In contrast to healthy individuals, resting NK cells from HCV infected patients displayed significantly reduced antiviral activity after incubation at 5% or 1% O₂. When cytokine-activated NK cells were observed, antiviral activity of healthy NK cells was reduced only after incubation at 1% O₂, while HCV NK cells responded already at 5% O₂ culture conditions. In line with that finding we observed significantly reduced IFN-γ secretion of resting and activated HCV NK cells cultured under hypoxic conditions. Of note, hypoxia-induced reduction of IFN-γ production was also observed in intrahepatic NK cells.

Next, we found that peripheral and intrahepatic NK cells from HCV patients displayed significantly reduced expression levels of the NK cell receptors Nkp46 and NKG2D when cultured at 5% and 1% O₂ whereas no such effects were observed for NK cells from healthy donors.

However, neither in healthy controls nor HCV infected donors anti-fibrotic NK cell activity was affected by low oxygen tension.

CONCLUSIONS: Taken together, our results indicate that in HCV infected patients, anti-viral but not anti-fibrotic activity of NK cells is markedly reduced under the low oxygen tension levels which are typical for liver tissue. Importantly, this held true also for intrahepatic NK cells.

Thus, low oxygen tension in the liver tissue must be considered as a novel additional mechanism contributing to insufficient intrahepatic anti-viral immune responses in chronic hepatitis C. Transferring experimental systems to hypoxic (or liver-normoxic) conditions is a new approach to gain insights into the physiological function of innate immunity against HCV.

P114

Immunoglobulin purified from antibody associated virus from hepatitis C viraemic sera exhibit selective targeting of homologous HCV genotype

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The host immune system responds to viral infection by producing neutralising antibodies (NAbs). However, the mutations of the immune dominant epitopes in viral envelope protein make them recalcitrant towards previously produced NAbs. Fractionation of virus into antibody associated (AAV) and an antibody free (AFV) population, followed by analysis at the molecular signature level presents an opportunity to enhance our understanding about evolution of viral envelope proteins and humoral immune escape. The objective of the current study was to analyse hypervariable region 1 (HVR1) of glycoprotein of hepatitis C virus (HCV) quasispecies in AAV and AFV fraction from sera of chronically infected hepatitis C patients. In the present study we have used serum derived HCV (sdHCV) to study the permisiveness of homologous HCV genotype in Huh7 when mixed with immunoglobulins purified from AAV.

Briefly eighteen serum samples from a panel of viraemic sera positive for different HCV genotypes were randomly selected. The serum was fractionated into AAV and AFV on protein G sepharose columns. Direct sequencing analysis of both the fractions was performed (Eurofins MWG). Immunoglobulins purified from genotype 1b and 3a AAV fraction were mixed with previously antibody negative homologous HCV to study the infection efficiency in comparison with sdHCV in Huh7. Interference in virus infectivity was evaluated by HCV 5'-UTR qRT-PCR assay.

Out of 18 samples used in this study; (n = 3/8) genotype 1b, (n = 3/3) genotype 1a, (n = 2/3) genotype 3a and (n = 1/4) genotype 4a were positive for a 306 bp region encompassing HVR1 in AAV fraction. qRT-PCR results showed that antibody binding to homologous HCV reduces infectivity of sdHCV. This trend of reduced infectivity was evident across genotypes 1b and 3a.

We report the differential quasispecies segregation in AAV and AFV fractions of sdHCV. Furthermore, our data supports the principle that serum derived antibodies that target HCV reduces the infectivity quotient and over time can contributes to the extinction of particular lineages.

P115

Human pDC potentially carrying two alleles with reconstituted IFN-α4 gene function show reduced IFN-α induction upon stimulation with HCV infected hepatoma cells

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Worldwide approximately 160 million people are chronically infected with hepatitis C virus (HCV). Hallmarks of
HCV are its genetic variability and the diverge courses of hepatitis C progression in patients. To assess whether intra-genotypic HCV variations affect the triggering of host innate immunity, we stimulated human primary plasmacytoid dendritic cells (pDC) with crude preparations of different cell culture-derived genotype 2a HCV variants. These experiments revealed that parental JFH1 did not induce IFN-α, whereas the intra-genotypic chimera Jc1 triggered massive IFN-α responses. Furthermore, efficient virus particle formation, but not virus infectivity, determined the magnitude of IFN-α responses. Notably, co-culture of pDC with HCV infected hepatoma cells retrieved the capacity to induce IFN-α, while Jc1 infected cells still triggered stronger responses than JFH1 infected ones. Recently, within the IFN-α locus several single nucleotide polymorphisms (SNP) were detected. One of these, rs12979860, showed high linkage disequilibrium with a SNP that reconstituted IFN-α4 gene function. Analysis of pDC derived from rs12979860 CC/CC (major allele) or TT/TT (minor allele) donors revealed that the genotype did not affect IFN-α responses against Jc1. On the contrary, hepatoma cells infected with Jc1 triggered strong IFN-α responses only in CC pDC, but not in TT ones. These results are striking since TT/TT patients with chronic HCV infection respond less efficiently to IFN-α/ribavirin therapy than CC/CC patients.

P116
The Impact of obesity-related hormones on the HCV life-cycle
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Obesity is associated with increased viral load during HCV-infection and non-response to IFN/Ribavirin-based therapy. Fat tissue is known to produce a variety of cytokines and hormones leading to increased levels of pro-inflammatory markers in sera of obese individuals. In this study we assessed the impact of several adipokines on cell-culture derived full-length HCV life cycle.

A few tested hormones showed moderate antiviral activity in vitro (IL-6, TNF-α). However, a promising effect was observed for the adipokine Chemerin.

Chemerin is a hormone that is mainly expressed by fat-tissue and the liver. It is elevated in obese compared to lean individuals, with serum levels of ~300 to ~200 ng/mL respectively, and has been recently described for the first time. By binding to its main receptor - the G-protein-coupled Chemokine-like receptor 1 (CMKLR1) - , which is mainly expressed by macrophages, pDCs/mDCs and NK-cells, Chemerin serves as a chemo-attractant. However, little is known about its impact on non-immune cells.

In cell-culture derived full-length HCV screen we observed a dose-dependent antiviral effect of this hormone (IC50 at 1000 ng/mL). We excluded a modulation of HCV-entry and confirmed an inhibition of replication with sub-genomic replicons of two different HCV genotypes (1a, 2a). Treatment of the target cells with Chemerin prior and post infection with HCV caused a robust inhibition of viral replication. Modulation of CMKLR1 did not alter the inhibition of viral replication by Chemerin in Huh-7.5 cells. Transcriptomic analysis of Chemerin-treated Huh-7.5 cells revealed an upregulation of distinct ISGs and also non-ISGs. A pathway analysis showed no clear correlation to already described antiviral pathways, pointing to an unique mode of action of this hormone. Chemerin was also able to inhibit propagation of Coronavirus in Huh-7.5 cells, although to a lesser extend.

We proofed that Chemerin acts antiviral in primary human hepatocytes and confirm induced expression levels for IFI-6 and ISG-15 in these cells by real-time PCR.

In conclusion we describe for the first time an antiviral activity of Chemerin in hepatoma- as well as primary human hepatocytes. The precise mechanism and signaling pathways of Chemerin are currently further evaluated in more detail.

P117
Hepatitis C Virus mediates NRG1-dependent down-regulation of ErbB3, thereby modifies ErbB receptor family composition at the cell surface
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BACKGROUND: Recently, both EGF receptor and EGFR-dependent signalling have been shown to play a role in HCV entry and replication. However, to what extent HCV also may interfere with expression of other ErbB receptor family members is still unknown. In this study we analyzed the influence of HCV on ErbB3 expression and the consequences for surface expression of other ErbB receptors.

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