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-a, whereas the intra-genotypic chimera Jc1 triggered massive IFN-a 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-a, 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.
METHODS: Huh7 cells either infected with the HCVcc strain JC1 or harbouring the HCV1 subgenomic replicon were subjected to targeted siRNA-mediated gene knockdown followed by rtPCR, immunoblot analysis and immunocytochemistry.

RESULTS: Our data show that HCV down-regulates ErbB3 expression via induction of Neuregulin (NRG1) expression and a NRG1-mediated autocrine circuit as well as activation of Akt. The upregulation of the expression of NRG1 by HCV involves the transcription factor Sp1. Consistently, knock-down of Sp1 using specific siRNA abrogates enhanced NRG1 expression and results in reconstitution of ErbB3. Likewise treatment with either NRG1 siRNA, the Akt inhibitor Triciribine or Methylycyclidine, a polymerase inhibitor, results in reconstitution of ErbB3 expression in HCV infected cells. Compared to acute infection, a chronic Infection of cells with HCV results in ErbB3 down-regulation which is even more pronounced. Most interestingly, reduction of ErbB3 expression either by HCV or by ErbB3 specific siRNA results in enhanced surface expression of EGFR and ErbB2. This mechanism may support viral replication as suggested from studies using pre-treatment with NRG1 or knock-down of ErbB3 expression by specific siRNA prior to infection with the HCVcc strain JC1.

DISCUSSION: The data presented provide evidence that HCV enhances surface expression of EGFR and of ErbB2 by reduction of ErbB3 protein levels via up-regulation of the expression of the ErbB3 ligand NRG1 and NRG1-mediated down-regulation of ErbB3, which further involves activation of Akt. Since EGFR is a co-factor for viral entry one may speculate that this pathway enables HCV to increase susceptibility of neighbouring non-infected cells by a NRG1-mediated paracrine mechanism.

P118
The Hepatitis C Virus (HCV) facilitates CXCR2 ligand expression in response to the Epidermal Growth Factor (EGF) and is able to induce the EGF production by an autocrine circuit in its host cell

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INTRODUCTION: To establish persistent infection with ongoing replication HCV requires strategies to escape antiviral immunity, to modulate the inflammatory response of the host and to utilize host infrastructure without largely impeding viability of the infected cell. Chemokines are most critical for the formation of the inflammatory and immune microenvironment. It is therefore well conceivable that HCV has evolved mechanisms to gain access to the control of these factors. The present study determines the influence of HCV on the EGF-induced CXC chemokine expression in its host cell.

METHODS: Huh-7 cells either infected with the HCV cc strain JC1 or harbouring the HCV subgenomic replicon were used and the impact of HCV on cellular signalling and gene expression was analysed employing targeted gene knockdown by siRNA, inhibitor-studies, rtPCR and Immunoblot-analysis.

RESULTS: Expression of CXCR2 ligands such as CXCL1, 2, 3 and CXCL8 in response to EGF was significantly enhanced in the presence of the HCV replicon or JC1. Most interestingly gene knockdown by siRNA suggest that HCV itself induces production of these chemokines via induction of an autocrine loop of EGF expression in its host cell. Furthermore inhibitor studies and gene knockdown by siRNA suggest that the CXCL8 expression requires an activation of MEK1 and needs the sensitization of the EGF receptor (EGFR) tyrosine kinase activity by an HCV-mediated reduction of TC-PTP expression. However ERK1/2, Jnk, and p38 are not involved in CXCL8 production. In contrast, production of CXCL1-3 is regulated independently of the MAPK/ERK-pathway.

CONCLUSION: The study provides evidence, that HCV has substantial impact on the release of mediators that mediate the inter-cellular communication and control recruitment of immune cells in its infected host cell. HCV modifies these inter-cellular communications by utilization of EGFR and EGFR-dependent signalling. The data suggest, that HCV enhances basal chemokine expression by an EGF-dependent autocrine circuit.

P119
Hepatitis C virus inhibits IL-1β-induced IκB expression and thereby antagonizes mechanisms which may affect cell proliferation

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BACKGROUND: Hepatitis C virus (HCV) infection is a major cause of chronic liver disease worldwide, leading to end-stage cirrhosis and development of hepatocellular carci-