OP06 TIM3 DOWN-REGULATION RENDERS CD4 EFFECTOR CELLS LESS SUSCEPTIBLE TO T-REG CONTROL IN PATIENTS WITH AUTOIMMUNE HEPATITIS

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Introduction In autoimmune hepatitis (AIH), the extent of CD4 effector immune responses is associated with defective $CD4^{pos}CD25^{pos}$ regulatory T-cells (T-regs). Engagement of T-cell-immunoglobulin-and-mucin-domain-3 (Tim3) on Th1-cells by galectin-9, expressed by T-regs, induces Th1-cell apoptosis. In AIH, Tim3 is down-regulated on CD4 effector cells. Whether the reduced Tim3 expression renders CD4 T-cells less susceptible to T-reg control is unknown.
Aim To evaluate whether Tim3 expression is associated with the ability of CD4 effector cells to be regulated by T-regs in AIH.
Method 18 ANA/SMA+ patients (median age: 14.9 years, 11 females) and 6 healthy subjects (HS, median age: 28.4, 5 females) were studied. T-regs and $CD4^{pos}CD25^{neg}$ effector cells were purified from PBMCs using immunomagnetic beads. $CD25^{neg}$ cells were further purified into $Tim3^{pos}$ and $Tim3^{neg}$ cell fractions and used as targets in co-culture experiments with T-regs. Target cell proliferation was assessed after 5-day culture by ^{3}H-thymidine incorporation and expressed as mean cpm count.
Results Proliferation of unfractionated $CD25^{neg}$ cells (HS: 37 745±5015; AIH: 6160+714) was lower than that of $Tim3^{neg}$ (HS: 115 488±8348, p=0.001; AIH: 9659+1041, p=0.02) and higher than that of $Tim3^{pos}$ (HS: 29 382±2323, p=0.03; AIH: 4604±1177, p=0.004) cells. Addition of T-regs reduced cell proliferation by 51% (P<0.001) in HS and by 26% (p=NS) in AIH when unfractionated $CD25^{neg}$ cells were used as targets; by 25% (p=0.14) and 23% (p=NS) when $Tim3^{neg}$ cells were the targets and by 62% (p=0.04) and 43% (p=0.03) when the targets were $Tim3^{pos}$ cells. Since $Tim3^{pos}$ cells produce higher levels of IFN-gamma than $Tim3^{neg}$ cells, we investigated the effect of IFN-gamma neutralisation on the ability of $Tim3^{pos}$ and $Tim3^{neg}$ cells to be regulated by T-regs. While treatment with anti-IFN-gamma neutralising antibodies did not change $Tim3^{neg}$ cell susceptibility to T-reg control, it decreased it in $Tim3^{pos}$ cells, inhibition of proliferation being 17% in HS and nil in AIH following T-reg addition.
Conclusion Compared to unfractionated $CD25^{neg}$ and $Tim3^{pos}$ cells, $Tim3^{neg}$ effectors display higher ability to proliferate and are less susceptible to T-reg control especially in AIH. Down-regulation of Tim3 renders CD4 effectors less amenable to immune regulation and therefore more likely to inflict and perpetuate liver damage. The decreased ability of T-regs to suppress proliferation of anti-IFN-gamma-treated $Tim3^{pos}$ cells suggests that IFN-gamma production is needed in order for these cells to be effectively regulated.

Basic science

OP07 MACROPHAGE CELL THERAPY CAUSES THE HEPATIC RECRUITMENT OF HOST EFFECTOR CELLS AND IMPROVES STRUCTURE AND FUNCTION IN A MURINE MODEL OF CHRONIC LIVER DISEASE

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Introduction Bone marrow (BM) cell populations have a number of roles in the development and resolution of chronic liver disease. Clinical trials of BM cell therapy have already begun. These have generally employed mixed cell populations often enriched for adult stem cells. Such cells may have a range of phenotypically diverse progeny. The identification of a defined cell type with beneficial effect will provide the basis of rational and predictable therapy. We have previously shown that macrophages are key mediators of scar remodelling. Iterative injury with carbon tetrachloride (CCl4) results in a well characterised model of murine hepatic fibrosis.
Aim We sought to determine whether bone marrow derived macrophages (BMMs) could be used as cell therapy for liver fibrosis.
Method Liver fibrosis was induced in female C57/Bl6 mice by 12 weeks i.p. carbon tetrachloride (CCl4). Macrophages were derived from the bone marrow of age-matched syngeneic mice cultured for 7 days under low adherence conditions in macrophage colony stimulating factor conditioned media. 8 weeks into the CCl4 injury protocol, mice received either 106 BMMs via the hepatic portal vein (n=8) or control medium (n=8). Serum was analysed for albumin and livers were analysed for mediators of inflammation, fibrosis and regeneration. To track donor cells, male (C57Bl/6) or transgenic green fluorescent protein+ (CBA) BMMs were delivered to strain-matched fibrotic wild type mice.
Results BMMs were 88% F4/80+/CD11b+, possessed characteristic morphologic and phenotypic features, and expressed the chemokines MCP-1, MIP-1α and MIP-2. At 12 weeks, C57Bl/6 mice receiving the macrophage injection had 32% less fibrosis (mean±SEM: 2.5±0.4 vs 3.7±0.3%, p<0.05) and higher serum albumin levels (46±2.6 vs 39.9±0.86 g/l, p=0.05). Significant improvements in fibrosis and serum albumin were also demonstrated in CBA mice. Donor macrophages transiently engrafted the scar increasing hepatic levels of macrophage (MCP-1), and neutrophil (MIP-1α, MIP-2 and KC) chemoattractants (p<0.05). This enhanced recruitment of host macrophages and neutrophils to the hepatic scar areas with associated increases in MMP-13 and MMP-9 (p<0.05). A 60% reduction in myofibroblast staining (p<0.05) followed. The early influx of host leukocytes was accompanied by a 346% increase in hepatic levels of the anti-inflammatory cytokine IL-10. Donor BMMs expressed high levels of the progenitor cell mitogen TWEAK. Macrophage recipients upregulated hepatic TWEAK by 216% with a 40% increase in the number of liver progenitor cells (p<0.05). Hepatocyte proliferation was not significantly affected.
Conclusion BMM therapy decreases fibrosis and increases regeneration improving clinically meaningful parameters of chronic liver disease in this model. The actions of the donor BMMs are amplified through paracrine signalling to numerically greater endogenous cell populations. Importantly, these effects are mediated by a single differentiated donor cell type, bringing clarity to the cause-effect relationship.

OP08 EVIDENCE FOR EARLY ASTROCYTE ACTIVATION, CELLULAR STRESS AND COMPENSATORY MICROGLIAL RELATED TRANSFORMING GROWTH FACTOR-α RESPONSES IN BILE-DUCT LIGATED RATS

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Introduction Inflammation and ammonia are important mediators in the pathogenesis of hepatic encephalopathy and though the mechanisms are unclear astrocytes are thought to have a central role. Recently Microglia, which also mediate brain inflammation, were implicated in the brain effects of acute liver failure; however their influence in chronic liver disease is unknown.
Aim The aim of this longitudinal study was to characterise the early brain responses in bile-duct ligated (BDL) rats in the 4-weeks following ligation.
Method Twenty-four male Sprague-Dawley rats were studied after sham-operation or BDL and sacrificed at either 1-day or, 1-, 2- or 4 weeks post-surgery (n=4/group). Consciousness, brain water content, arterial ammonia, plasma biochemistry and proinflammatory (IL-6, TNF-α and γ-IFN) and antiinflammatory (IL-4