12

Host defense

12-01/P

THE DIFFERENT EFFECT OF INTERFERON-LAMBDA ON HEPATITIS B VIRUS REPLICATION IN TWO HUMAN HEPATOMA CELL LINES

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Hepatitis B virus (HBV) replicates preferentially in human hepatocytes. We analyzed the effect of interferon-lambda (IFN-λ) on HBV replication in human hepatoma cell lines and the effect of HBV replication on the expression of CRF2-12, a subunit of IFN-λ receptor complex. We analyzed HBV replication in WT10 and PEB8 human hepatoma cell lines treated with IFN-λ by real-time PCR and Southern blotting. PEB8 produces HBV of *adw*R9 subtype, while WT10 produces HBV of *adr* subtype. HBV replication in PEB8 but not in WT10 was suppressed by IFN-λ treatment. In both cell lines, similar amount of CRF2-12 to that in their parental cell line was expressed, and similar amount of transcripts of MxA as well as 2'5'-OAS were induced by IFN-λ. In PEB8, neither HBV transcripts nor secretory antigen was affected by IFN-λ treatment. In conclusion, HBV replication in human hepatocytes may be suppressed by IFN-λ but the antiviral activity of IFN-λ may vary with the cellular and/or viral factors. CRF2-12 expression in hepatocytes may not be regulated by HBV replication.

12-02/P

RESISTANCE TO TYPE I INTERFERON CONTRIBUTES TO REPLICATION FITNESS AND VIRULENCE OF WEST NILE VIRUS

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Interferon α/β (IFN) defenses impart control of virus replication and spread. The emergence of West Nile virus (WNV) into the Western Hemisphere is marked by the spread of pathogenic lineage I strains that differ from typically avirulent lineage II strains. To begin to understand how IFN interactions may influence the phenotypic properties of divergent lineage I and II viruses, we compared the genetic, pathogenic, and IFN-regulatory properties of a new lineage I isolate from Texas, WNV-TX 2002-HC (WNV-TX02), which was recovered during the WNV expansion across the U.S. with a lineage II isolate from Madagascar (WNV-MAD78). The WNV-TX02 genome sequence clustered with emergent North American isolates and is most closely related to other Texas strains. Compared to WNV-MAD78, WNV-TX02 replicated to higher levels in cultured human cells, resisted the antiviral actions of IFN *in vitro* and demonstrated greater virulence in mice. Cell-based studies revealed that WNV-TX02 disrupted IFN-induced Jak-STAT signaling during infection by preventing Tyk2 activation and the downstream phosphorylation and nuclear translocation of STAT1 and STAT2. WNV-MAD78 was defective in these properties, but its replication was rescued in cells with a nonfunctional IFN α/β receptor (IFNAR). Consistent with this, virulence of WNV-MAD78 was recovered upon infection of mice lacking the IFNAR. Thus, control of the host cell response to IFN is a key feature of WNV pathogenesis and replication fitness that may underlie the emergence of epidemic strains. Our results provide the first characterization of an avirulent, non-emergent form of WNV that is attenuated in its ability to block type I IFN signaling involved in host defense. The attenuated phenotype of WNV-MAD78 may provide a starting point for vaccine approaches exploiting the link between viral stimulation of host defenses and immunity to infection.

12-03/O

VIRAL AND THERAPEUTIC CONTROL OF INTERFERON PROMOTER STIMULATOR 1 DURING HEPATITIS C VIRUS INFECTION

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Viral signaling through retinoic acid inducible gene-I (RIG-I) and its adaptor protein, interferon promoter stimulator 1 (IPS-1), induces interferon regulatory factor-3 (IRF-3) activation and the interferon-α/β (IFN) host response that limits virus infection. Previously, we have shown that these RIG-I mediated pathways are essential in determining host permissiveness to Hepatitis C virus (HCV) RNA replication, and that viral expression of the HCV NS3/4A serine protease abrogates RIG-I signaling. Recent studies show that NS3/4A is able to cleave IPS-1 to block RIG-I signaling. Here, we show that HCV infection of cultured cells transiently induce IRF-3 activation in a manner dependent on RIG-I and IPS-1. These responses limit HCV production and constrain cellular permissiveness to virus replication. Biochemical studies reveal that HCV shuts off this host response early in infection by NS3/4A cleavage of IPS-1 at C508, resulting in its release from the mitochondrial membrane and loss of interaction with RIG-I, thereby preventing downstream activation of IRF-3. Confocal microscopy of HCV infected cells and patient liver tissues demonstrate a subcellular redistribution of IPS-1 within infected hepatocytes, whereas protein expression analyses show that IPS-1 cleavage correlates with a lack of ISG15 expression and conjugation of target proteins *in vivo*. Furthermore, HCV protease inhibitors effectively prevented IPS-1 cleavage by NS3/4A and restored RIG-I signaling of IFN-β induction. Our results suggest a dynamic model in which early activation of IRF-3 and induction of the host antiviral response is reversed by IPS-1 proteolysis and abrogation of RIG-I signaling as NS3/4A accumulates in newly infected cells. We have now identified additional IPS-1 interacting proteins whose roles in RIG-I signaling of the IFN host response, and regulation by HCV will also be discussed. Our studies accentuate the importance of both RIG-I and IPS-1 in the host response to viral infections.

12-04/P

OMEGA INTERFERON THERAPY VERSUS ANTI RETRO VIRAL TRITHERAPY AGAINST HIV/AIDS, ASTHMA AND CANCER

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The present invention concerns an immunotherapeutic agent based on Interferon Omega associated to vasodilators, antioxidatives agents and anticoagulants against HIV/AIDS, asthma and cancer, also its use inhuman and veterinary medicine. This therapy is the new way of therapeutic transduction of the targeted and controlled intranuclear signal wich will lead to the modulation of polyaminic metabolism, foundation of the new continue immunotherapeutic processes based on our invention. This new pharmaceutical composition injected sub cutaneously and intramuscularly in well determined sites of the lymphoid system and ganglionic areas of the patient's body produces a progressive and perennial immunotherapeutic effect both cellular and humoral. The pharmaceutical formula of our composition is based on lymphocytar Interferon Omega associated with adjuvants actions like: vasodilators, antioxidatives agents, anticoagulants and corticosteroids. The synergistic action of cytokines and adjuvants is meant for therapies as follow:
- chronic viral affections especially HIV I, HIV 2, AIDS and viral hepatitis,
- affections associated to intracellular parasites like: Leishmaniosis, Trypanosomiasis, bacterial,
- tuberculosis, syphilis, Chlamydiae and Mycoplasmosis.
Chronic allergic affections like asthma treatments of lymphoid and mesodermic cancerous processes We here present our pharmaceutical composition named ACTIVAX witch will be proved effective at at least 89% complete immunotherapeutic by activating both cellular and humoral immunity in human and veterinary medicine, its protocol of use and its therapeutic indications. Our invention and its directives of use are meant for the treatment of the above affections both in human and veterinary medicine.

12-08/P

STIMULATION OF ANTIVIRAL DEFENCE MECHANISMS BY ACYCLIC NUCLEOSIDE PHOSPHONATES

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Acyclic nucleoside phosphonates (ANPs) are antivirals effective against replication of both DNA-viruses and retroviruses, and thus have become important therapeutic means in combating some life-threatening diseases such as AIDS (*tenofovir*; Viread) and hepatitis B (*adefovir*; Hepsera).The major mechanism of antiviral action of ANPs is the inhibition of virus-induced DNA polymerases or of reverse transcriptases. The aim of our work is a development of new generation of antivirotics that would also augment natural defence mechanisms of the host. We have investigated possible immonostimulatory potential of newly developed ANPs, derivatives of adenine (A) and 2,6-diaminopurine (DAP) differing at the N^9-side chain represented by 9-[2-(phosphonomethoxy)ethyl] (PME) and 9-[2-(phosphono-methoxy)propyl] (PMP) moieties, and modified by various substitutions at the N^6-amino function of the heterocyclic base. Immunobiological activity of compounds was evaluated under *in vitro* conditions using rat resident peritoneal macrophages. A number of compounds were found to stimulate macrophages for a) high-output nitric oxide (NO) production (determined by Griess reagent), b) high secretion of TNF-α, c) modest, though statistically significant secretion of IFN-γ (assayed by ELISA). All NO, IFN-γ, and TNF-α possess key roles in antiviral defence. It is suggested that the NO-upregulation is largely due to the synergistic action of IFN-γ and TNF-α. The most effective ANPs proved to be N^6-cyclooctyl-PMEDAP, N^6-isobutyl-PMEDAP, non-substituted (*R*)-PMPDAP, N^6-cyclopropyl-(*R*)-PMPDAP, and N^6-cyclopentyl-(*R*)-PMPDAP. Their immunostimulatory effects were significant at concentrations of 10-25 μM. Expression of immunobiological potential of ANPs depends on activation of NF-κB.
This work was supported from the Centrum for New Antivirals and Antineoplastics (no. 1M6138896301).

12-09/P

MICRO-RNA EXPRESSION PROFILES IN NAÏVE AND HEPATITIS C VIRUS REPLICON CONTAINING LIVER CELLS.

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MicroRNAs (miRNAs) are small RNAs of 18–25 nucleotides in length that regulate a variety of biological processes. Recent studies show that cellular miRNAs may serve to control the replication of viruses in cells, and virus itself can also generate viral miRNAs and interact with cellular mRNAs, suggesting the important role of miRNAs in virus-host interactions. To study whether HCV genes could change the miRNA expression profile in human liver cells, we used a newly developed high throughput microarray method (Invitrogen), to test the expression levels of all known (about 329 confirmed and 144 predicted) human miRNAs in naïve human hepatoma cells Huh7, and Huh7 cells harboring HCV subgenomic replicons. The results from

the average of four separate miRNA microarrays show that, about 60 different microRNAs were detected significantly above (at least 10-fold) background in Huh7 cells, and HCV subgenomic genes could down and up-regulate 18 and 6 miRNAs. miR-122, which was confirmed to be liver specific and required for HCV replication, was slightly down-regulated. The fact that the top up- and down-regulated miRNAs (miR-197 and miR-377, respectively) are the same as in those seen following Interferon treatment (unpublished data), strongly suggests the importance of those miRNAs in regulating HCV and host cell interaction. Further experiments are on going to identify target sequences for the miRNAs and their role in Hep C infection and interferon treatment.

12-10/O

WEST NILE VIRUS EVADES ACTIVATION OF RIG-I AND MDA5-DEPENDENT PATHWAYS WITHOUT ANTAGONIZING HOST DEFENSE SIGNALING

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The ability of viruses to control and/or evade the host antiviral response is critical to the establishment of a productive infection. We have previously shown that the ability of WNV-NY to delay the activation of interferon regulatory factor 3 (IRF-3), a transcription factor critical to the initiation of the antiviral response, is essential for WNV-NY to achieve maximum virus production. Furthermore, WNV-NY utilizes a unique mechanism to control activation of IRF-3. In contrast to many other viruses, which impose a nonspecific block to the IRF-3 pathway, WNV-NY eludes detection by the host cell at early times post-infection. To better understand this process, we assessed the role of the pathogen recognition receptor (PRR), RIG-I, in sensing WNV-NY infection. RIG-I null mouse embryo fibroblasts (MEFs) retained the ability to respond to WNV-NY infection; however, the onset of the host response was delayed compared to WT MEFs. This suggests that RIG-I is involved in initially sensing WNV-NY infection while other PRRs sustain and/or amplify the host response later in infection. The delayed initiation of the host response in RIG-I null MEFs correlated with an increase in WNV-NY replication, indicating that activation of the host response by RIG-I early in infection is important for controlling replication of WNV-NY. The involvement of the RIG-I homolog MDA5, which has also been shown to be involved in activation of the host antiviral response, in sensing WNV-NY infection was also examined. Disruption of signaling through both MDA5 and RIG-I completely abrogated the host response to WNV-NY, suggesting that MDA5 is responsible for the residual activation of the host response observed in RIG null cells and a key component in the host's defense against viral infection.

12-11/P

IN VITRO INDUCTION OF CYTOKINES IN LYMPHOCYTES BY HUMAN HERPESVIRUS 6B INFECTION

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Both A and B variants of human herpesvirus 6 (HHV-6) and HHV-7 establish latency in CD4 immune cells, but upon reactivation they induce different disorders. These might – probably - depend on different mediator patterns produced by infected lymphocytes. After studies on cytokines induced by HHV-6A and HHV-7, recently we have investigated cytokine production of CD4+ MOLT-3 cells during the complete course of viral replication cycle. HHV-6B was concentrated by ultracentrifugation subsequently, cells were infected at high multiplicity for synchronized infection. At different time intervals supernatant and cell samples were collected until cells died due to cytopathic effect. Cytokine secretion was measured by sandwich ELISA while the intracellular mRNA content by real time RT-PCR. Mock infected cells were controls. It was found that, HHV-6B first increased subsequently decreased interleukin (IL)-2 and interferon (IFN)-γ production, its effect on IL-12 secretion was the opposite. Disintegration of their synergism leading to immune suppression might be augmented by increased level of tumour growth factor (TGF)-β. Decreased production of IL-15 inhibits activity of natural killer cells. Severe suppression of granulocyte-monocyte colony stimulatory factor (GM-CSF) and IL-3 secretion might disturb B cell maturation leading to lymphomagenesis. Diminished IL-4 secretion results in weak antibody production and anti-tumour activity, while suppressed IL-10 level retards B cell maturation. Low levels of TNF-α and β explain why HHV-6B is not able to activate human immunodeficiency virus (HIV). High IL-1β level could be responsible for high fever in acute herpesvirus infections. HHV-6B seems to affect both Th1 and Th2 cytokine systems. Acute diseases normalise rapidly but chronic infections might be devastating.

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12-12/O

LOSS OF STAT1 BUT NOT STAT2 OR IRF9 RESULTS IN LETHAL LYMPHOCYTIC CHORIOMENINGITIS VIRUS INFECTION IN MICE

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Functional interferon (IFN) signaling is critical for the host response against viruses. While type I IFN (α/β) signaling is mediated through STAT1, STAT2 and IRF9, type II IFN (γ) signaling uses STAT1. Here we examined the role of STAT1, STAT2 and IRF9 in the host response to viral infection in the CNS. Wild-type (WT) mice and mice lacking STAT1, STAT2 or IRF9 were infected intracranially (i.c.) with lymphocytic choriomeningitis virus (LCMV). In WT mice, a lethal meningoencephalitis occurred by day 7 with characteristic cerebral seizures and LCMV being largely confined to the CNS. LCMV-infection of STAT2 KO and IRF9 KO mice caused a transient non-fatal clinical disease and virus spread to peripheral organs. Furthermore, deficiency in either STAT2 or IRF9 resulted in viral persistence and a chronic inflammatory response in the brain and periphery that was associated with focal T-cell infiltrates and persistent expression of various cytokine and chemokine genes. By contrast, in STAT1 KO mice, LCMV infection led to the death of the animals without cerebral seizures at 8-10 days. Similar to STAT2 KO and IRF9 KO mice LCMV spread to peripheral organs in STAT1 KO mice but inflammatory lesions were far more extensive in these mice. Surprisingly, LCMV infection *via* intra-peritoneal (i.p.) injection was also lethal in STAT1 KO mice which showed pathology similar to STAT1 KO mice infected i.c. By contrast, i.p. infection of WT mice was non-eventful and non-fatal. The findings point to fundamental differences in the roles of STAT1 *versus* STAT2 or IRF9 in controlling the host response to viral infection. We suggest that STAT1, STAT2 and IRF9 are all required for limiting LCMV replication and spread (likely *via* type I IFN action). However, STAT1 has an additional function to moderate an otherwise lethal host response in disseminated LCMV infection.

12-13/P

CROSS-TALK BETWEEN PML AND P53 DURING POLIOVIRUS INFECTION : IMPLICATIONS IN ANTIVIRAL DEFENCE

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PML nuclear bodies (NBs) are dynamic intranuclear structures harbouring numerous transiently localised proteins. PML, the NBs organiser, is directly induced by interferons and its expression is

critical for antiviral defence. Another protein induced by interferon implied in this effect is p53. We demonstrate the molecular events following poliovirus infection that lead to PML-dependent p53 activation and protection against virus infection. Poliovirus infection induces PML phosphorylation through the ERK pathway, increases PML SUMOylation, and induces its transfert from the nucleoplasm to the nuclear matrix. These events result in the recruitment of p53 to PML NBs, p53 phosphorylation on serine15 and activation of p53 target genes leading to the induction of apoptosis and inhibition of viral replication. This effects, which requires the presence of PML, is transient as poliovirus targets p53 by inducing its degradation in proteasome and MDM2 dependent manner. Moreover, the knock down of p53 by siRNA results in higher poliovirus replication suggesting that p53 participes in antiviral derense. Our results provides evidence of how poliovirus counteracts p53 antiviral activity by regulating PML and NBs thus leading to p53 degradation.

12-14/O

POXVIRUS SEQUESTRATION OF PATHOGEN-ASSOCIATED MOLECULAR PATTERNS AND THE HOST RESPONSE

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Cells have evolved elaborate mechanisms to counteract the onslaught of viral infections. To activate these defenses, the viral threat must be recognized. Danger signals, or pathogen-associated molecular patterns, that are induced during the replication of viruses include dsRNA, and possibly, Z-form nucleic acid. Understanding the signal transduction pathways activated and host gene expression induced by these danger signals is vital to understanding virus-host interactions. The vaccinia virus E3L protein is involved in blocking the host antiviral response and increasing pathogenesis, functions that map to dsRNA and Z-DNA binding motifs. Viruses containing mutations in E3L allow modeling of the role of dsRNA and Z-form nucleic acid in the host response to virus infection. Deletions in the Z-DNA or dsRNA-binding motif led to activation of signal transduction cascades and up-regulation of host gene expression, many involved in the inflammatory response. These data suggest that poxviruses actively inhibit cellular recognition of viral danger signals and the subsequent cellular response to the viral threat.

12-15/P

EVASION OF THE INTERFERON-MEDIATED IMMUNE RESPONSE BY VARICELLA-ZOSTER VIRUS

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Interferon is a cytokine that acts rapidly *via* the Jak/STAT pathway to induce either an antiviral state in uninfected cells, or cell death in those cells already infected with virus. Viruses have devised a number of means to avoid the Interferon (IFN) system, including targeting components of this signaling pathway. Using a screening system based on the 2FTGH cell line producing the Guanine/Phosphoribosyltransf erase gene product (Gprt) and negative-selection with 6-Thioguanine (6-TG), we can rapidly screen for viral gene products that functionally inhibit the IFN signaling cascade. By introducing a genetic library from Herpesviruses, specifically Varicella-Zoster Virus (VZV), this approach has lead to the identification of those genes involved in the inhibition of the IFNα signaling pathway. Further analysis, *via* micro-array, qt-PCR and expression studies have been undertaken. The advantage of our system is that it allows characterization of novel genes or novel functions of previously known genes where traditional approaches based on sequence homology would fail. This aims to provide a better understanding of the host defense response.

12-16/P

ROLE OF TOLL-LIKE RECEPTOR 2 AND-9 IN A HERPES SIMPLEX VIRUS INFECTION.

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In a murine model of a systemic Herpes Simplex Virus 2 (HSV-2) infection, we have investigated the role of Toll-like receptor (TLR) 2 and -9 in the innate and adaptive immune response to virus infections. Innate resistance, in term of viral loads in organs was examined in C57bl/6j (w.t.) TLR2 and -9 knock out (k.o.) mice. To evaluate the role of TLRs in the adaptive immune response, mice were infected with an attenuated HSV TK- strain and challenged 7 or 90 later with wt virus. Viral load, serum antibody levels, and T cell activity was measured. Finally, virus-induced expression of pro-inflammatory cytokines in dendritic cells (DCs) from wt, TLR2 and -9 k.o. was measured. There were no differences in innate resistance to HSV-2 infection in 8-10 week old WT, TLR2 and -9 k.o. mice. However, in 4-5 week old mice, TLR2 and -9 k.o. mice both displayed reduced viral load in the brain as compared to wt mice. With respect to development of immunity after immunisation with TK-, no difference between wt and the TLR k.o. mice strains were observed. Despite this, we observed that expression of TNF-α and IL-6 by DCs during HSV-2 infection was dependent on TLR2, where as production of the Th1-driving cytokine IL-12 was dependent on TLR9. Therefore, TLR2 and TLR-9 differentially affect the host response to HSV-2 infection *in vivo*. However, deficiency in either gene does not compromise innate or adaptive immune defence against HSV-2. This suggests that pattern recognition receptors work in cooperative and redundant ways to sense virus infections and mediate both immediate and long lasting protection.

12-17/P

IMPAIRMENT OF LIPOPOLYSACCHARIDE-ACTIVATED SIGNALING PATHWAY BY HIV TAT PROTEIN

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HIV infection is characterized by cytokine dysregulation and severe immune defects that allow pathogens including bacteria and cytomegalovirus to cause life-threatening infections. In healthy individuals, macrophages/monocytes are activated by bacteria through Toll-like receptor recognition of the lipopolysaccharides (LPS) to trigger innate immunity. However, in HIV-infected patients, this immune response is impaired. Tat, a transactivating protein for HIV replication, has been suggested to play a role in mediating the virus-induced immunosuppressive effects. We hypothesize that HIV Tat interferes with the LPS-induced cytokine expression leading to impaired immune response of macrophages/monocytes. To examine the effects of Tat on LPS-induced signaling, primary human blood monocytes were pretreated with recombinant Tat prior to the addition of LPS. The kinetics of LPS-induced cytokine expression was analyzed by quantitative RT-PCR. With Tat pretreatment for 1 to 4 hr prior to LPS addition, the HIV protein had differential effects on the expression of proinflammatory cytokines including TNF-α, IFN-β, and IL-6. We showed that Tat pretreatment did not have effect on the subsequent LPS-induction of TNF-α mRNA but differentially caused an up-regulation of IL-6 expression. In contrast, following Tat pretreatment, the cells became deficient in LPS-induction of IFN-β expression. The results were further confirmed by ELISA which showed that the levels of the LPS-induced IL-6 protein were elevated. Since MAPKs are signaling kinases known to regulate IL-6 expression, we examined whether Tat effects on the LPS-activated phosphorylation of ERK, p38 kinase and JNK by Western blotting. We showed that concomitant with cytokine dysregulation, Tat inhibited the LPS-activated phosphorylation of ERK but not on JNK or p38 kinases. Using Western blotting and electrophoretic mobility shift assays, we demonstrated Tat suppressed the LPS-induced activation

of NFκB. Taken together, Tat protein perturbs the LPS-induced cytokine expression in monocytes through the impairment of the LPS-activated ERK phosphorylation and NFκB activation. (Supported in part by Hong Kong Research Grants Council, HKU7408/04M).

12-18/P

DEVELOPMENT OF SCREENING ASSAYS FOR THE IDENTIFICATION OF NEW ANTIVIRALS TARGETING VIRAL INTERFERON ANTAGONIST PROTEINS

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Type I interferon (IFN) plays a major role as a first line of defense against viral infections. However, many of the regulatory mechanisms that modulate this response are still unknown. We have developed a reporter cell line that mimics the transcriptional induction of an IFN-regulated gene during viral infection and/or IFN treatment. This cell line contains a reporter gene consisting of a red fluorescence protein (RFP) fused to chloramphenicol acetyltransferase (CAT) under the control of an IFN stimulated response element (ISRE). The cells express high levels of RFP-CAT upon viral infection with Sendai virus (SeV) or IFN treatment and this can easily be monitored either by a fluorescence reader or by CAT assay. We propose to use this cell line to develop assays for the screening of cellular libraries and small molecule compounds with the ability to either induce or repress the IFN response. Molecules with the ability to induce the IFN response could have applications as potential immunomodulators *in vivo*. Molecules with the ability to repress the IFN response can be potential negative regulators of the IFN response. In addition, this cell line could be used to screen for compounds and cellular genes with the ability to inhibit viral IFN antagonist proteins. These viral proteins are good targets for the development of antiviral drugs because reducing the anti-IFN activity of the invading virus may allow the host innate immune response to control viral replication. With this concept, we have stably expressed different IFN antagonist proteins from influenza, arena and hepatitis C viruses in our reporter cell line. Stable expression of the NS1 protein of influenza virus, the NP protein of LCMV and the NS3-4a protein of hepatitis C virus results in block of RFP-CAT expression after stimulation with SeV infection. We predict that compounds with the ability to inhibit these viral products will restore RFP-CAT expression and will be potential inhibitors of viral infection. These new compounds may represent new classes of antiviral drugs.

12-19/P

ATTENUATION OF INTERFERON PATHWAY BY DENGUE VIRAL PROTEASE THROUGH A RIG-I-DEPENDENT MECHANISM

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Intracellular viral infection is sensed by the cellular RNA helicase RIG-I, which then turns on the type I interferon (IFN) response by activating the transcription factors IRF3 and NF-κB. Recently, accumulated evidences have revealed the important role of RIG-I antiviral pathway and virus appears to counteract this pathway. The adaptor protein MAVS (also known as Cardif, IPS-1, and VISA) of RIG-I pathway was found to be targeted by hepatitis C virus protease NS3-4A. Here we report that the protease NS2B-3 encoded by dengue virus serotype 2 (DEN-2) interferes with IFN response by targeting RIG-I, as IRF3 activation triggered by virus infection and the N-terminal CARD domain of RIG-I, but not by the downstream molecules such as MAVS and TBK1, were blocked by DEN-2 protease. Overexpression of the wild-type but not a catalytically inactive NS2B-3 reduced the protein level of RIG-I, and a protein-protein interaction of RIG-I and DEN-2 NS2B-3 was noticed. The virus-induced IRF3 and NF-κB activation was also blocked in cells overexpressing DEN-2 NS2B-3. Collectively, our data show that DEN-2 protease modulates the IFN production by counteracting RIG-I, suggesting that members of the Flaviviridae family might adapt different mechanisms to attenuate the IFN response.

12-20/P

CCHFV REPLICATION DOWN-REGULATES THE PRODUCTION OF INTERFERON DURING THE INNATE IMMUNE RESPONSE

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In the combat against viral infections, mammalian cells elicit innate responses, characterized by the induction of type I interferons and the subsequent up-regulation of interferon stimulated genes (ISGs). To counter-act the cellular defence, many viruses encode interferon antagonists with the ability to down-regulate the early immune responses. However, no interferon antagonist has, up to this date, been identified for Crimean-Congo Hemorrhagic Fever Virus (CCHFV), a Nairovirus of the family *Bunyaviridae* and the causative agent of severe hemorrhagic fever in humans. To investigate if CCHFV has the ability to interfere with the immune defence, we used UV-light to produce replication deficient CCHFV and compared the interferon response following infection, to that of an infection with native CCHFV. In cells infected with UV-treated virus, up-regulation of ISG56 mRNA was observed already after three hours. However, for the native virus, ISG56 up-regulation was not observed until 24 hours post infection. As expected, expression of CCHFV NP mRNA was only observed in cells infected with the native virus. In addition, the ability of native and UV-treated virus to induce nuclear translocation of the transcription factor IRF-3 was investigated. We found IRF-3 in the nucleus already after three hours in cells infected with UV-treated virus but not until 24 hours post infection for the native virus. Taken together, our observations indicate that replicating, native CCHFV expresses an interferon antagonist with the ability to down-regulate the interferon response during the early phase of infection, possibly by interfering with the activation pathway of the transcription factor IRF-3.

12-21/P

TOLL-LIKE RECEPTOR 2- AND -9-INDEPENDENT ANTIVIRAL CYTOKINE RESPONSE IN HUMAN MONOCYTE-DERIVED MACROPHAGES AND DENDRITIC CELLS AFTER HERPES SIMPLEX VIRUS INFECTION

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We have previously shown that cellular recognition of herpes simplex virus (HSV) proceeds through both Toll-like receptor (TLR)-dependent and TLR-independent mechanisms in murine macrophages. Here, we present new data on recognition of HSV infection in human monocyte-derived macrophages and dendritic cells (DCs). We found that in human macrophages and DCs, a wide spectra of antiviral cytokines and interferons (IFNs) are produced, including the newly discovered type III IFNs IL-29 (IFN-lampda1) and IL-28 (IFN-lambda2/3). For other cell types than human monocyte-derived cells reports have evidenced TLR2- and TLR9-mediated recognition of HSV. In the human monocyte-derived macrophages and DCs, however, we observed TLR2- and TLR9-independent cytokine production. Furthermore, the cytokines response was dependent on virus replication. We suggest an intracellular recognition mechanism dependent on HSV-derived transcriptional products. Possible HSV-recognition receptors are the RNA helicases melanoma-differentiation-associated gene 5 (Mda-5) and retinoic-acid-inducible protein I (RIG-I), which were both shown

to be unregulated after HSV infection. Furthermore, our studies show a new viral evasion mechanism for HSV. Virus deficient in infected cell protein (ICP)27 strongly induces expression of IFNs and cytokines compared to the wild type virus. The ICP27-deficient virus result in increased activation of the cytokine-regulating transcription factors IRF3 and NF-kappaB. Collectively, our data show that HSV-recognition in human macrophages and dendritic cells proceeds independent of TLR2 and TLR9, but dependent on virus replication. Viral ICP27 is added to the list of viral proteins that inhibit the antiviral cytokine response generated during HSV infection.

12-22/P

THE KERATINOCYTE RESPONSE TO VACCINIA VIRUS INDUCED PATHOGEN-ASSOCIATED MOLECULAR PATTERNS

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Vaccinia virus (VV) is the agent currently used in smallpox vaccinations. Vaccinations are given by scarification, which delivers the virus by breaking the skin. Keratinocytes provide an impermeable layer of protection to the body as these cells are located on the surface of the skin. During VV vaccination keratinocytes are most likely one of the first cell types infected. Cells have evolved multiple mechanisms to counter viral infections. Pathogen-associated molecular patterns, such as dsRNA, are by-products of virus replication and allow the cell to recognize the viral infection and mount a defense against the virus. The signal transduction cascades activated and the corresponding host gene expression profile in response to viral infection are crucial to the understanding of virus-host interaction. The VV E3L protein is involved in overcoming the host antiviral response by sequestering dsRNA and is a vital factor contributing to pathogenesis. Infection of primary keratinocytes with viruses containing mutations in the dsRNA binding domain of E3L led to activation of many signal transduction cascades and caused increased expression of many host genes involved in the inflammatory and antiviral response. The antiviral response in keratinocytes was very robust supporting the role of these cells in the host's primary defense. These data suggest a model for pathogenesis seen during vaccinations and may also provide the reason why viruses containing mutations in the dsRNA binding domain of E3L prove to be good candidates for future vaccines.

12-23/P

THE ROLE OF TYPE I INTERFERON IN LEGIONELLA INFECTION OF MURINE MACROPHAGES

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Legionella pneumophila is an intracellular bacteria that upon aerosolization can be inhaled by humans, infect alveolar macrophages and cause a severe pneumonia called Legionnaire's Disease. Once in the cell, Legionella is able to prevent the phagosome lysosome fusion and forms a unique ER-studded replicative vesicle. Macrophages from most inbred strains of mice resist Legionella replication with the exception of A/J mice.
The unique susceptibility of macrophages from A/J mice to Legionella infection has been mapped to Naip5 (Neuronal Apoptosis Inhibitory Protein 5, also called Birc1e), a member of the NBS-LRR (Nucleotide-Binding Site and Leucine-Rich Repeat) family of pattern recognition molecules. More recent studies suggest that wild-type Naip5 (e.g., from C57Bl/6J mice) recognizes Legionella flagellin and directs the activation of the inflammasome, culminating in the production of IL-1β and IL-18, and potentially apoptosis. Although flagellin deficient Legionella fail to activate the inflammasome, they still serve to robustly activate NFκB (nuclear factor kappa B) and IFN-I (Type I Interferon) expression. Consistent with an important role for IFN-Is in the innate response to Legionella, IFNAR-1 (Interferon-alpha Receptor 1) null macrophages are readily infected with this pathogen. Moreover, a single treatment with IFN-I effectively blocks Legionella growth in susceptible A/J macrophages. As IFN-I treatment does not alter Naip5 expression, our studies suggest that the protection afforded by IFN-I is independent of Naip5. Current studies are focused on understanding how IFN-I expression is stimulated and how IFN-I mediates its protective effect.

12-24/P

INHIBITION OF TOLL-LIKE RECEPTOR-DEPENDENT AND -INDEPENDENT IMMUNE SIGNALLING PATHWAYS BY VACCINIA VIRUS PROTEINS

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Vaccinia virus (VV) is a double-stranded DNA virus that has evolved a range of immune evasion strategies. We have previously identified the VV proteins A46 and A52 as inhibitors of Toll-like receptor (TLR) signalling. Toll-like receptors trigger innate immunity in response to the recognition of pathogen-associated molecules. The discovery that A46 and A52 block TLR signalling was the first evidence for a role for TLRs in anti-viral immunity. Although both proteins share some homology and inhibit TLR-induced signals, they seem to target the signalling pathways at different levels and to different extents. We have identified the intracellular targets of A46R and A52R, and have characterized their effects on the different signalling pathways emanating from TLRs as well as on TLR-induced gene induction. The first step of TLR signalling is mediated by homotypic interactions between the Toll-Interleukin-1-Resistance (TIR) domains of the receptors and the adaptor molecules. While A46 contains a TIR domain and binds directly to host TIR-domain containing proteins, A52 interacts with the downstream signalling molecules TRAF6 and Irak2. Both A46R and A52R independently contribute to virulence. More recently the cytoplasmic RNA-helicase RIG-I (retinoic-acid inducible gene-1) has been identified as an important TLR-independent mediator of anti-viral immunity. We have now identified other vaccinia virus open reading frames (ORFs) that interfere not only with TLR-signalling but also shut down signals emanating from RIG-I. Therefore the actions of these ORFs seem to be broader and are likely to be more potent than the effects of A52 or A46. We have set out to explain the differences in specificity and potency of these VV proteins by looking for additional targets of the novel ORFs and have found an interaction between one of these VV proteins and a host helicase, which could account for some of the additional effects observed.

12-25/O

INTERFERON-Λ CONTRIBUTES TO INFLUENZA VIRUS RESISTANCE

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A broad range of different IFN subtypes are secreted by virus-infected cells which in turn trigger the synthesis of antiviral factors like Mx and PKR that confer resistance to influenza A virus (FLUAV). IFN-α, -β, -ω, -ε/τ, -κ and limitin (type I IFN) and IFN-λ (type III IFN) activate similar STAT signaling cascades although they engage distinct cell surface receptors on target cells. Since mice lacking

functional type I IFN receptors (IFNAR$^{0/0}$) are highly susceptible to various viral diseases, it remained unclear if IFN-λ also contributes to virus resistance. We report here that IFNAR$^{0/0}$ mice possessing a functional *Mx1* gene (Mx-IFNAR$^{0/0}$) were more resistant to intranasal infection with FLUAV than IFNAR$^{0/0}$ mice with defective *Mx1* genes. Enhanced resistance of Mx-IFNAR$^{0/0}$ mice was most striking (lethal dose ≈ 100-fold higher) if infections were performed with a mutant FLUAV that triggers a more vigorous innate immune response than wild-type virus due to a C-terminal truncation of the IFN antagonistic factor NS1. Interestingly, resistance of Mx-IFNAR$^{0/0}$ and IFNAR$^{0/0}$ mice did not differ substantially if a hepatotropic, Mx-sensitive orthomyxovirus (Thogotovirus) was used for infection, indicating that a protective factor distinct from type I IFN was active in infected lungs but not liver. To determine if the unknown protective agent was IFN-λ, we applied recombinant IFN-λ by the intranasal route a few hours before FLUAV challenge. Treatment with either IFN-λ2 or -λ3 resulted in robust protection of Mx-IFNAR$^{0/0}$ but not IFNAR$^{0/0}$ mice against highly pathogenic wild-type FLUAV, demonstrating that IFN-λ can mediate FLUAV resistance. The activity of virus-induced IFN-λ might further explain why FLUAV mutants lacking NS1 are benign in IFNAR$^{0/0}$ mice but virulent in STAT1$^{0/0}$ and PKR$^{0/0}$ mice. Our results collectively indicate that the type III IFN system may protect the organism from viruses like FLUAV which enter the body *via* mucosal surfaces.

12-26/P

INTERFERON-ANTAGONISTIC ORTHOMYXOVIRUS PROTEIN TARGETS TFIIB: SELECTIVE SUPPRESSION OF IRF-MEDIATED GENE EXPRESSION

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The tick-transmitted orthomyxovirus Thogoto virus (THOV) has an anti-interferon (IFN) strategy remarkably different from the related influenza A virus (FLUAV). To interfere with the induction of the antiviral type I IFNs, FLUAV uses the nonstructural protein NS1 which binds dsRNA and blocks the nuclear export of host mRNAs. THOV, by contrast, has no nonstructural proteins but a protein termed ML, a splice variant of the structural matrix protein, which strongly interferes with the activation of promoters dependent on IRF-1, -3, -7, and -9. For IRF-3 and IRF-7, we could show that ML prevents their dimerisation while not affecting nuclear translocation. This suggests that ML directly interferes with formation of the transactivating complex on interferon stimulated response element (ISRE) dependent promoters. Indeed, using tandem affinity purification we identified the general RNA-PolII transcription factor TFIIB as an ML-associated protein . The interaction was confirmed by coimmunoprecipitation and *in vitro* binding assays and may explain the observation that ML, besides its strong anti-IRF effect, also has a weak general impact on RNA-PolII-dependent mRNA transcription. TFIIB is known to physically and functionally interact with IRF-1. Therefore, we are currently analyzing the effect of ML on the interplay of TFIIB with IRF-1 as well as other members of this important transcription factor family. In summary, we hypothesize that binding of the orthomyxoviral pathogenicity factor ML to core transcription factor TFIIB interferes with the function of IRFs on ISREs and thus prevents the induction of antiviral genes.

12-27/P

TEMPLATE LENGTH-DEPENDENT INHIBITION OF INFLUENZA A VIRUS MINIREPLICONS BY ANTIVIRAL MX1 GTPASE

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The interferon-induced Mx1 GTPase is a potent inhibitor of influenza A virus (FLUAV) in mice. Here, we investigated the mode of action of the mouse Mx1 protein against FLUAV at the molecular level. It has previously been shown that viral primary transcription is inhibited by Mx1. To further analyze the mechanism of action, we compared the Mx1 sensitivities of different FLUAV strains. In general, avian FLUAV isolates were more sensitive than human strains to the antiviral effect of Mx1. An interesting exception was the human strain A/WSN/33 which also showed a high Mx1 sensitivity. Therefore, we used a FLUAV minireplicon system based on strain A/WSN/33 to analyze the mechanism of Mx1 action. Surprisingly, no inhibition of reporter gene expression was detected with a minigenome coding for chloramphenicolacetyltransferase (CAT, 670 nucleotides (nt)). In contrast, strong inhibition was observed when luciferase (1650 nt) or a CAT-CAT construct containing two tandem CAT sequences (1340 nt) served as reporter genes. Thus, the length of the reporter gene seemed to determine Mx1 sensitivity of FLUAV minireplicons. Our data suggest that mouse Mx1 protein inhibits *in vivo* reconstituted FLUAV polymerase complexes in a template length-dependent manner.

12-28/P

NEUTRALIZATION OR ABSENCE OF THE IL-23 PATHWAY DOES NOT COMPROMISE IMMUNITY TO MYCOBACTERIAL INFECTION

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IL-23, a member of the IL-12 family, is a heterodimeric cytokine that is composed of the p40 subunit of IL-12 plus a unique p19 subunit. IL-23 is critical for autoimmune inflammation, partially by its induction of the pro-inflammatory cytokine IL-17A. It is less clear, however, if IL-23 is required during the immune response to pathogens. We have examined the role of IL-23 during *Mycobacterium bovis* BCG infection. We find that IL-23 reduces bacterial burden, promotes granuloma formation, and influences inflammatory cytokine expression when IL-12 is absent. However, IL-23 does not contribute substantially to host resistance when IL-12 is present, as the ability to control bacterial growth and form granulomas is unaffected in IL-23p19-deficient mice and mice treated with a specific anti-IL-23p19 antibody. IL-23p19-deficient mice are also able to mount an effective memory response to secondary infection with BCG. While IL-23p19-deficient mice do not produce IL-17A, this cytokine is not necessary for effective control of infection. This data suggests that IL-23 by itself does not play an essential role in the protective immune response to BCG infection; however, the presence of IL-23 can partially compensate for the absence of IL-12. Furthermore, neutralization of IL-23 or IL-17A does not increase susceptibility to mycobacterial infection.

12-30/P

OXIDIZED PHOSPHOLIPIDS INHIBIT PHAGOCYTOSIS AND IMPAIR OUTCOME IN GRAM-NEGATIVE SEPSIS IN VIVO

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Oxidized phospholipids that are generated during inflammation exert anti-inflammatory properties and prevent death during murine endotoxemia. Oxidized 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine (OxPAPC) inhibits the interaction of lipopolysaccharide (LPS) with LPS-binding protein (LBP) and CD14. We here determined the functional properties of OxPAPC and potential interference with CD14 during abdominal sepsis caused

by *Escherichia (E.) coli*. Administration of OxPAPC rendered mice highly susceptible to *E. coli* peritonitis, as indicated by an accelerated mortality and enhanced bacterial outgrowth and dissemination. $CD14^{-/-}$ mice also displayed increased mortality and bacterial outgrowth and OxPAPC did not further impair host defense in these animals. The mechanisms by which OxPAPC and CD14 deficiency impaired the immune response differed: whereas $CD14^{-/-}$ mice demonstrated a strongly reduced recruitment of phagocytes to the site of the infection, OxPAPC did not influence the influx of inflammatory cells but strongly diminished the phagocytosing capacity of neutrophils and macrophages by a CD14 independent mechanism. These data suggest that oxidized phospholipids such as produced during inflammatory reactions may contribute to mortality during Gram-negative sepsis *in vivo via* impairment of the phagocytic properties of professional phagocytes.

12-31/P

PREDICTIVE MODEL AND SUSTAINED VIROLOGIC RESPONSE FOR PEG-IFN-α-2 + WEIGHT-BASED RIBAVIRIN NONRESPONDERS RE-TREATED WITH IFN ALFACON-1 + WEIGHT-BASED RIBAVIRIN

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Background: HCV patients who fail to achieve ≥2 $\log_{10}$ reductions in HCV RNA after 12 weeks of Peg-IFN-α-2 + ribavirin (RBV) therapy have a ≈ 3% chance of obtaining a sustained virologic response (SVR), so therapy is often halted at that point. Since all activities of IFN-α are mediated by the production of IFN-stimulated genes (ISG), lack of response to peg-IFN-α-2 might be attributed to lack of adequate ISG induction. IFN alfacon-1 (Infergen®) is a bioengineered type 1 IFN that displays 10- to 100-fold higher biological potency, and can hyperstimulate ISG production when compared to naturally occurring IFNs. We hypothesized that switching to IFN alfacon-1 in patients who failed to achieve ≥2 $\log_{10}$ reductions in HCV RNA after 12 weeks of Peg-IFN-α–2 + RBV therapy might result in increased SVR.

Methods: We conducted a retrospective review of 137 consecutive patients. All patients previously received 12 weeks of Peg-IFN-α-2b 1.5 μg/kg SC every week + weight-based RBV and did not have a ≥2 $\log_{10}$ reduction in HCV RNA. With no washout, these patients were retreated with IFN alfacon-1 at 15 μg QD and weight-based RBV for 12 weeks. Subsequently the dose of IFN alfacon-1 was reduced to 15 μg TIW for the remainder of the 48 weeks. HCV RNA was assessed at baseline and at weeks 12, 24, 48, and 72 (6 months posttherapy). A logistic regression model was constructed to determine variables that predicted SVR to IFN alfacon-1 + RBV therapy.

Results: The percentage of patients who became HCV RNA negative at weeks 12, 24, 48, and 72 was 23%, 31%, 43%, and 37% (SVR) respectively. In a multivariate model that assessed the predictive value of viral and demographic factors and SVR to retreatment with IFN-alfacon-1 + RBV, only the $\log_{10}$ reduction in HCV RNA from prior Peg-IFN-α-2 + RBV therapy was an independent predictor of outcome ($P < 0.001$). Specifically, the mean reduction in HCV RNA after 12 weeks of Peg-IFN-α-2 + RBV for patients who achieved SVR to IFN alfacon-1 + RBV was 0.5 ± 0.05 $\log_{10}$ copies/mL *versus* 0.18 ± 0.04 $\log_{10}$ copies/mL for non-responding patients. Neither HCV genotype, presence of fibrosis, viral copy number at baseline, nor sex were significant predictors of SVR to retreatment with IFN alfacon-1 + RBV.

Conclusions: In these difficult-to-treat patients, retreatment with IFN alfacon-1 + RBV resulted in a clinically significant rate of SVR (37%). Log_{10} reductions during prior peg-IFN-α-2 + RBV was a significant predictor of SVR to retreatment with IFN alfacon-1 + RBV. These initial data are highly promising and warrant further study.

12-32/O

NOVEL CPG OLIGODEOXYNUCLEOTIDE-BASED STRATEGIES FOR INDUCTION OF MUCOSAL IMMUNITY IN THE FEMALE GENITAL TRACT

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Intravaginal administration of non-replicating antigens elicits only weak, non-disseminating immune response. The development of a vaginal immunostimulatory adjuvant capable of generating a potent specific immune response towards antigens derived from sexually transmitted pathogens would therefore have profound implications for the prevention of sexually transmitted diseases. In our laboratory, we have recently documented the ability of CpG oligodeoxynucleotide (ODN), a Toll-like receptor 9 ligand, for generation of a rapid and potent response of Th1-associated cytokines as well as CC and CXC chemokines in the murine female genital tract and the draining lymph nodes. We could also show that CpG ODN can function as a potent Th1-tilting adjuvant for vaginal immunization of mice. Thus, intravaginal vaccination with recombinant gD protein from herpes simplex virus type 2 in combination with CpG ODN gave rise to a strong antigen-specific Th1-like immune response in the genital lymph nodes as well as the spleens of the vaccinated mice. Further, such an immunization scheme conferred protection against an otherwise lethal vaginal challenge with the virus. We have also developed a novel, rationally designed immunostimulatory adjuvant based on chemical conjugation of CpG ODN to the non toxic B subunit of cholera toxin (CTB). We could demonstrate that optimal human or murine CpG ODN chemically linked to CTB elicits robust CC chemokine responses in both human and murine immune cells *in vitro*. Further, mucosal administrations of mice with CTB-CpG were found to elicit potent innate chemokine responses in the female genital tract mucosa. Importantly, we could show that CTB-CpG can serve as a powerful Th1-tilting adjuvant for induction of antigen-specific acquired immunity in mice. Interestingly, we could show that the conjugation of CTB to CpG ODN can override species-specificity of CpG ODN. These preclinical proof-of-concept studies suggest a novel CpG ODN-based strategy by which to counter sexually transmitted diseases in humans.

12-33/O

IC31™, A NOVEL ADJUVANT BASED ON A CATIONIC PEPTIDE DELIVERY SYSTEM, IN PRECLINICAL AND CLINICAL STUDIES

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There is a great need to improve vaccination techniques, especially for the management of cancer and pathogens with an intracellular life cycle. For the control of the underpinning diseases the induction of a strong cellular immune response is particularly required, in contrast to classic vaccines that mainly seem to address humoral immunity. Therefore it is necessary to develop adjuvants that drive the immune system into the desired path. IC31™ combines a negatively charged synthetic oligodeoxynucleotide (ODN1a) with a positively charged peptide (KLK). KLK enhances the uptake of antigens by APCs and facilitates also the uptake of ODN1a, which acts *via* the intracellularly located TLR9 / MyD88-dependent signaling pathway of the innate immune system. Further analyses have shown that the immunostimulatory effect of IC31™ is also mediated by a depot formation at the injection site, caused by KLK. IC31™ leads to a sustained activation of antigen presenting cells (APCs) which is characterized by a broad mechanism of action resulting in the induction of potent effector and memory antigen-specific immune responses. Both, $CD4^+$ helper T cells as well as $CD8^+$ cytotoxic T cells are induced. In addition, strong humoral immune responses with increased antibody titers are generated by IC31™. All together, IC31 is a strong immunostimulator with a wide spectrum of applications. Beside its broad mechanisms of action, IC31™ convinces by an excellent safety profile. IC31™ has been tested in a variety of preclinical vaccine studies with promising results and has entered clinical trials concerning a novel protein subunit vaccine against TB. Thus, our data strongly suggest that IC31™-induced adaptive immunity is suitable for the design of vaccines against infectious and neoplastic diseases.

12-34/O

ADJUVANT ACTIVITY OF INTERFERON ALPHA IN INFLUENZA VACCINATION

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Influenza vaccination is only partially effective in at-risk populations including the elderly and immuno-compromised and there is a need for non toxic adjuvants to enhance protection. IFNα produced primarily by plasmacytoid dendritic cells (DCs) as part of the innate immune response to infectious agents, is a polyclonal B-cell activator that induces a strong primary humoral immune response characterized by isotype switching and protection against virus challenge. IFNα also acts as a powerful adjuvant when ad-mixed with influenza vaccine and injected intramuscularly (im). Oro-mucosal (om) administration of IFNα also enhances the humoral response to concomitant im influenza vaccination even though IFN was administered separately from the vaccine. IFN treatment increased both virus-specific IgG1 and IgG2a characteristic of a mixed Th1/Th2 response, and secretory IgA associated with resistance to infection. The use of transgenic mice expressing an IFN-regulated EGFP reporter gene showed that IFN activated DCs, are present in the peripheral circulation of mice 4 hours after influenza vaccination and that the number of circulating IFN-activated DCs increased markedly when IFN was administered om concomitantly with im injection of influenza vaccine suggesting that trafficking of APCs to the site of vaccination may explain in part the mechanism(s) of the adjuvant activity of IFNα. A randomized double-blind clinical trial was initiated to evaluate the efficacy of om administration of IFN α as an adjuvant to influenza vaccination in an elderly institutionalized population. Healthy subjects without neoplastic or autoimmune disease were vaccinated with a standard influenza sub-unit vaccine and then randomized to two groups and treated orally with either 10^7 IU of Intron A in saline or saline alone. Expression of ISG15 was determined in epithelial cells recovered from the oral cavity to control for IFN uptake. The virus-specific antibody response in the two groups was determined at 15, 21, 90, and 180 days.

12-35/O

A HUMAN PROTEIN USED AS A VACCINE ADJUVANT TO INDUCE T-CELL RESPONSES

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A consensus has emerged that one of the key factors in bringing effective therapeutic vaccines to patients will be the choice of the right adjuvant. This is not to say that the selection, design and optimisation of antigens are not vital. Or that there will be no place for vectorisation systems especially those able to increase antigen uptake by dendritic cells. But without some way of decisively intervening in the way those antigens are processed inside the dendritic cell, how that cell matures, migrates and, finally, how it presents the antigen to potential effector T cells, we will never be able to make therapeutic vaccines work.
The above is even more important for therapeutic vaccines against cancer. Although vaccine adjuvants are being extensively tested in pre-clinical and clinical approaches, their mechanisms of action are not yet completely understood. Most of them are TLR ligands that work mainly by activating and licensing dendritic cells (DC) to present antigens with greater efficacy to naïve or memory CD8 cells. The ability to induce a strong immune response that includes the generation and expansion of tumour-specific cytotoxic T lymphocytes (CTL) is indeed the first requirement for a clinically effective cancer vaccine. Recent advances in knowledge have enabled us to understand that what we thought of only a few years ago as motley collections of bacterial debris, are in fact ligands for a coherent set of pathogen detecting receptors. This seductive discovery is, however, in danger of making us think that the TLR ligands are the only solution to the adjuvant question. We propose that they may have limitations and that a human protein acting by a totally different mechanism might be more effective in eliciting those sought-after CTLs.