thermore, apoptosis may contribute to ethanol-induced liver injury via complement activation. Supported by NIH F31-AA016434, RO1-AA011975.

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PP2-196 Nonstructural protein 4B of hepatitis C virus inhibits interferon responses

Hui-Ju Wu, Pong-Yu Huang, Lih-Hwa Hwang, Poster Presentation II

Nonstructural protein 4B of hepatitis C virus inhibits interferon responses

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Interferon (IFN) administration is the main therapy for HCV-infected individuals, but still a fraction of them are not completely responsive to the treatment. Several HCV proteins such as core, E2, and NS5A proteins have been demonstrated to attenuate IFN responses through different mechanisms. In the present study, we examined whether HCV NS4B, a membrane-bound protein whose functions are mostly unknown, exerted any effects on IFN-induced antiviral responses. From the reporter assay, we showed that NS4B significantly suppressed IFN-induced activation of IFN-stimulated responsive element (ISRE) promoter activity. We further examined the ability of HCV NS4B to confer IFN resistance on VSV, an originally IFN-sensitive virus, by a trans-rescue assay. The viral titer was low when cells were treated with IFN-α, which, however, was significantly increased by HCV NS4B. We also examined the effects of HCV NS4B on the transcription of interferon-stimulated genes (ISGs) by qRT-PCR and found that the IFN-induced expression of ISG15, ISG54, ISG56, and OAS2-p69 was significantly downregulated by NS4B. The results were confirmed by chromatin immunoprecipitation (ChIP) assay . The levels and the activation status of the interferon-stimulated gene factor 3 (ISGF3), known to regulate the IFN pathway, were also noticeably reduced by NS4B. Taken together, these results strongly suggest that, in addition to the above mentioned HCV proteins, HCV NS4B may also play a role in IFN antagonism.

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PP2-197 Role of fusion activity in cross-presentation of influenza nucleoprotein-derived antigens

Natalija Budimir, Tjarko Meijerhof, Jan Wllschut, Anke Huckriede, Aalzen de Haan, Poster Presentation II

Role of fusion activity in cross-presentation of influenza nucleoprotein-derived antigens

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Recently, interest in influenza vaccination strategies aiming at the induction of cross-protective immune response against heterosubtypic virus strains has revived. These strategies target activation of cross-protective cytotoxic T lymphocyte (CTL) responses directed against conserved viral proteins, i.e. nucleoprotein (NP). In this respect whole inactivated virus (WIV) vaccine can be considered a good vaccine candidate. Stimulation of MHC class I-restricted CTL activity by non-infectious vaccines, such as WIV, could be achieved through cross-presentation by dendritic cells (DCs). This process involves endocytosis of antigen, processing of antigen by cytosolic proteasomes and transportation through the transporter associated with antigen processing (TAP) into MHC class I-loading compartments. Due to the fusogenic capacity of the virus, delivery of the nucleocapsid (and NP), into the cytosol is an active process mediated by the virus itself. Since virus inactivation protocols used to produce WIV vaccine can compromise viral fusion activity, they potentially could diminish WIV immunogenicity in terms of CTL induction. We therefore investigated to what extent fusion activity contributes to cross-presentation using untreated and 0.5% formaldehyde (FA) treated WIV as a model for fusion active and inactive particles, respectively. We found that fusion inactivation did not reduce *in vitro* maturation of bone marrow-derived DCs in response to WIV uptake nor upregulation of IL-12 gene expression and IL-12p70 production. Fusion inactivation of WIV did not reduce MHC class I-restricted presentation of a NP-derived epitope to previously primed CTLs *in vitro*. However, fusion activity played a significant role in priming of NP-specific CTLs *in vivo*, as demonstrated by *in vivo* cytotoxicity assay. From these data we conclude that fusion activity of WIV is beneficial in priming of CTL in unprimed individuals (the very young), but does not contribute to boosting of CTL responses in individuals primed for influenza by infection earlier in life (adolescent, adults, the elderly).

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PP2-198 Human metapneumovirus blocks the induction of type I interferon

B. van den Hoogen, M. Zahira, F. van Hagen, A. Andeweg, A. Osterhaus, R. Fouchier, Poster Presentation II

Human metapneumovirus blocks the induction of type I interferon

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Rapid induction of type I interferon (IFN) expression is a central event in the establishment of the innate immune response against viral infection, and requires the activation of multiple transcriptional proteins following engagement and signaling through Toll-like receptor-dependent and -independent pathways. Many viruses therefore encode factors that subvert the IFN system to enhance their virulence. Most viruses belonging to the family *Paramyxoviridae* encode nonstructural proteins to subvert this innate immune response. For this purpose viruses belonging to the subfamily paramyxovirinae use proteins encoded by alternative reading frames within the phospoprotein (P) gene. Members of the subfamily pneumovirinae (such as RSV) use the NS1 and NS2 proteins as antagonistic proteins. The human metapneumovirus (hMPV) is a causative agent of severe respiratory tract illness, and belongs to the family of *Paramyxoviridae*, subfamily pneumovirinae. HMPV does not encode a P gene with alternative reading frames or proteins related to the NS1 and NS2 of RSV. So far, little is known about the interaction between hMPV and the innate immune system. We demonstrate that hMPV blocks the IFN production pathway at early time points after infection. Upon infection of A549 cells with the prototype strain (hMPV NL/1/00) transcripts for the RNA sensors RIG-I and MDA-5, as well as transcripts for the IFN genes were not up regulated. In addition, the virus did not induce translocation of IRF3 to the nucleus, and the virus was able to block IRF3 translocation induced by Sendai virus infections. Thus, like other paramyxoviruses , hMPV counteracts the innate immune system at early stages after infection, however hMPV must use a novel mechanism to do so.

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PP2-199 Type III interferon activity in the brain

Prasanthi Bandi, Nyree Maes, Minjung Han, Anthony van den Pol, Michael D. Robek, Poster Presentation II

Type III interferon activity in the brain

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The type III interferon (IFN) family (IFN-λ1, 2, and 3) elicits an antiviral response that is nearly identical to that evoked by IFN-α/β, but these cytokines signal through a receptor that displays a more tissue-specific distribution in vivo. The rapid non-cytolytic IFN-α/β-mediated inhibition of virus replication is particularly important in the central nervous system (CNS), where virus- or immune-mediated destruction of infected neurons is detrimental to the host. However, not much is known regarding the antiviral activity of IFN-λ in the brain. Although IFN-λ has little antiviral activity against certain mouse pathogens in the brain, human neurons have been reported to be capable of both producing and responding to this cytokine. We found that intranasal or intracranial infection of mice with vesicular stomatitis virus (VSV) induced IFN-λ2 mRNA expression in the brain, and that human IFN-λ1 and λ2 mRNA expression were induced by VSV infection in cultured primary human brain cells. Although the relative magnitude of IFN-λ induction was substantial, its expression level was still nevertheless low on an absolute basis. In addition, we found that IFN-λ induced the expression of IFN-stimulated genes (ISG) in multiple human CNS cell types, including primary neurons, astrocytes, and choroid plexus epithelial and endothelial cells. However, the magnitude of ISG expression induced by IFN-λ was lower than that activated by IFN-α, and as a consequence, provided a lower level of protection against subsequent virus challenge. These results show that while IFN-λ may provide some protection against virus infection in human brain cells, it appears to play a minor role compared to IFN-α/β. These studies also further support the idea that due to its modest activity in the brain, therapeutic use of IFN-λ for chronic HCV infection may cause fewer of the neurological side effects that are associated with IFN-α therapy.

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PP2-200 Interferon response in murine plasmacytoid dendritic cells after SARS coronavirus infection

Anna de Lang, Corine H. Geurts van Kessel, Albert D.M.E Osterhaus, Bart L. Haagmans, Poster Presentation II

Interferon response in murine plasmacytoid dendritic cells after SARS coronavirus infection

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The pathogenesis of severe acute respiratory syndrome coronavirus (SARS-CoV) is likely mediated by disproportional immune responses and the ability of the virus to circumvent innate immunity. Although, SARS-CoV is able to block the production of type I interferons (IFNs) in most cell types, human plamacytoid dendritic cells (pDCs) have been shown to produce IFNα upon SARS-CoV infection. In contrast, little is known about type I IFN production in SARS-CoV infected mice. In vivo a modest upregulation of IFNβ mRNA but no induction of IFNα is observed in BALB/c mice. To examine the IFN response after SARS-CoV infection in mice, murine pDCs derived from BALB/c and BL6 mice were infected with SARS-CoV after which IFN responses were analyzed using RT-PCR and ELISA. The mRNA levels for IFNα, IFNβ and IFNλ were upregulated 10–100 times in SARS-CoV infected pDCs, suggesting a potent IFN response in these cells. At the protein level, however, only IFNα could be detected after SARS-CoV infection. Similar results were obtained with heat inactivated SARS-CoV and influenza virus. In contrast, the specific pDC stimulator CpG-ODN induced IFNα, IFNβ and IFNλ both at the mRNA level and at the protein level, showing that these cells are capable of producing these different IFN proteins. Interestingly, pDCs derived from BL6 mice infected with SARS-CoV produced more IFNα protein than pDCs from BALB/c mice, consistent with higher IFNα mRNA levels. This study shows that murine pDCs are able to produce IFNα after SARS-CoV infection but the production of IFNβ and IFNλ seems to be blocked at the translational level. The tight regulation of IFN production in pDCs with respect to IFN subtypes and genetic background may be important in the pathogenesis of SARS-CoV.

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PP2-201 Identification of new regulators of the innate antiviral response using a genome-scale lentiviral-based shRNA screen

Martin Baril, Daniel Lamarre, Poster Presentation II

Identification of new regulators of the innate antiviral response using a genome-scale lentiviral-based shRNA screen

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The immediate-early phase of the host innate response to viral infection is initiated through pathogen recognition and expression of early protective genes such as type I interferon (IFN). Here, we performed a genome-scale RNA interference screen to identify positive and negative regulators of the innate response to Sendai virus infection. In a primary screen, an individually arrayed library of lentiviral-based short hairpin RNA (shRNA) targeting 16,000 human genes was used to knock down each gene (in pools of 3 shRNA per gene) of a 293T cell line stably expressing the luciferase gene under the control of the IFN-β promoter. Using the strictly standardized mean difference (SSMD) as a ranking metric, we selected 600 candidate genes (300 positive and 300 negative regulators) whose silencing significantly modulated the reporter activity. This cell-based assay was validated by identifying known positive regulators such as IRF3, MAVS, c-Rel, RelA/p65 and IKKβ. Bioinformatics analysis of gene ontology (GO) biological process and molecular function categories identified significantly enriched terms, such as eight members of the IκB kinase/NF-κB cascade among 300 positive regulators, representing a fold enrichment of 3.7 ($P = 0.006$). All candidate genes are being re-confirmed and tested in secondary screens using individual shRNA-expressing lentiviruses (minimum of 5 shRNA per gene) to minimize the selection of potential off-target hits. The secondary screens were designed to define the step in which newly identified regulators are positioned in the antiviral signaling cascade. The approach described in this study will provide significant insight for novel regulators of the host innate response to viral infection. Data from primary and secondary screens will be presented.

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PP2-202 The relative antiviral activity of human alpha interferons on primate and mouse alpha interferons on hamster and rat cell lines

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The Type I IFN subtypes are often relatively species selective, which makes cross-species experiments difficult to interpret. The Syrian golden hamster and Norwegian rat are common rodent models, and two macaques, rhesus and cynomolgus, are common non-human primate models for a variety of viral infections. The alpha interferon subtypes from these species are mostly unavailable and hence either mouse or human IFN subtypes will frequently be used in these model systems. In order to understand which mouse or human alpha IFN subtypes could be used in cross-species experiments, we have established antiviral cytopathic effect inhibition assays on rhesus macaque (LLC-MK2/VSV), cynomolgus macaque (JTC-12/EMCV), Syrian golden hamster (BHK-21/VSV) and norwegian rat (C6/VSV) cell lines. All 14 mouse alpha IFNs were tested on the hamster and rat cells and all 12 of the human subtypes were tested on the rhesus and cynomolgus cell lines. In general, most of the mouse IFN-alpha subtypes exhibited activity on the other rodent cells and most of the human IFN-alpha subtypes displayed activity on the macaque cells. However, there are several notable exceptions with certain subtypes having little of no activity in the cross-species assay. The results of this study may be used to identify and select IFN-alpha subtypes that can be best used in the relevant animal model system.

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PP2-205 A carcinogenic heterocyclic amine, 2-amino-1-methyl-6-phenylimidazol [4,5-b]pyridine (PhIP), attenuates lipoteichoic acid-stimulated TNF-α expression

Jintaek Im, Hyung Shim Choi, Sun Kyung Kim, Sang Su Woo, Young Hee Ryu, Seok-Seong Kang, Cheol-Heui Yun, Seung Hyun Han, Poster Presentation I

A carcinogenic heterocyclic amine, 2-amino-1-methyl-6-phenylimidazol[4,5-*b*]pyridine (PhIP), attenuates lipoteichoic acid-stimulated TNF-α expression

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2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), a heterocyclic amine with strong carcinogenic and mutagenic potential, is created abundantly in the over-cooking of meat and fish. Carcinogenic toxicants are often implicated in immunosuppression, where cancer cells are not easily eliminated by the host immune system. Here, we investigated the effect of PhIP on tumor-necrosis factor-α (TNF-α expression by a murine macrophage cell-line, RAW 264.7, stimulated with lipoteichoic acid (LTA) which is a major virulence factor of Gram-positive bacteria. Upon exposure to LTA purified from *Staphylococcus aureus*, TNF-α expression was substantially induced, whereas pretreatment with PhIP significantly inhibited LTA-induced TNF-α expression. LTA is known to activate Toll-like receptor 2 (TLR2) and NF-κ B, resulting in TNF-α expression. Interestingly, PhIP did not interfere with LTA-binding to TLR2, its stimulation of TLR2, or the DNA binding activity of NF-κ B. However, treatment with actinomycin D facilitated the PhIP-induced attenuation of TNF-α mRNA expression, implying that PhIP might decrease TNF-α mRNA stability rather than its biosynthesis. Furthermore, Western blot analysis demonstrated that PhIP reduced the phosphorylation of ERK1/2 and JNK but not p38 kinase in LTA-stimulated cells. The addition of a protein kinase C (PKC) activator, phorbol 12-myristate 13-acetate, rescued PhIP-inhibited TNF-α expression in LTA-stimulated cells. These results suggest that PhIP down-regulates TNF-α expression in LTA-stimulated macrophages by decreasing TNF-α mRNA stability and signaling pathways related to PKC, ERK1/2, and JNK activation.

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