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HEPATIC ANTIGEN-PRESENTING CELLS BIAS CD4+ T CELLS TO A TH-17 FATE

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Introduction: The liver is a site in which some infections evade immunity, and these include Hepatitis C Virus and the liver stage of the malaria parasite. The explanation for such "liver tolerance" is not fully explained, but it has been attributed either to a bias in T cells primed in the liver towards Th2 rather than Th1 function, or alternatively to the induction of T-regulatory cells (Tregs). Using highly purified liver antigen-presenting cells (APCs); we have refuted both of these models. Methods: We developed a novel multistep flow cytometry sorting technique to characterize and isolate specific population of liver cells based on different markers including CD11c, F4/80, CD11b, CD146, Tie2, VEGFR2 and retinol fluorescent. Each subset of cells was isolated from B6 mice, and pulsed with exogenous soluble ovalbumin as a source of the CD4 T cell epitope, or alternatively was isolated from an ovalbumin-transgenic mouse, in which the target antigen was expressed endogenously. We addressed the role of each costimulatory molecule, IL-6, IL-10 and LIF using blocking antibodies or relevant knockout mice strains. Signature T cell response was studied based on activation markers, cell proliferation, transcriptional profile (FoxP3 vs ROR-gt) and cytokine production. Characteristic Th1, Th2, Th17 cytokines were assayed using xMAP multiplex technology. Results: Several subsets of liver cells were competent APC, but none was as effective as classical spleen DC. Interestingly, kupffer cells (KCs) were powerful APC for cell-intrinsic antigens, while LSECs were more effective in presenting an exogenous protein antigen. In contrast to spleen DC, liver APCs were much more dependent on ICAM-1 for T cell-APC interaction. This was confirmed using ICAM-1 -/- mice. Spleen DC, KC and LSEC all induced ROR-gt expression in CD4 T cells and promoted IL-17 secretion. However, in contrast to spleen DC, neither Th1 cells nor Th2 cells were strongly induced, nor was there evidence for induction of FoxP3+ Tregs by hepatic APCs. In fact, Th17 differentiation was the only aspect of CD4 T cell function that was induced as effectively by the liver APCs as by spleen DCs. Conclusion: This is the first study that has systematically characterized antigen presentation to CD4 cells by various liver APCs. Our data argue that the susceptibility of the liver to viral and malarial parasites is not likely to be due to a general "immunosuppressive milieu". Instead, the overall bias of liver APC was towards the development of a Th-17 response at the expense of other CD4 T cell fates. In addition, liver APCs use a different set of co-stimulatory pathways to instruct T cells

Disclosures:

The following people have nothing to disclose: Mohammad R Ebrahimkhani, Isaac Mohar, Ian N. Crispe

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ETHANOL INHIBITS PRESENTATION OF ALLOGENEIC AND EXOGENOUS ANTIGENS BY DENDRITIC CELLS (DC)

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Background: Recently, we demonstrated in a murine chronic ethanol model that specific cytotoxic T cell activity generated by genetic immunizations against hepatitis C virus nonstructural protein 5 (NS5) was substantially reduced; however, complete restoration of this defect could be achieved by adoptive transfer of DCs from control but not ethanol-fed mice. These observations suggest that the depressed effector T cell responses may be due, in part, to intrinsic defects associated with antigen presenting and/or T-cell priming functions by DCs. Here we performed experiments aimed at exploring the cellular mechanisms of these effects of ethanol through analyzing the processing and MHC Class II presentation of exogenous antigens by DCs. Methods: 6-8 weeks old Balb/c or CBA/caj mice were fed ethanol or isocaloric control diet for 8 weeks. Twelve and 7 days before sacrifice mice were injected with Flt3L expression plasmid to expand the splenic DC population. DCs were purified with CD11c+ microbeads and utilized for antigen presentation, processing and peptide-MHC Class II (pMHCII) complex detection assays. IL-2 was measured via ELISA in T cell-DC co-cultures as an effective indicator of antigen presentation. Antigen processing and pMCHII complexes were detected by flow cytometry. Results: We observed that ethanol not only suppresses allogeneic peptide presentation to T cells by both Balb/c and Cba/caj DCs but also alters presentation of exogenous ovalbumin (OVA) to both OVA specific DO11 T cell line and OVA sensitized primary T cells. In contrast, ethanol does not appear to inhibit processing of exogenous antigens within the endocytic pathway. However, we found lower peptide-MHC class II complexes on the DC cell surface of ethanol-fed mice suggestive of a peptide loading or transport defect. When DCs were stripped of the contributing cytokine signals following fixation, there appeared to be no difference in DC function between ethanol and control-fed mice regarding T-cell activation. Likewise, neither supplementing the DC-T cell co-culture with DC derived cytokines such as TNF-α, IL-6, 12 and IFN-γ nor neutralizing the IL-10 with antibodies was able to restore impaired antigen presentation. Conclusion: Ethanol inhibits exogenous and allogeneic antigen presentation by DCs. This inhibition is mediated by synergistic effects of reduced formation MHC Class II complexes with antigenic peptides and intrinsic defects in DC cytokine secretion. Therefore, DCs are a critical target cell for the suppressive effect of ethanol on the host immune response.

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OXIDATIVE STRESS UP-REGULATION OF OSTEOPONTIN DRIVES COLLAGEN I INDUCTION IN HEPATIC STELLATE CELLS BY BINDING $\alpha V\beta 3$ INTEGRIN AND SIGNALING VIA THE PI3K/PAKT PATHWAY

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Background: Increased myofibroblasts and up-regulation of fibrillar collagen I are key events leading to liver fibrosis. Osteopontin (OPN), is a matricellular cytokine that binds integrins and CD44, participates in cell adhesion, invasion, matrix remodeling, metastasis, and inflammation; however, its role in stellate cell (HSC) activation and collagen I deposition has not been evaluated yet. **Aims**: Since we have previously demonstrated that OPN is constitutively expressed in cells within the periportal region and it is highly induced in CCl_4-induced liver injury, we believe that OPN triggers signals that are required for fibrogenesis to occur. Specifically, we hypothesize that: 1) Oxidative stress will induce OPN expression; and 2) OPN will up-regulate collagen I in HSC acting as a feed-forward mechanism to promote scarring. **Methods**: Primary human HSC and

mouse HSC from WT and *Opn*$^{-/-}$ mice were isolated. To determine the role of oxidative stress on OPN induction in HSC, pro-oxidants (0-200 μM H_2O_2 or 0-0.25 mM BSO) and antioxidants (0-200 U/ml catalase or 0-2 mM GSH-EE) were used alone or in combination. To study the pro-fibrogenic potential of OPN, HSC were incubated with OPN (0-10 nM) and intra- and extracellular collagen I expression was analyzed by Western blot. To identify the receptors and signaling pathways involved in the effects mediated by OPN, neutralizing antibodies to αvβ3 integrin and CD44, or specific kinase inhibitors, were added prior to OPN treatment. **Results**: 1) Both H_2O_2 and BSO, known to deplete GSH, significantly increased OPN expression in a time- and dose-dependent fashion and these effects were prevented by catalase and GSH-EE; 2) OPN did not induce phenotypic changes in HSC; however, it increased cell proliferation; 3) OPN up-regulated intra- and extracellular collagen I in a time-dependent manner, while no effects were observed in MMP1/2/9, with collagenolytic activity; 4) HSC from *Opn*$^{-/-}$ mice showed decreased cellular activation and less collagen I expression than those from WT mice, demonstrating also a role for intracellular OPN in mediating scarring; 5) Neutralizing antibodies to αvβ3 integrin but not to CD44 prevented the OPN induction of collagen I; suggesting a role αvβ3 integrin in these effects; 6) OPN treatment increased PI3K and phosphorylation of Akt; and 7) Co-incubation with wortmannin, a PI3K inhibitor, blunted the effects of OPN on collagen I, identifying the PI3K-pAkt pathway as implicated in the pro-fibrogenic effects of OPN. **Conclusion**: oxidative stress-driven induction of OPN plays a key role in modulating HSC activation and collagen I induction via αvβ3 integrin-PI3K-pAkt signaling.

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SPECIFIC DELIVERY OF AN ALK5-INHIBITOR TO HEPATIC STELLATE CELLS REDUCES EXTRACELLULAR MATRIX DEPOSITION IN FIBROTIC MICE

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Transforming growth factor-β (TGF-β) is a major pro-fibrogenic cytokine. Its binding to the type-II TGF-β receptor on hepatic stellate cells (HSC) leads to heterodimerization of this receptor with the type-I TGF-β receptor, also known as ALK5. ALK5 is then transphosphorylated, which leads ultimately to HSC activation and production of extracellular matrix. TGF signaling takes place mainly via the Smad-signaling pathway, but other pathways have been found to be involved as well. Apart from its pro-fibrogenic properties, TGF-β has systemic effects, mainly on other immunecompetent cells. This prevents administration of high dosages of ALK5-inhibitor, however, at low dosages ALK5-inhibitor can not induce sufficient anti-fibrogenic effects. We therefore propose that targeted delivery of ALK5-inhibitor to HSC will specifically inhibit fibrosis-related TGF-β signaling. We synthesized a conjugate of ALK5-inhibitor coupled to mannose-6-phosphate human serum albumin (M6PHSA), which is known to be taken up specifically in activated HSC. The ALK5-inhibitor was coupled to M6PHSA through the Universal Linkage System (ULS™). In vitro effect studies were performed on isolated rat HSC. The effect and the distribution of the conjugate in vivo were determined using an acute CCl_4-induced liver injury model in mice. Binding of the conjugate to activated HSC was specifically through the target-receptor. Drug could be released from the conjugate in vitro by chemical replacement. In activated HSC, the ALK5-conjugate inhibited deposition of collagen I and III and attenuated mRNA expression of the fibrotic markers α-smooth muscle actin and collagen 1A1. We also examined TGF-β dependent signaling in vitro and found that the ALK5-conjugate inhibited the Smad signaling pathway, as evidenced by a significant reduction in Smad promoter dependent luciferase expression. IVIS imaging showed accumulation of the M6PHSA conjugate in the fibrotic liver and HSA staining showed that the conjugate localized in the non-parenchymal cells of the liver. In a dose finding study using an acute CCl_4-induced liver injury model in mice, ALK5-conjugate reduced the deposition of collagen at two different doses, while free ALK5-inhibitor did not give this effect even at the highest dose injected. We conclude that we can specifically deliver ALK5-inhibitor to HSC using the M6PHSA carrier and that this targeted drug effectively reduces fibrotic parameters in vivo and is more effective than free ALK5-inhibitor.

Disclosures:

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AUTOPHAGY REGULATES HEPATIC STELLATE CELL ACTIVATION AND FIBROGENESIS

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Introduction: Features of hepatic stellate cell (HSC) activation during hepatic fibrosis are well characterized and tightly coordinated, including loss of retinoid-containing lipid droplets, induction of cytokines and their receptors, enhanced immune signaling, altered apoptotic potential, regulation by extracellular matrix (ECM), and increased metabolic activity. Remarkably, these same features are also hallmarks of autophagy, a highly regulated cellular response evolved to maintain energy homeostasis during conditions of cellular stress or enhanced metabolic demand. We therefore hypothesized that autophagy contributes to, or regulates, HSC activation. Aim: To evaluate the role of autophagy in HSC activation in hepatic fibrosis. Methods: Autophagy was blocked in both immortalized and primary murine HSCs by the established inhibitors 3-methyladenine (3MA, 10 mM) or lentiviral silencing of Atg7. A panel of HSC markers was quantified using qRT-PCR and western blot, and lipid content was analyzed by Oil red O staining. Autophagy was also induced by nutrient deprivation for 6h and 12h. LC3B-I conjugation to LC3B-II and ultrastructural evidence of autophagolysosomes, both markers of autophagy, were documented by western blot and electron microscopy (EM), respectively. Results: Blockade of autophagy by either 3MA or siAtg7 in primary and immortalized HSCs significantly inhibited HSC activation based on both reduced expression of mRNAs for Colα1 (I), Colα2 (I), β-PDGFR, MMP2, and marked induction of adipose differentiation related protein (ADRP). Western blot for collagen (I) also confirmed reduced protein expression. Autophagy inhibition also markedly increased intracellular lipid content, suggesting reversion to a more quiescent phenotype. Conversely, nutrient starvation stimulated HSC activation and fibrogenic gene expression. The appearance of