Conclusion HCV core Els—44 inhibits NK cell activity via two distinct mechanisms, directly via KIR2DL2/S, and synergistically via the CD94-NKG2A receptor. This synergistic interaction at CD94-NKG2A represents a novel mechanism for inhibiting NK cells. It demonstrates the sensitivity of NK cells to small changes in the peptide content of HLA class I, and is thus potentially a sensitive mechanism for viral escape from the host innate immune response.

**P92 CD161+ γδ T CELLS: DEFINING THEIR ROLE IN PATIENTS WITH AND WITHOUT CHRONIC HEPATITIS C**

doi:10.1136/gutjnl-2011-300857a.92

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**Introduction** γδ T cells have been found in blood/liver of patients infected with hepatitis C (HCV). CD161+ is a C-type lectin found on the surface of T cells in humans. HCV specific T cells have been found to express CD161 with enrichment within the liver. The role & function of CD161+ γδ T cells has not been established in HCV.

**Aim** To explore the phenotype and function of CD161+ γδ T cells in humans, and to assess the impact on these cells of HCV infection.

**Method** Whole blood/Peripheral Blood Mononuclear cell (PBMC) antibody staining with subsequent flow cytometry was performed to assess phenotype. Function was examined by Intracellular Cytokine staining (ICS). Intrahepatic lymphocytes (IHLs) were isolated from patients having liver biopsies for clinical indications with paired blood samples. Cord blood samples were used after ethical approval gained and consent from mothers.

**Results** In healthy controls, the CD161 subset encompassed a mean of 52.1 (±12.8)% of γδ cells. The CD161 subset expressed more CXCR3/CCR6/IL-18R (p=0.03/0.01 and 0.05 respectively) than the CD161- subset. The CD161+ cells expressed significantly more IFN-γ/ TNF-α (p=0.001 respectively) than the negative subset, and more Granzyme A, B and K and perforin in keeping with a Th1 phenotype. 20% of γδ cells from cord blood samples expressed CD161, suggesting it is an innate feature but expanded throughout life.

**Conclusion** These data suggest that STAT2 is a critical component of the TLR signalling response to early inflammatory stimuli, in particular through maintaining the normal phosphorylation of IkB.
Methods 48 CYP-Treg cell lines were obtained from 12 AIH-2 patients positive for the predisposing HLA-DR7 and DR3 alleles; 36 Treg cell lines specific for a DR7 or DR3-restricted influenza-haemagglutinin (HA) peptide were generated from 9 DR7+ or DR3+ healthy subjects (HS) and used as controls. CYP- and HA-Treg were obtained after co-culture with peptide-pulsed semi-mature DCs. Treg were expanded for 2 weeks in the presence of: (1) IL2 (500 U/ml); (2) IL2+rapamycin (RP) to enhance Treg function; (3) IL2+IL6/IL1b, cytokines mimicking the proinflammatory milieu of AIH-2. Treg phenotype was determined by flow cytometry; frequency of cytokine-producing cells by intracellular staining.

Results Before expansion, the frequency of CD127- and FOXP3+ cells exceeded 80% in both CYP- and HA-Treg. Compared to HA-Treg, CYP-Treg contained higher numbers of IFNg+ cells (6.4 ± 6.9 vs 3.6 ± 1.2; p = 0.09), IL2 (9.5 ± 2.6 vs 2.6 ± 0.5; p = 0.02), IL17 (7.1 ± 1 vs 3 ± 1.2, p = 0.026), IL10 (9.1 ± 2 vs 3.5 ± 2.6, p = 0.05) and TGFb (10.4 ± 2 vs 3.6 ± 0.7, p = 0.001) producing cells. After expansion with IL2, CYP- and HA-Treg maintained a similarly high frequency of FOXP3+ and CD127+ cells, while frequency of IFNg+ cells increased markedly (CYP-Treg: from 6.5 ± 1 to 27 ± 5, p < 0.001; HA-Treg: from 3.8 ± 1.5 to 11 ± 1.3, p < 0.01). Exposure to RP decreased the frequency of IFNg+ cells by 36% (p = 0.04) in HS and by 30% (p = 0.15) in AIH-2. Exposure of Treg to IL6/IL1b had no effect on their phenotype and cytokine production.

Conclusion Compared to HA-Treg, CYP-Treg contain higher numbers of cytokine-producing cells, possibly reflecting a higher activation state of their precursors. After expansion, antigen-specific Treg retain a classical T-reg phenotype (CD127+ and FOXP3+) even upon exposure to pro-inflammatory stimuli, but contain a high proportion of IFNg+ cells. Reduction of IFNg+ cells in the presence of RP suggests a role for this drug in the expansion of antigen-specific T-reg for immunotherapy in AIH-2.

Abstract P95 Figure 1 Phosphoflow experiments investigating NF-kBp65 and MAPKp38 expression following LPS challenge in study groups.

Abstract P95 Figure 2 Phosphoflow experiment investigating STAT-1 and STAT-3 expression in study groups at baseline and following exogenous IL-10 administration.

Molecular mechanisms of monocyte reprogramming in acute liver failure: importance of hepatically derived anti-inflammatory mediators

doi:10.1136/gutjnl-2011-300857a.95

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Introduction Monocytes from patients with acetaminophen-induced acute liver failure (AALF) bear striking phenotypic and functional similarities with endotoxin tolerant (ET) monocytes and may account for the marked predisposition to sepsis and increased mortality in AALF. ET reprograms monocyte responses in response to lipopolysaccharide (LPS) stimulation by reducing expression of pro-inflammatory (e.g., TNF-z, IL-6) and augmenting the production of anti-inflammatory cytokines (e.g., IL-10). At a molecular level, reductions in positive regulators (e.g., NF-kBp65) of toll-like receptor (TLR)-4 dependent signalling pathway typify ET. Soluble anti-inflammatory mediators, such as IL-10 and SLPI, exert negative regulation of TLR responses via STAT3 and NF-kBp65 dependent signalling pathways respectively.

Aim To delineate the molecular mechanisms and functional consequences of ET in AALF.

Method Following TLR-4 (LPS; 100 ng/ml), IFN-z (10 ng/ml) and IL-10 (50 ng/ml) stimulation, phosphoflow technique was used to identify changes in regulators of TLR (NF-kBp65, MAPK p38), STAT-1 and STAT-3 signalling pathways in ex-vivo CD14+/CD3+ monocytes in eight AALF patients and 10 healthy volunteers (HC). Results expressed as MFI and ratio of activation (MFI following stimulation/MFI in baseline). Serum TNF-z, IL-10 and SLPI were measured by ELISA (pg/ml) in 34 AALF patients and 15 healthy volunteers (HC). Regional levels of TNF-z, IL-10 and SLPI (portal vein [PV], hepatic vein [HV]) were determined in a further five AALF patients at time of liver transplantation (LT). Hepatic expression of TNF-z, IL-10 and SLPI (all pg/ml below) was determined using whole liver tissue homogenates from seven AALF explants and eight controls. Ex-vivo monocyte phagocytosis of FITC-labelled Escherichia coli was determined in five AALF and 10 healthy volunteers (HC) using FACS analysis.

Results In contrast to HC, TLR-4 stimulation markedly reduced NF-kBp65 expression, while MAPKp38 signal transduction responses remained similar to that of HC (Abstract P95 figures 1–2). Baseline STAT-3 expression was significantly elevated in AALF patients compared to HC whereas no differences in STAT-1 expression was detected (Abstract P95 figure 2). Increase in STAT-3 expression following IL-10 stimulation was similar between AALF patients and HC.

AALF patients had significantly higher serum concentrations of TNF-z (21 vs 1.5; p < 0.001), IL-10 (170 vs 40; p < 0.001) and SLPI (71 200 vs 43 310; p < 0.001) compared to HC. A trans-hepatic (HV > PV) gradient was seen for IL-10 and SLPI but not for TNF-z in 4 out of 10 patients positive for the predisposing HLA-DR7 and DR3 alleles and 17 without a predisposing allele.

Abstract P95 Figure 3 Regional levels of SLPI and IL-10 in five patients with AALF.

AALF patients had significantly higher serum concentrations of TNF-z (21 vs 1.5; p < 0.001), IL-10 (170 vs 40; p < 0.001) and SLPI (71 200 vs 43 310; p < 0.001) compared to HC. A trans-hepatic (HV > PV) gradient was seen for IL-10 and SLPI but not for TNF-z in 4 out of 10 patients positive for the predisposing HLA-DR7 and DR3 alleles and 17 without a predisposing allele.