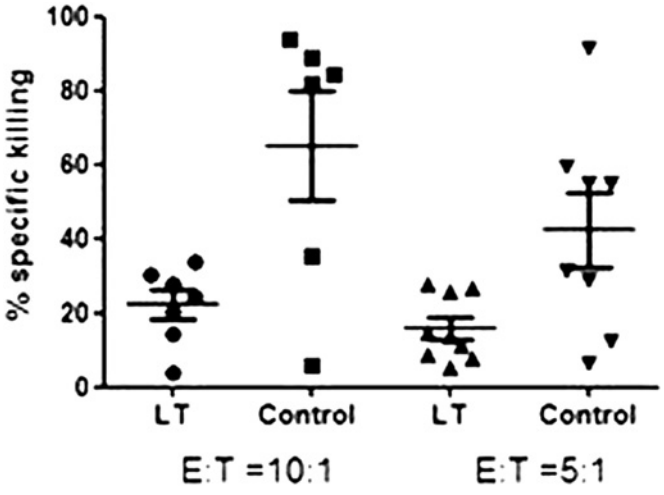

Abstract P100 Figure 2 NK cell Killing of K562 target cells.

lyse target cells that do not express self HLA class I molecules (the "missing-self" model). As liver transplants are not matched for HLA, significant NK cell alloreactivity is expected, but is not seen in practise.

Aim This aim of this study is to investigate the effect of LT on recipient NK cell reactivity.

Method Whole blood was collected from 16 liver transplant patients and 10 controls. The transplant patients were all on calcineurin inhibitor-based immunosuppression. NK cells from the peripheral blood were analysed for expression of the cell surface inhibitory receptors CD158a/b (killer cell immunoglobulin-like receptors (KIR) specific for HLA-C), and the activating receptors NKp30, NKp46 and NKG2D. Following overnight incubation with IL-15 NK cell function was assessed using a flow cytometry-based killing assay.

Results There was significantly reduced expression of NKp30 and NKp46 in post LT patients compared with controls ($p<0.001$ for both, Student t test), but no reduction in NKG2D expression (Abstract P100 figure 1). There was no difference in KIR expression or segregation of activating receptor expression with expression of specific KIR. However, consistent with the phenotyping results there was a significant reduction of NK cell killing of target cells in post LT patients compared with controls as shown in Abstract P100 figure 2 ($p=0.011$, Student t test).

Conclusion Following liver transplantation there is down-regulation of activating NK cell receptors and suppression of NK cell activity. We propose that this suppression helps to maintain tolerance of the HLA-mismatched liver allograft.

P101 REGULATORY T CELLS EXHIBIT REDUCED PHENOTYPIC STABILITY UPON PRO-INFLAMMATORY CHALLENGE IN AUTOIMMUNE HEPATITIS

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Introduction In autoimmune hepatitis (AIH) $CD4^+CD25^{high}$ regulatory T-cells (Tregs) are numerically defective and fail to suppress T-cell-mediated immune responses. Expression by Tregs of the ectoenzyme CD39 contributes to their ability to suppress by initiating an ATP hydrolysis cascade which leads to the production of adenosine, a molecule with inhibitory properties. Recently expression of CD39 has been associated with T-reg phenotypic stability under inflammatory conditions.

Aim To investigate the frequency and phenotypic stability of $CD39^+$ Tregs in AIH.

Method 24 AIH patients (23 ANA/SMA$^+$, 1 LKM-1$^+$; 12 females, median age: 15 years) and 24 healthy subjects (HS; 16 females, median age: 35 years) were studied. The phenotype of circulating Tregs was assessed by flow cytometry using monoclonal antibodies to CD4, CD25, CD127, CD39 and CD73, an ectonucleotidase that in mice is expressed by Tregs and works in tandem with CD39. The frequency of IFNγ, IL10 and IL17-producing cells within Tregs was determined by intracellular staining. Analysis was performed at baseline and after exposure to anti-CD3/CD28 T-cell expander or to the pro-inflammatory cytokines IL1β and IL6 (IL1β+IL6).

Results At baseline, $CD39^+$Tregs were less numerous in AIH (8.78±0.77) than HS (11.93±1.04, P=0.019) and displayed a trend towards higher CD127 expression (7.41±3.25 vs 2.75±1.33, p=0.13) and reduced FOXP3 mean fluorescence intensity (1230±260 vs 997±232, p=0.09). CD73 expression on $CD39^+$Tregs did not differ between the two groups. Exposure to T-cell expander increased the frequency of $IFN\gamma^+CD39^+$Tregs in AIH (from 7.37±2.15 to 24.3±13.37, p=0.043) but not in HS (12.4±2.9 to 6.6±2.4, p=NS). Although the frequency of $IFN\gamma^+CD39^+$Tregs augmented after treatment with IL1β+IL6 in both AIH (from 7.37±2.15 to 72.9±9.42, $p<0.001$) and HS (12.39±2.94 to 56.51±16.12, $p<0.001$), the increase was higher in the former than in the latter (10-fold vs fivefold). IL1β +IL6 increased the frequency of $CD127^+CD39^+$Tregs in AIH (from 4.95±2.23 to 19.6±11.43, p=0.05) but not in HS (2.7±1.3 to 1.3±0.8 p=NS). No change in the frequency of $IL10^+$ and $IL17^+CD39^+$Tregs was noted upon T-cell expander or IL1β+IL6 stimulation in AIH and HS.

Conclusion Compared to HS, Tregs from AIH patients display lower CD39 expression and are more prone to become activated upon exposure to pro-inflammatory stimuli, a finding which indicates reduced phenotype stability. A decrease in CD39 expression and in phenotypic stability may contribute to impaired Treg suppressive function in AIH.

P102 CLEVER-1 MEDIATES THE TRANSMIGRATION OF B CELLS ACROSS HUMAN HEPATIC SINUSOIDAL ENDOTHELIUM

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Introduction Lymphocytes are recruited via the unique hepatic sinusoidal channels during chronic inflammatory liver diseases. This low shear vascular bed is lined by hepatic sinusoidal endothelium (HSEC) which lacks certain conventional adhesion molecules leading us to look for novel receptors involved in lymphocyte recruitment. HSEC express several scavenger receptors including CLEVER-1 which we have recently shown mediates regulatory T cell recruitment to HSEC. B cells have been implicated in the pathogenesis of liver disease and driving liver fibrosis.

Aim B cells must be recruited from the peripheral circulation into liver tissue but the molecular mechanisms that mediate this process are not known. Our aim was to study if CLEVER-1 plays a role in this process.

Method We used isolated HSEC in flow adhesion assays to study the functional role of CLEVER-1 in lymphocyte subset recruitment. Immunofluorescent staining and confocal microscopy were used to characterise the transmigration of lymphocytes across HSEC under conditions of flow. Time lapse video recordings and Image J software was used to compare T cell and B cell recruitment via HSEC monolayers under conditions of flow.