Since HSV 1 can persistently infect the enteric nervous system (ENS) causing gut dysmotility[1], we investigated the role of Toll like receptor (TLR)2 in HSV 1 induced ENS alterations.
C57/Bl6 (WT) and TLR2KO mice were inoculated with HSV 1 or replication deficient HSVdeltaICP27 intranasally and 4 weeks (W) later intragastrically (IG). Macrophages or CD8 lymphocytes were depleted by clodronate liposomes or anti CD8. One 2W after viral IG injection we determined in the ileum a) monocyte chemotactic protein 1 (MCP 1) level by ELISA, b) infiltrating immune cells by fluorescent activated flow cytometry, c) ENS integrity by immunohistochemistry and by changes in isometric muscle tension following electric field stimulation.
HSV 1 or HSVdeltaICP27 IG injection in WT mice caused loss of neuronal HuC/D and peripherin, altered cholinergic transmission, increased MCP 1 and recruited activated macrophages (CD11b+F4/80+CD80+). Clodronate mediated macrophages depletion entirely prevented HSV 1 induced ENS anomalies. TLR2KO mice failed to up regulate MCP 1 and to recruit macrophages following HSV 1 exposure but HSV 1 reactive CD3+CD8+INFgamma+ lymphocytes infiltrated the myenteric plexuses. CD3+CD8+ cells but not macrophages depletion prevented HSV 1 induced ENS alterations in TLR2KO mice.
Our study provides evidences that TLR2 signaling is essential for the optimal development of anti viral immune responses following HSV 1 infection of ENS. However, TLR2 driven recruitment of inflammatory cells causes neuronal damage and gut dysfunction.

1. Brun, *et al. Gastroenterology* 201 ; 138: 1790.

REF O28

Ross River Virus is able to persist in tissues of infected macaque similarly to Chikungunya virus despite lower viremia during acute phase

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The chikungunya virus (CHIKV) and the Ross River virus (RRV), two Alphaviruses provoke persistent myalgia/arthritis in humans. We showed that chronic CHIKV infection in macaque (Macaca fascicularis) involved macrophages. To better characterize the alphavirus arthritis development mechanism in human, we compared RRV and CHIKV infections within primate model. We infected macaques with an infectious molecular clone of the strain LR2006 OPY 1, we previously used, and with two strains of RRV: a) T48, a molecular clone derived from a mosquito isolate obtained in 1948 and b) QML 1, an 1999' Australian clinical strain. We then followed the clinical, viral and immunological parameters in peripheral blood at different time point during the infection and on the tissues at the euthanasia. In situ Hybridization and Immuno histochemistry allowed to 1) identify sites of viral replication and viral reservoirs tissue at the early and late infection associated with CHIKV and RRV and viral titration and Q PCR together with Cytokine follow up by ELISA 2) assess the mechanisms associated with arthralgia and/or persistence of these alphaviruses. Our study is the first comparison of in vivo CHIKV and RRV infections in primates, showing that despite different replication kinetic, both pathogens are able to induce in vivo local inflammation and tissue damage during arthralgia. Different pro inflammatory cytokines balances showed that processes however might be distinct. Our alphaviruses chronic infection model can therefore be used to assess treatment strategy for these specific tissue damages.

REF O29

MicroRNA expression signatures in chronic viral hepatitis progression

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Several studies investigated miRNA expression in hepatocellular carcinoma, but little is known on the changes in liver miRNA expression during the different stages of chronic viral hepatitis. This study, which investigated miRNA expression profile by microarray analysis in liver biopsies from 40 patients with chronic B and C viral hepatitis, identified a coherent picture of miRNAs that were significantly associated with viral aetiology and with histological features of chronic liver disease. In fact, data on differentially expressed miRNAs that were identified in this study were consistent with validated targets and activity of each miRNA. Among modulated miRNAs, the miR 200a/200b/429 cluster appeared relevant in liver disease and its up regulation was associated with different histological markers of disease progression (i.e., fibrosis, inflammation and necrosis, steatosis); few miRNAs were differentially expressed in HBV and HCV infected livers and were conducible to specific viral activity; advanced fibrosis/cirrhosis was associated with the overexpression of fibrosis related miRNAs, the under expression of miRNAs with tumor suppressor activity, and with a miRNA signature consistent with retention of the epithelial phenotype and inhibition of epithelial to mesenchymal transition. The most significant miRNAs associated with severe fibrosis were investigated and confirmed in a mouse model of acute liver fibrosis. In conclusion, this study described miRNAs signatures associated with chronic viral hepatitis progression that could be potentially used as markers of disease.

REF O30

Characterisation and functional analysis of the murine gammaherpesvirus 68 microRNAs

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MicroRNAs (miRNAs) regulate gene expression post transcriptionally by altering the stability of their target mRNA. Murine gammaherpesvirus 68 (MHV 68) encodes at least 15 miRNAs derived from a cluster of 8 tRNA like molecules. The miRNAs are located in a genomic region known to encode latency associated transcripts, but their functions are unknown. MHV 68 naturally infects murid rodents and is closely related to Kaposi's sarcoma associated herpesvirus and Epstein–Barr virus which are associated with lymphoproliferative tumours in humans. The strict species specificity of gammaherpesviruses has limited research on the human viruses mainly to in vitro studies. Infection of mice with MHV 68 provides a unique small animal model to investigate in vivo pathogenic features that are difficult to assess in humans. During latency, MHV 68 expresses a protein encoded by ORF73 named murine latency associated nuclear antigen (mLANA). In this study, a recombinant MHV 68 virus containing beta lactamase gene fused to ORF73 was used to detect and isolate latently infected mouse splenocytes by flow cytometry. This system facilitates the characterisation of viral miRNAs expression in latently infected cells. At day 14 post infection which coincides with a peak splenic viral load, ten viral miRNAs were detected in mLANA positive splenocytes with variable expression levels. In order to investigate the functions of these miRNAs, a mutant virus that lacks the miRNAs was constructed. The phenotypic analysis of this mutant virus will allow us to investigate the role of viral miRNAs in pathogenesis.