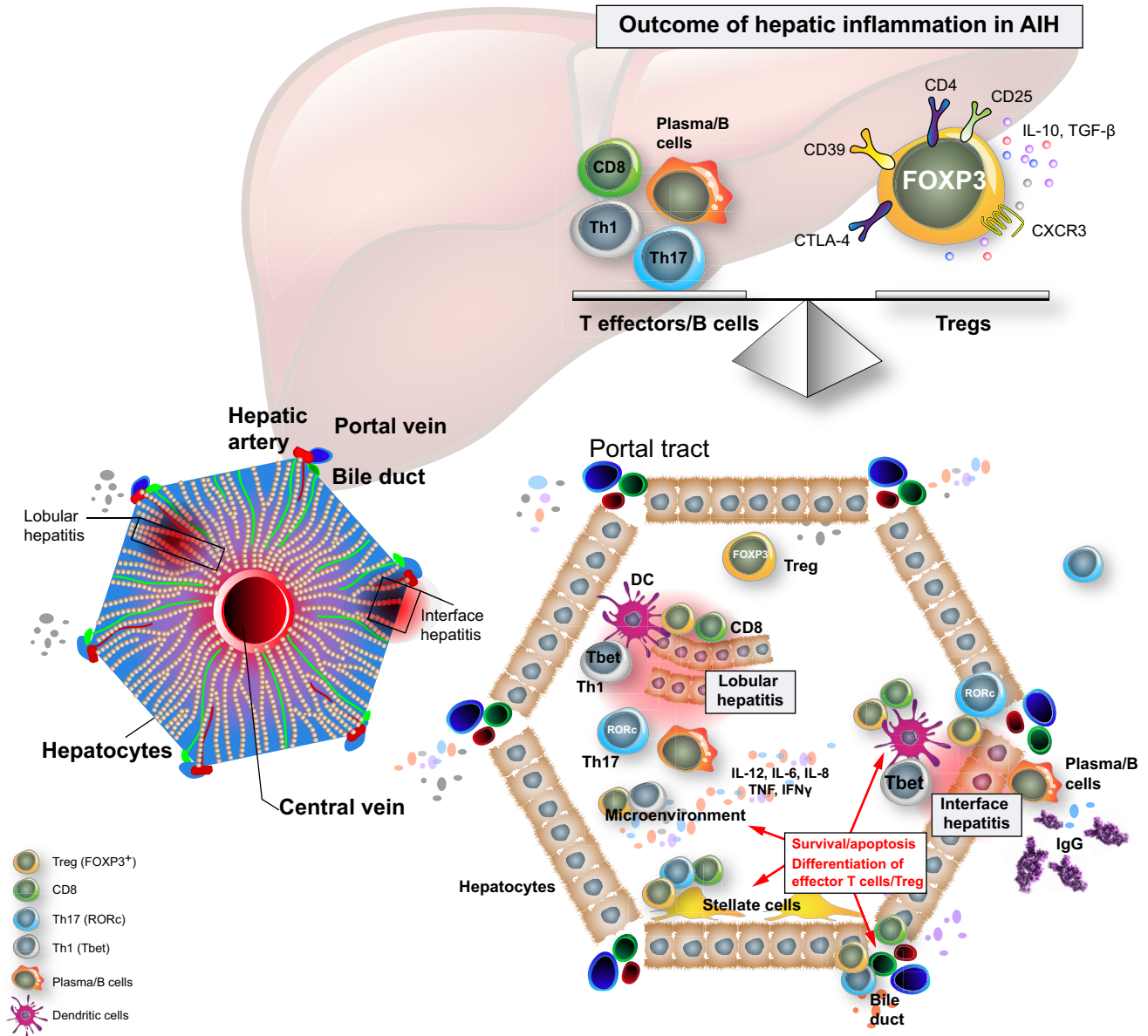


Fig. 1. Intrahepatic effector lymphocytes include Tbet transcription factor expressing Th1 cells, RORc transcription factor expressing Th17 cells, CD8 T cells, and immunoglobulin G (IgG) secreting plasma cells are involved in hepatic inflammation (lobular hepatitis and interface hepatitis) in AIH. FOXP3⁺ regulatory T cells (Tregs) suppress effector lymphocyte proliferation and cytokine secretion to control ongoing hepatitis. Intrahepatic Tregs express CD4, CD25, CTLA-4, CD45RA, liver homing chemokine receptor CXCR3 and secrete IL-10 and TGF-β. The hepatic microenvironment is enriched with pro-inflammatory cytokines such as IL-6, IL-12, TNF, IFN-γ, and professional antigen presenting dendritic cells. Survival, differentiation and apoptosis of both effector lymphocytes and regulatory T cells are dependent on the intrahepatic microenvironment, stellate cells, biliary epithelial cells and dendritic cells. The balance of T effector/B cells vs. Tregs generally dictates the outcome of autoimmune hepatitis.

data, showing that Tregs are more susceptible to particular forms of immunosuppression, suggest we should consider drugs that more specifically suppress effector cell responses. These observations are relevant for future therapy with infused Tregs and would suggest that Treg infusions will be most effective if used at induction or instead of maintaining immunosuppression. The current study reports that steroid and azathioprine therapy has a greater effect on intrahepatic Tregs compared with T effector cells [1,9]. An important immunosuppressive mechanism of action of prednisolone/glucocorticoid is the inhibition of *IL2* gene expression and two other commonly used immunosuppressive drugs, cyclosporine and tacrolimus; also reduce IL-2 production

[16]. Tregs are highly dependent on IL-2 and this may explain the particular susceptibility of Tregs to treatment in the present study. Thus, it may be logical to use immunosuppressive drugs that preserve IL-2 mediated signalling in AIH.

We have previously reported similar frequencies of intrahepatic effector and regulatory lymphocytes in liver tissue removed at transplantation from patients with autoimmune hepatitis [6] but the present study is the first to describe this in steroid-naïve patients. The consistent frequencies of intrahepatic Tregs show that there is no failure of Treg recruitment to the liver in AIH, and suggest it is the balance of Tregs and effector cells that will dictate the outcome. It is thus important to understand the

factors within the hepatic microenvironment that determine differential survival or activation of Tregs and effector cells (Fig. 1).

A further observation in the present paper was that the frequency of intra-portal B cells correlates with levels of serum IgG suggesting that the elevated IgG levels that are characteristic of active AIH may be a consequence of local intrahepatic production. More research is required to understand the functional consequences of local B cell activation in the liver and the nature of the IgG secreting cells. The fact that IgG secretion falls on rituximab therapy despite the fact that CD20 is not expressed on plasma cells suggests that IgG autoantibodies may be secreted by memory B cells within inflammatory niches in tissue consistent with the findings of the present study [17].

Another important conclusion from the present study is that studies of Tregs in blood may not reflect what happens in tissues. Studies have reported different findings regarding the frequency and function of Tregs in the peripheral blood of patients with autoimmune hepatitis [18–20], which emphasizes the importance of studying intrahepatic Tregs and not over-interpreting findings from blood. Finally, analysis of the makeup of hepatic infiltrates in AIH may provide useful to predict outcome and response to therapy as well as helping to predict which patients can have immunosuppression withdrawn without a high risk of relapse.

In conclusion, the present study provides more evidence on the important role of intrahepatic Tregs vs. effector T cells and B cells in autoimmune hepatitis. A detailed study of Tregs in the liver tissues and how inflammation effects their local survival and function will provide closer understanding of Tregs in autoimmune hepatitis (Fig. 1). Functional rather than phenotypic studies are required to gain the necessary insights into the pathogenesis but they are difficult to perform. Correlation of immunophenotypic data with clinical data is helpful as suggested in the current study. Immunoregulatory mechanisms in the human liver are more complicated than previously thought and understanding these aspects will allow us to develop more specific therapy in AIH that gets away from blanket immunosuppression.

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Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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