05

Interferons

05-01/P

N-GLYCOSYLATION OF MURINE INTEFERON-BETA IN A PUTATIVE RECEPTOR BINDING REGION

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Type-I interferons (IFNs) are crucial for resistance of organisms to viral infections. They also have an important antiproliferative activity and modulate the adaptive immune response. Human and mouse genomes encode a large family of type-I IFNs such as IFN-α, IFN-β, IFN-ε, IFN-κ, IFN-ω (human), and limitin (mouse). Part of these IFNs are glycosylated. Although the aminoacid sequences of these various IFN subtypes differ markedly, they are all considered to share a common 3-D structure and to bind the same heterodimeric receptor, composed of the IFNAR1 and IFNAR2 subunits. Interaction between human IFN-α2 and IFN-β, and IFNAR2 was reported to involve helix E and part of the AB loop of the IFN molecule. Analysis of available mammalian IFN-β sequences showed that they all carry 1 to 5 predicted N-glycosylation sites. Interestingly, murine IFN-β contains three predicted N-glycosylation sites (N29, N69, N76), one of which (N29) being located in the AB loop, in a region predicted to interact with the type-I interferon receptor. The aim of this work was to test whether this site is indeed N-glycosylated and whether glycosylation of this site would affect IFN antiviral activity. In this work, we showed that all three N-glycosylation sites predicted from the sequence, including N29, carry N-linked sugars. Mutation of individual N-glycosylation sites had a weak negative influence on IFN antiviral activity. In contrast, the complete loss of glycosylation dramatically decreased activity. The presence of a glycosylation site (N29) in the AB loop of murine IFN-β suggests that interaction of this IFN with the IFNAR receptor could partly differ from that of human IFN-α2 and human IFN-β, in spite of the strong similarity of the 3D structures of these molecules.

05-03/P

RNASE L IS A TARGET OF ISG15 CONJUGATION

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Interferon-α/β (IFN) serves a critical role in anti-viral innate immunity and in modulating the adaptive immune response to infection and tumor development. In response to infection or Toll-like receptor agonists, IFN is produced and subsequently leads to the upregulation of hundreds of genes. One of the most highly induced genes is ISG15, a small ubiquitin-like protein that acts within cells to post-translationally modify cellular proteins. ISG15 conjugation (ISGylation) to substrate proteins occurs in a manner similar to ubiquitin conjugation by utilizing activating, conjugating, and ligating enzymes to facilitate the addition of ISG15 to specific lysine residues. We have recently identified the IFN regulated protein RNase L, as a potential target for ISG15 conjugation. RNase L is an endoribonuclease activated by 2',5'-oligoadenylates (2-5A) generated by a family of IFN induced 2-5A synthetases. It has been well documented to possess potent antiviral, pro-apoptotic, and anti-proliferative activity mediated by the degradation of viral and cellular RNAs and transcription regulation. In addition, RNase L has more recently been identified as a tumor suppressor associated with prostate cancer. Our studies analyzing the ISGylation of RNase L will continue to focus on mapping of the conjugation site and determining the functional implications of RNase L conjugation. These findings may add new insight into the regulation of RNase L's multiple functions, as well as provide evidence for the role of ISGylation in the cellular response to IFN.

05-04/P

HISTONE DEACETYLASES ARE NOVEL ISGYLATION TARGETS

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ISG15 is an interferon (IFN)-regulated ubiquitin-like protein that is implicated in the host immune response. ISG15 is covalently linked to lysine residues in target proteins (ISGylation) by enzymes analogous to those that mediate ubiquitylation. ISGylation is a dynamic process, with the ISG15 isopeptidase, UBP43, catalyzing the removal of ISG15 from its substrates. In a screen for candidate ISGylation substrates and UBP43 regulators, we identified histone deacetylase 2 (HDAC2) as a novel UBP43 interacting protein. Histone deacetylases (HDACs) are a family of proteins that mediate removal of acetyl groups from lysine residues in histone and non-histone proteins, and are classified into three families based upon homology to yeast proteins. To determine if HDAC2 is an ISGylation substrate, 293T cells were transfection with UBE1L, UBC8, ISG15, and HDAC2 expression plasmids. Immunoprecipitation of HDAC2 and Western blot analysis with anti-ISG15 revealed a novel immunoreactive band at approximately 80kD, the predicted size for a mono-ISGylated HDAC2. In contrast, this band was absent in transfection that lacked ISG15. Analysis of other HDAC family members in the ISGylation transfection system revealed multiple HDACs were ISGylated. Our subsequent analysis focused on HDAC6, as this member interacts with HSP90 and maintains HSP90 in a deacetylated state, which is specifically required for HSP90 interaction and stabilization of client proteins. HSP90 has been implicated in IFN action through TBK-1 stabilization and IRF-3 activation, therefore ISGylation of HDAC6 may modulate this activity. Deletion analysis suggested the presence of two ISGylation sites in HDAC6, and further mapping of the ISGylated residues will permit the functional analysis of this modification. HDACs modulate diverse cellular functions, thus their regulation by ISG15 may mediate, in part, the biological activities of IFN.

05-05/O

INTERFERONS: NATURAL DEFENSE AGAINST ANTHRAX AND OTHER BACTERIAL INFECTIONS *IN VIVO*

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Interferons (IFN) play a role in innate immunity during many viral, bacterial, and protozoal infections. During bacterial infections, production of interferons and protection by interferons has been reported for streptococci, staphylococci, *Haemophilus*, *Shigella*, tubercle bacilli, *Pseudomonas*, and *Bacillus anthracis*, *in vitro* and *in vivo*. *In vitro* IFN is reported to activate and protect macrophages against anthrax infection. We addressed the hypothesis that, *in vivo*, interferon also plays a defensive role against inhalation anthrax. First, we studied the protective effect of interferon and an interferon inducer during experimental anthrax in a murine model. In addition, we studied infection of IFN type 1 receptor KO mice (IFNAR KO). Adult Swiss-Webster mice were pretreated with either recombinant murine interferon β (rMuIFNβ) injected i.p. or with the double-stranded RNA IFN inducer Poly I:CLC i.m. and/or i.n., or with IFNγ i.p. 24 hr prior to intranasal challenge with *Bacillus anthracis* Ames spores. Mice pretreated with Poly I:CLC were more resistant to progression of lethal infection with *B. anthracis,* as shown by significant delay in time to death. To determine the role of IFN, other groups of mice were treated with rMuIFNβ or rMuIFNγ prior to nasal instillation of anthrax spores. Protection occurred with the type 1 IFN rMuIFNβ, but, surprisingly, not with type 2 rMuIFNγ. Combining Poly I:CLC and rMuIFNγ was not synergistic. The results suggest that the type 1 IFNs may be more important than type 2 IFN in innate immunity to anthrax. Consistent is the preliminary finding that IFNAR KO mice develop an accelerated disease. Studies of IFN production during infection are ongoing. Thus it is likely that the IFN type 1 component of the innate immune response is responsible for stimulating a significant protection against anthrax *in vivo*, as IFNs do with other bacteria. Clinical usefulness may be possible.

05-06/P

SOFT MEDICAL FORMS OF VIFERON® IN VIRIOUS INFECTION DISEASES

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Viferon® suppositoria contains recombinant interferon alpha-2b (IFN- 2b), vitamins E and C, as antioxidant. Viferon® ointment contains IFN- 2b, vitamins E. Viferon® gel contains IFN- 2b, antioxidants (vitamin E, methionine, benzoic and citric acids) and excipients. Treatment with Viferon® suppositoria in the complex therapy infants with clinical manifestations of viral-bacterial infectious diseases resulted in degrease of duration of infectious toxicosis, length of desease, period of antibacterial treatment. Treatment with Viferon® ointment of patients with recurrent herpes virus infection (RHI) – 4 times daily during 7 days - resulted in complete clinical recovery in 75% patients (60% in the control group with Zovirax®). Treatment with Viferon® gel of children with recurrent stenosing laryngotracheobronchitis (SLTB) – 5 times daily for one week and then 3 times daily for three weeks - resulted in decrease of clinical symptoms (duration of intoxication syndrome decreased by a factor of 2,4, duration of laryngitis and rhinitis decreased by a factor of 2,3 and 1,8, respectively).

05-07/P

THE SCAFFOLDING ADAPTOR PROTEIN GAB2 IS STABILIZED BY THE INTERFERON-RECEPTOR IFNAR1 CHAIN

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Interferons (IFNs) are pleiotropic cytokines involved in the regulation of antiviral, antitumor, and immune responses as well as cell differentiation. Upon interaction with their specific receptors on target cells, type I IFNs induce the Jak/STAT signalling pathway. This cascade leads to transcription of IFN sensitive genes which encodes proteins involved in the various biological activities of type I IFNs. Numerous studies have provided evidence, however, that the activation of the Jak/STAT pathway alone cannot account for the wide range of IFN's biological effects. Interestingly, several proteins not involved in the Jak/STAT signalling cascade are tyrosine phosphorylated in response to IFN stimulation, but the mechanisms leading to their activation remain to be defined. In order to better understand the role of alternative signalling pathways in the type I IFN response, we used a proteomics approach to identify novel proteins tyrosine-phosphorylated upon IFN-alpha 2 stimulation. This study led to the identification of several proteins including the Grb2-associated binder 2 (Gab2). Gab2 belongs to a family of scaffolding proteins capable of assembling multimeric signalling complexes involved in several growth factor and cytokine signalling pathways. We have shown that Gab2 is tyrosine-phosphorylated in response to type I IFNs and is constitutively associated with the type I IFNs receptor subunit, IFNAR1. We also report that this interaction stabilizes Gab2 and that the overexpression of IFNAR2, the second subunit of the type I IFN receptor, shifts the Gab2 adaptor away from IFNAR1. The role of Gab2 stabilization in IFN signalling pathways is currently under investigation. This question is important in the light of the involvement of both Gab2 and type I IFNs in osteoclastogenesis and oncogenesis.

05-08/P

INVESTIGATION OF INTERFERON STIMULATION OF DENDRITIC CELLS FOR INFLUENZA VIRUS VACCINE DEVELOPMENT

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Avian influenza presents the most significant global health challenge since AIDS. To date 100s of millions of birds have been culled and over 100 human deaths have occurred with a mortality rate of 50%. New influenza viruses emerge through mutation and gene reassortment of different virus strains and their ability to jump species barriers. The challenge is to achieve effective vaccine control as biosecurity measures breakdown and antiviral drug resistance has developed. Current inactivated and live attenuated virus vaccines are not globally effective as the virus frequently changes giving rise to new strains of unpredicted virulence. The advent of DNA vaccines incorporating cytokine adjuvants enables better vaccine solutions. We present data on the differential efficacy of type I interferon (IFN) subtypes in a DNA vaccination/challenge mouse model. BALB/c mice were treated with IFN transgenes (IFNA1, A2, A4, A5, A6, A9, or B) alone or co-immunised with HA, NP or M genes derived from influenza A/PR/8/34 (H1N1), delivered intramuscularly via a mammalian expression vector. Results show that IFN-alpha 6 is a potent cytokine that enhances protective immunity in conjunction with HA and NP vaccines. Although IFN induces dendritic cell (DC) maturation and stimulation, the role of IFN subtypes and their interaction with DC, the body's first line of defense to virus infection of mucosal surfaces in the conducting airways, is not well understood. DC subsets play a crucial role in linking the innate and adaptive immune responses with activation of cytotoxic CD8+ T lymphocytes and immune memory. The aim of this part of the study was to compare the efficacies of IFN subtypes in enhancing antigen-processing and immune-stimulating capacity of DC. The outcome will be to recommend strategies for improved avian influenza vaccine development in order to reduce the impact of a pandemic threat if more efficient human-to-human transmission occurs.

05-09/P

TYPE I INTERFERON PRODUCTION BY NEURONS IN THE COURSE OF VIRAL ENCEPHALITIS

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Type I interferons, also referred to as IFNs-alpha/beta, are cytokines playing a crucial role in resistance to viral infections. In the periphery, plasmacytoid dendritic cells (pDCs) are considered to be the major type I IFN producers. Constitutive expression of IRF-7 enables pDCs to rapidly synthesize large amounts of IFNs after viral infection. In the central nervous system (CNS), pDCs are considered to be absent from the parenchyma, and little is known about the cells producing IFN. Our study aimed to identify the cells producing IFN in the CNS *in vivo*, following infection by neurotropic viruses. Expression of IFN-beta, some IFN-alpha subtypes, and limitin, but not of IFN-epsilon or IFN-kappa, was upregulated in the CNS of mice infected with Theiler's virus and La Crosse virus. Double immunofluorescent labeling and in situ hybridization combined with immunohistochemistry showed that both infected cells and cells with undetectable infection produced IFN. Interestingly, besides macrophages and ependymal cells, neurons turned out to be important producers of both IFN-alpha and IFN-beta. Expression of IRF-7 was also examined as this factor is central to IFN genes activation. In situ hybridization failed to show cells with detectable IRF-7 expression in non-infected mice. Upon viral infection, IRF-7 expression was upregulated more than 100-fold and detected in many cell types, including neurons, albeit it was more prominent in inflammatory cells. Our data show that, *in vivo*, neurons take an active part to the antiviral defense by being both IFN producers and responders. Our current efforts aim to define the events involved in the initiation of IFN production in the CNS.

05-10/P

LOW INDUCTION OF INTERFERONS IN HUMAN LEUKOCYTES BY SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS

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The interferons (IFNs) are key mediators of the innate immune response against viral infections. It is a common opinion that the ability of viruses to circumvent IFN responses plays a crucial role in determining the outcome of infection.
The goal of the current study was to examine the changes of IFNs (Type I and II) expression that occur over the first 48 hours after infection with SARS coronavirus (strain hsr1) of lymphomonocytes (PBMC) and to compare these changes to those induced by well-established IFN-inducing viruses, such as Vesicular Stomatitis (VSV) and Newcastle (NDV) viruses. The experiments have been performed by using PBMC of 10 differents healthy donors.
The results showed that VSV and NDV induced high titers of "IFN bioactivity" although the amounts of IFNs produced varied extensively according to both the virus and the individual PBMC donors. In contrast, there was minimal or no detectable total IFN activity in the supernatant of SARS-CoV-infected PBMCs. This parallels the finding that SARS-CoV induces a lower synthesis of IFN alpha, beta and gamma proteins compared to VSV and NDV. Characterization of the profile of IFN alpha subtypes genes expression in SARS-CoV-infected PBMCs showed that the level of IFN alpha 2 subtype was higher than other IFNs alpha subtypes (IFN alpha-1, -2, -5, -6, -8, -10, -13, -17,-21). In addition the results showed that IFN-inducing activity of SARS-CoV was dependent on viability of the virus, because UV-inactivated virus induced a significantly lower IFNs induction compared to live virus. In conclusion the data showed that SARS-CoV is a lower inducer of IFNs compared to VSV and NDV and raise the possibility that the IFN system has a limited direct role in early host defence against SARS-CoV infection.

05-11/P

IFN INDUCED GENE EXPRESSION IN CELLS FROM NASAL WASHES OF INFANTS WITH ACUTE BRONCHIOLITIS

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Objective. The aim of the study was to evaluate the activation of IFN-induced genes [2'-5' oligoadenilate synthetase (2-5OAS), protein kinase R (PKR) and P56] in children suffering from acute respiratory infections. *Methods*. Children with acute bronchiolitis (n = 36) were enrolled in this study. Nasopharyngeal washes were collected in the first 48 hours of admission to Policlinico Umberto I Hospital and were analyzed for common respiratory viruses [Respiratory syncytial virus (RSV), Influenza A and B, Coronavirus OC43, 229 E, NL63, HKU1, Metapneumovirus, Adenovirus, Rhinovirus and Parainfluenza 1, 2,3,4] by using RT-PCR. Levels of expression of 2-5OAS, PKR and P56 mRNAs were determined by Real time PCR. RESULTS: A viral infection was detected in 83% of the specimens of children with acute bronchiolitis. Specifically RSV was the most common virus agent detected (58%), followed by Rhinovirus (25%) and OC43 (3%). In the children with viral infections compared to uninfected infants, elevated levels of 2-5OAS [median 54 (range 20-8719)] *versus* 9 (6-19), PKR [median 17 (range 4-1782] *versus* 3 (range 2-14) and P56 [median 232 (range 28-20594)] *versus* 9 (range 34-75) were found. In addition there was a trend towards greater IFN-induced genes expression in RSV infection compared with Rhinovirus infection. *Conclusion*. These results indicate that in children with acute bronchiolitis there is a strong activation of IFN system induced at least by RSV, Rhinovirus

and OC43 viruses. It is possible that the extent of activation of IFN system may vary depending on the specific type of virus.

05-12/P

NEUTRALIZING INTERFERON ANTIBODIES IN A CHRONIC HEPATITIS C PATIENT NON RESPONDER TO PEGYLATED INTERFERON

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We here describe the clinical course of a patient with chronic hepatitis C in whom the lack of response to pegylated interferons (PEG-IFN) seems due to the presence of neutralizing antibodies (Nab) to PEG-IFN. Briefly a 52-year-old male with chronic hepatitis C ($_{\text{genotype 2a/2c}}$) was treated for 7 months with PEG-IFNa2b at 120 mcg/week subcutaneously (s.c.) plus oral ribavirin 800 mg/day for 24 weeks with no virological and biochemical response. No significant changes in serum HCV-RNA and ALT levels were observed even after during treatment a further six months of therapy with PEG-IFNa2a (at 180 mcg/week s.c), plus ribavirin (1000mg/d), with amantadine (200 mg/d). Surprisingly, no adverse events were observed, nor flu-like symptoms related to IFN treatment. A possible explanation was the development or presence of Nab to IFN alpha. A search for Nab to IFN alpha2a and PEG-IFNa2a was performed and Nab-positivity was recorded in all available serum samples collected during PEG-IFN therapy and later. The titer of NAb was higher when the neutralizing activity was measured against rIFNalpha 2a (range: 209-1031 $t_{1/10}$) than against PEG-IFN (range:33-133 $t_{1/10}$). This finding should be expected, given the different specific activities of the two IFN alpha preparations. No neutralizing activity was recorded (<10$t_{1/10}$) against nIFN alpha thus indicating that nIFN alpha, which is constituted of different IFN alpha subtypes, may overcome the inhibitory activity of NAb when directed to only one subtype, namely IFN alpha 2. The PEG-IFNa2a was then switched to Leukocyte IFNalpha (Alfaferone, 9 MU/d, LeIFNalpha) plus ribavirin at 1000 mg/d. This further treatment led to a significant antiviral response; indeed, the serum HCV-RNA became negative and ALT levels normalized. The NAb titres decreased until becoming negative during treatment with LeIFNalpha. After four months of LeIFNalpha therapy, while demonstrating an optimal antiviral response, the patient developed generalized myasthenia gravis.

05-13/P

STRUCTURE-FUNCTION ANALYSIS OF THE HUMAN INTERFERON ALPHA CARBOXYL-TERMINUS

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The hybrid interferon alpha (IFN-alpha) molecules derived from different IFN-alpha subtype exhibited marked differences from their parental molecules in their antiviral, immunological and antiproliferative activity. Studies using hybrid interferon alpha 2c and alpha 21b have shown that the N-terminal region between amino acid residues 81-95 and the hydrophobic residues at position 86 and 90 are important for binding and antiproliferative activity of human IFN-alpha. However the significance of the C-terminus of the IFN-alpha molecules in their biological activity remains to be determined. In this paper the structure-function relationships for the human IFN-alpha C-terminus were studied using recombinant variants. A total of seven novel hybrid clones derived from parental IFN-alpha 2c and alpha 21b clones were constructed and introduced into the T7 expression vector for protein production using Gateway recombination cloning. All residues changed in these hybrid clones are located on the E-helix and C-terminus tail of IFN-alpha molecule. Of the seven clones, two have an additional substitution at residue position 114 and 139, respectively. The clone plasmids were transformed into *E. coli* cells and the soluble 6×His-MBP-TEV-interferon fusion proteins were prepared from cell lysates using IMAC purification. After TEV protease digestion, the products were tested for antiviral activities in both human WISH cells and bovine MDBK cells. In comparison with IFN-alpha 2c and IFN-alpha 21b clones, the relative antiviral activities of these new constructs vary depending on the cell type tested and the structure of the clones.

05-14/P

ENHANCED ACTIVATION OF IFN-GAMMA SYSTEM MAY PLAY A MAJOR ROLE IN THE GBV-C-DRIVEN PROTECTION AGAINST HIV-1 INFECTION

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GB virus C (GBV-C) co-infection is associated with a better prognosis in HIV-infected persons and IFN activation can be one of the possible mechanisms involved in this protection. In this study, we compared the endogenous activation of the IFN system in PBMC from GBV-C positive and negative patients infected with HIV-1.
The study included 23 GBV-C positive and 23 GBV-C-negative HIV-infected patients, comparable in terms of CD4 cell counts and HIV viral loads. The levels of mRNA for IFN-alpha, IFN-gamma, PKR, IFNAR-1, 2-5-OAS and MxA were measured by real time RT-PCR in PBMC, using β-actin as internal control.
The endogenous levels of IFN-gamma and PKR specific RNA in HIV/GBV-C co-infected patients were significantly higher than in HIV mono-infected subjects, while IFNAR-1 and 2OAS levels tended to be higher, although not reaching statistical significance. MxA and IFN-alpha mRNA were comparable. Direct positive correlation was found between IFN-gamma, PKR, IFNAR-1 and 2-5-OAS, suggesting a coordinated activation of the IFN-gamma-related system, while no correlation was found with MxA and IFN-alpha. A subset of patients were re-sampled at a subsequent time point, showing that, in those with unchanged GBV-C state, the level of mRNA for IFN-gamma and PKR remained unchanged, while in those with reversal of their state for GBV-C (either positive-to-negative or viceversa), there was a consistent change in the mRNA levels of both IFN-gamma and PKR.
These findings, from both cross sectional and longitudinal observation in HIV-1 infected patients, strongly suggest that GBV-C enhances the activation of IFN-gamma response. On the whole, our results may be relevant to understand the GBV-C-driven protection against HIV, suggesting that the endogenous activation of the IFN system can contribute to the control of HIV replication.

05-15/P

IMPORTANCE OF COMPETITION FOR INITIAL STEP OF IFN RECEPTOR BINDING: NEW INSIGHTS

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In our previous projects, 14 different IFN-α21b/α2c hybrids and their mutants were expressed and purified, their antiviral and antiproliferative activities were examined, competitive binding experiments were performed, and all constructs were compared antigenically. Because very poor competition between hybrid molecules and parental IFNs was observed, we tried to find answers to the following questions: What could be the reasons for poor competition? Could poor competition be explained by the existence of multiple IFN-binding sites on IFNAR2-EC? To answer these questions, several experiments were per-

formed including competitive binding ELISA, native electrophoresis followed by Western blot, BIACORE analysis, microarray analysis, as well as neutralization of antiproliferative activities of IFNs on Daudi cells with anti IFN mAbs. A comparison of antiproliferative activities of single interferons *versus* interferon combinations on Daudi cells was also done. Based on our results we concluded, that IFN-α2c and CM3 bind to IFNAR2-EC competitively with distinct binding centers. Low competitive binding between IFN-α2c and CM3 observed earlier in *in vivo* experiments could be a consequence of better binding properties of IFN-α2c, that influence signal transduction in Daudi cells.

05-16/P

PURIFICATION AND CHARACTERIZATION OF A NEW CLASS OF TYPE I INTERFERONS

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A novel interferon (IFN) was found by our laboratory in a survey of many mammalian genomes. It exhibits some homology with IFN-a, IFN-b and IFN-w and is categorized as a new class of Type I interferons by the HUGO gene nomenclature committee (HGNC). This IFN was designated IFN-n. The primary sequences of IFN-n have very high similarity in different mammalian genomes and the primate IFN-n gene has been well conserved for forty-five million years. The IFN-ν gene is intact in the feline and other cat genomes, while in the human genome, a stop codon exists at amino acid position 55 in the putative mature peptide. We first modified human IFN-ν by changing the stop codon TAA into CAA (Gln). Both feline and the modified human IFN-ν cDNAs were obtained from the corresponding genomes by PCR. The cDNAs were expressed in E. coli, then purified by size-exclusion chromatography, and assayed for typical interferon activities. Both human and feline IFN-ν showed anti-proliferative activity. Human IFN-ν also exhibited antiviral activity. To minimize the presence of misfolding or oligomerization of IFN-ν during its purification, we mutated two pairs of the six cysteines (Cys1, 6, 27, 96, 137 and 156 in mature peptide) in human IFN-ν. One pair of the cysteines (Cys6 and Cys156) does not exist in other type I interferons. The other pairs of cysteines (Cys1 and Cys96) exist in IFN-α but not in IFN-β. The remaining cysteines were Cys27 and Cys137. The mutated IFN-ν showed a better homogeneity and formed less oligomers in comparison with wild-type IFN-ν. This mutant retained the anti-proliferative and antiviral activity. The activity of IFN-ν was evaluated in a number of cell lines and a variety of assays. The results demonstrate that IFN-ν exhibited remarkable differences from the other type I interferons in a variety of assays.

05-17/P

INTERFERON-LAMBDAS –NOVEL INTERFERONS IN ANTIVIRAL DEFENCE

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For many years interferons (IFN) have been known to play a critical role in combating invading pathogens, especially viruses. IFNs are expressed as first line of defence against viral infections. Recently, three novel IFN-like molecules were discovered and named IFN-λ_1 & IFN-$\lambda_{2/3}$. IFN-λs have been shown to have antiviral activity against several RNA viruses *in vitro*; and recently, also against the DNA virus - herpes simplex virus type 2 (HSV-2) – *in vivo*. IFN-λ exerted antiviral activity both during a systemic infection and during a local genital infection. However, the mechanism behind the antiviral activity of IFN-λ is largely unexplored. To address this issue we have isolated various cell populations from mice and examined which cell types respond to IFN-λ and produce the cytokine during viral infection. Furthermore, the antiviral response against HSV-2 is being investigated in IFN-λ-receptor-knock-out mice in order to elucidate the roles for IFN-λ in antiviral defence. We are examining the roles of IFN-λ in two mouse-models of HSV-2 infection; a systemic infection and a local infection. The results will be presented at the meeting.

05-18/P

CHARACTERIZATION OF NEUTRALIZING ANTIBODIES TO INTERFERON BETA USING A NOVEL GENE-REPORTER ASSAY

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Production of neutralizing antibodies (NAB) to interferon beta (IFNβ) is associated with reduced clinical response in patients with multiple sclerosis. Currently, detection of NABs is based upon measurement of IFN activity using antiviral bioassays or induction of MxA. Bioassays are imprecise, only greater than two fold differences can be detected, give variable results, and take several days to complete. Measurement of MxA induction requires use of cell lines or peripheral blood, and subsequent evaluation of MxA levels by ELISA or measurement of mRNA. A highly sensitive and reproducible method for quantifying IFN activity was developed, based on human PIL5 cells, transfected with the luciferase reporter-gene controlled by an IFN responsive chimeric promoter, which allows IFN activity to be determined with a high degree of precision within a few hours. Vinblastin treated PIL5 cells can be stored frozen for several months without loss of IFN sensitivity or the need for cell culture. The PIL5 assay is highly sensitive (detects <1.0 IU/ml of IFNα or IFNβ), and reproducible (standard error +/- 15%), over a wide range of IFN concentrations (<1.0 to 100 IU/ml). The use of this assay to determine neutralizing titer using the Kawade method, which calculates the serum dilution that reduces IFN activity from 10 to 1 laboratory Units/ml, has shown that NABs detected in patients treated with IFN β-1a (Avonex or Rebif) neutralize both types of IFN to the same extent but exhibit markedly lower neutralizing titers against IFN β-1b (Betaferon). When the same sera were tested using a constant quantity (20 pg) of IFN protein, identical neutralizing titers were obtained for all three IFNβ subtypes. None of these sera neutralized IFNα 2b (Intron A). These data suggest that neutralization titer is dependent upon the specific activity of the IFN subtype used in the neutralization assay.

05-19/P

ROLE OF STAT3 IN ANTIVIRAL RESPONSE

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Type I interferons (IFNs) are critical cytokines for innate immunity to combat viral and bacterial infections. It has been well documented that signal transducer and activator of transcription (STAT) proteins, such as STAT1, STAT2, and STAT3, are activated upon IFN-α/β stimulation. While essential roles of STAT1 and STAT2 in IFN-α/β-mediated antiviral responses are demonstrated in gene targeting mice, the role of STAT3 remains unclear. Using STAT3KO mouse fibroblasts (MEFs) and macrophages, the role of STAT3 in IFN-α-mediated antiviral responses during encephalomyocarditis virus (EMCV) infection was investigated. The cytopathic effect (CPE) of EMCV was reduced in STAT3KO MEFs when compared to wild type (WT) MEFs. This was further supported by reduced apoptotic rate in EMCV-infected STAT3KO MEFs using propidium iodide (PI) staining. In addition, IFN-α-induced antiviral state was enhanced and viral titers were reduced in STAT3KO MEFs after EMCV infection when compared to WT MEFs. Likewise, antivirus-associated genes, such as interferon regulatory factor 1(IRF1) and inducible nitric oxide synthetase

(iNOS), were enhanced upon IFN-α stimulation and viral titers were reduced in EMCV-infected STAT3KO bone marrow-derived macrophages (BMMs). Interestingly, expression and activation STAT1 in STAT3KO BMMs were increased after EMCV infection. Taken together, these results suggest that STAT3 is a negative regulator for IFN-α-mediated antiviral responses in MEFs and BMMs.

05-20/P

DISTINCT DIFFERENCES BETWEEN TYPE I AND TYPE III INTERFERON SIGNALING

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Type I interferons (IFNα/β) are cytokines used for the treatment of hepatitis B and C and cancer. However, the beneficial effects of type I IFNs are also accompanied with deleterious side effects. IFN-λ is a new type III IFN that shares similar biological properties with type I IFNs. Unlike the type I IFN receptor, the IFN-λ receptor (IFNLR) is not ubiquitously expressed, yet both types of IFNs activate the JAK/STAT pathway that results in the assembly of the ISGF3 complex. To determine whether signaling differences exist between these two classes of IFNs, we stably transfected the mouse B16 melanoma cell line with the IFNLR1 subunit as these cells do not respond to IFN-λ. Our studies revealed that the kinetics of IFN-λ stimulated activation of the transcription factors, STAT1 and STAT2, are quite distinct from those of type I IFNs. While stimulation of B16 cells with type I IFNs resulted in a transient STAT activation that decayed after 2 hours, IFN-λ stimulation of B16 cells stably-transfected with the IFNLR1 resulted in prolonged STAT activation that was detectable at 24 hours. Consequently, in response to IFN-λ stimulation, we detected prolonged binding of the ISGF3 complex to DNA together with prolonged and higher levels of ISGF3-mediated gene expression as compared to type I IFN stimulation. Interestingly, while type I IFNs induced an antiproliferative effect in B16 cells, a very low amount of IFN-λ was sufficient to induce apoptosis. These effects were not cell-specific as they were also observed in human 2fTGH fibrosarcoma cells stably expressing the IFNLR1. Therefore, our data indicate that IFN-λ signaling appears to be distinct to that of type I IFNs. Given the restricted expression of the IFNLR, IFN-λ may represent an alternative choice to the existing type I IFN immunotherapy.

05-21/O

ANTIVIRAL RESPONSE TARGETS UBIQUITIN MEDIATED PATHWAYS BY INDUCTION OF UBIQUITIN LIKE PROTEIN – ISG15

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Interferon regulatory factor 3 (IRF-3) plays a critical role in the activation of the antiviral immune response. However the transcriptional activity of IRF-3 is tightly regulated by proteosome mediated degradation. We have shown that IRF-3 is ubiquitinated (Ub) and that IFN induced Ub like protein ISG15, inhibits Ub of cellular proteins including IRF-3 and subverts Ub mediated degradation of IRF-3 in infected cells. IFN treatment and NDV infection profoundly increases conjugation of ISG15 to cellular proteins including IRF-3. The half life and nuclear transport of IRF-3 were significantly increased in infected cells expressing ectopic ISG15, while relative levels of IRF-3 were substantialy lower in ISG15 null MEF. These results revealed that virus mediated subversion of the antiviral response by proteolysis of IRF-3 is counteracted by induction of ISG15 expression and that ISGylation provides a feedback mechanism which enhances the host antiviral response via IRF-3 stabilization.
ISG15 has also a critical role in the innate antiviral response to HIV-1 infection and we had identified ISG15 as a critical component in IFN mediated inhibition of HIV-1 release and assembly. Ectopic ISG15 mimics the IFN inhibition and inhibits release of HIV-1 virions without having any effect on the synthesis of viral proteins. Elimination of ISG15 expression by siRNA reversed the IFN mediated inhibition of HIV-1 replication. Addressing the molecular mechanism of HIV-1 inhibition, we have observed that ISG15 specifically inhibited Ub of Gag and Tsg101 and disrupted the interaction of Gag L domain with Tsg101. However conjugation of ISG15 to Gag or Tsg 101 was not detected. These data uncover a novel mechanism by which IFN, via induction of ISG15, targets the cellular endosomal trafficking pathway used by HIV-1 to exit the cell and advances the understanding of IFN mediated effects.

05-22/O

TYPE 1 INTERFERON RECEPTOR-DEPENDENT MODULATION OF MYELOID CELL ACTIVATION DETERMINES THE COURSE OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Multiple sclerosis (MS) is a chronic demyelinating disorder of the central nervous system (CNS) which is considered to be a T cell-mediated autoimmune disease. While treatment of MS patients with interferon-β (IFN-β) leads to a marked decrease in the exacerbation rate as well as to delayed sustained disease progression, the precise mechanisms of the beneficial effects of IFN-β are still enigmatic. In this study we show that type 1 interferon receptor-deficient mice (IFNAR$^{-/-}$) were highly susceptible to experimental autoimmune disease (EAE) and developed a more severe disease course with increased CNS inflammation, demyelination and lethality. Since IFNAR is expressed in all body tissues, little is known about the actual target tissue during disease. To clarify this, we used Cre/loxP-mediated gene targeting to investigate the cell-specific function of IFNAR *in vivo*. Mice with a specific IFNAR deletion in the central nervous system (Nestin-CreIFNARflox/flox) revealed no differences in the clinical course and showed compatible tissue damage in the CNS compared to WT controls, indicating that type 1 interferons do not have a direct protective impact on the CNS. Interestingly, neither T cell (CD4-CreIFNARflox/flox) nor B cell (CD19-CreIFNARflox/flox) -specific IFNAR deletion influenced the clinical course and cellular composition of infiltrating cells. However, IFNAR deletion on macrophages/neutrophiles (LysM-CreIFNARflox/flox) led to severe disease with an enhanced effector phase and increased disease lethality as seen in IFNAR$^{-/-}$ mice. Deletion of IFNAR on macrophages induced altered MHC class II expression and change of cytokine and chemokine production. In summary, we show that IFNAR triggering, specifically on myeloid cells, but not on lymphocytes or CNS cells, is crucial for immunomodulatory effects of type 1 IFN during autoimmune CNS disease.

05-23/P

MAY INTERFERON PLAY A ROLE IN THE MANAGEMENT OF HIV INFECTION?

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Controlled clinical trials designed to treat HIV infection with alpha IFN showed significant efficacy in delaying progression to AIDS-related events and in prolonging survival. These results were basically confirmed by other studies, but the therapeutic approach was soon abandoned, due to the advent of antiretroviral drugs, that, used in combination, enabled to provide a highly antiretroviral therapy (HAART). However, it is becoming increasingly difficult to maintain this treatment for long periods of time, due to the accumulation of side effects

that may become intolerable by the patients. For these reasons, protocols of "structured" interruptions" of therapy have been tested, usually with poor results, due to the return of viral replication and subsequent loss of CD4 T lymphocytes. Under these circumstances, an alternative therapy, capable of controlling the progression of the infection to allow the reversal of the side effects in the temporary absence of HAART would be desirable. We initiated a pilot study aimed at exploring the effect of Pegylated (Peg)-IFN alpha + Ribavirin towards markers of HIV infection in HIV-HCV co-infected patients who interrupted their antiretroviral therapy, to start IFN therapy to control their progressive liver disease. At this time, 19 patients have been included, with an average follow up of 4 months. So far, no significant increase of baseline HIV load, nor loss of CD4 T cells have been detected. Moreover, and perhaps more interestingly, 6 patients seem to show a decrease of HIV proviral DNA. If confirmed after a longer period of treatment, these findings seem to be promising in terms not only of a delayed progression, but even of a reduction of the latent infection compartment. In our opinion, Peg-IFN alpha is a promising candidate in the global management of HIV infection, mainly during HAART interruption.

05-24/P

PROTECTIVE EFFECT OF IL-12 AGAINST MRSA INFECTION IN IMMUNOCOMPROMIZED MODEL MICE

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Object. Multidrug-resistant *Staphylococcus aureus* (MRSA) is one of the main causative agents of nosocomial infection and is a lethal pathogen on immunocomplomized hosts. To prevent and protect from MRSA infection in immunocompromized hosts are very important for them to maintain quality of life. We have investigated the protective effects of several cytokines and biological response modifiers such as IL-2, IL-6, TNF-α, LPS and MDP against MRSA infection in immunocompromized hosts. Recently, productions of TNF-α and IFN-α were regulated by cytokine network and production of IL-12 was the key cytokine on the regulation. In this study, we have studied whether or not IL-12 have critical role on MRSA infection in immunocompromized hosts. *Materials and methods*. Immunocompromized conditions of Balb/c mice, Clea Japan, were induced by the injections of cyclophosphamide (CY; Shionogi, Japan) and carrageenan (CG; Sigma, USA). Recombinant human IL-12 (rhIL-12) and human IL-12-producing cell line, EDFT-IL-12 MBT2, were also used as immunomodulators. MRSA KU-01-06-37 strain was derived from Kyorin University Hospital. *Results and Discussion*. 1: The 50% lethal doses (LD_{50}) of KU-01-06-37 of normal control mice, CY-treated mice, CG-treated mice and CY- and CG-treated mice were 5.0×10^8, 5.5×10^6, 5.5×10^8 and 2.0×10^6 CFU/mouse, respectively. 2: Injection of rhIL-12 (2,000pg/ mouse) both in CG-treated mice and in CY-treated mice could not improve any protective effect since rhIL-12 have easily metabolized in the body. 3: To maintain the constant concentration of IL-12 in the body, we have used EDFT-IL-12 MBT2 cells. 4: Injection of EDFT-IL-12 MBT2 cells induced higher antibacterial activity in CY-treated mice than CG-treated or CY- and CG-treated mice. 4: Concentrations of IL-12 in mouse serum after *i.p.* inoculation EDFT-IL-12 MBT2 cells were maintained 20 pg/ml for a week and then reduced. 5: These results suggest that sustainable presence of IL-12 activated cytokine network and induce the IFN-α and TNF-α production from remaining cells such as NK and so on.

05-25/P

THE EFFECTS OF IFN-α/β RECEPTOR-RNA INTERFERENCE ON THE BIOLOGICAL ACTIVITIES OF IFN-α IN SENSITIVE AND TOLERANT CELLS

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Human interferon (IFN)-α 2c/21b and various IFN-α 2c/21b hybrids and mutants have been expressed, purified and characterized for both antiviral and antiproliferative activities using tumor cell lines including Daudi, OVCAR3, and A549 cells. These IFNs were found to differ in their antiproliferative activities in Daudi, OVCAR3, and A549 cells; Daudi and OVCAR3 cells were sensitive to the IFNs tested while A549 cells were tolerant to them. To elucidate the possible reasons for these differences, the relationship between cell lines which showed sensitivity or tolerance to the IFN-αs and the IFN-α/β receptor (IFNAR) was examined. The expression levels of IFNAR1 and 2 were measured by western blot and/or flow cytometry analysis in these cell lines. IFNAR1 and 2-RNA interference (RNAi) were transfected into OVCAR3 cells using lipofection. The effects on the expression of IFNAR1 and 2 on the antiproliferative activity were examined in OVCAR3 cells. The IFNAR1 and 2-RNAi substantially decreased the detection of IFNAR1 and 2 by western blot and flow cytometry, and abrogated the antiproliferative activity on OVCAR3 cells. In conclusion, knock down of IFNAR1 and 2 by RNAi decreased IFNAR1 and 2 on OVCAR3 cells and reduced the antiproliferative response to IFN-α.

05-26/P

USE OF CALF INTESTINAL EPITHELIAL (CIEB) CELLS TO MEASURE THE BIOLOGICAL ACTIVITY OF HUMAN INTERFERONS

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The natural HuIFN-α represents a heterologous mixture of different subtypes with more or less predominant antiviral, antiproliferative, antitumor and other biological activities. Even more than a quarter of a century has passed since the discovery of Interferons (IFN), still a universally accepted method for their biological assay is not generally approved. One of the reasons lies definitely in the fact, that IFNs are pleiotropic molecules showing not only antiviral but also other activities. At present the most widely used method for IFN quantitation is based on the measurement of various parameters of viral replication in IFN-treated cells. Among these parameters, the one based on cytopathic effect (CPE)- inhibition offers the most useful assay to determine the biological activity of IFN. Such an activity is expressed in I.U. (International Units)/ml. Although the diploid fibroblast cells seem to be the most sensitive, the use of different continuous cell line is more practical. The purpose of this study was to introduce the Calf intestinal epithelial (CIEB) cell line and to compare its sensitivity for HuIFN-α and HuIFN-γ of standard cell lines: WISH, HAC-3/T2 (Human amniotic cell lines) and MDBK (Bovine kidney cell line). During the experiments cells were cultivated in Eagle's Minimal Essential Medium (EMEM) supplemented with 8% of SR-2.0552P (Serum replacement based on Porcine ocular fluid) . On next day, different concentrations of HuIFN-α or γ were added. Cells were incubated overnight at 37°C, IFN samples were removed, and pre-titered VSV (Vesicular Stomatitis Virus) was added. After 18^h incubation at 37°C, the 50% CPE was scored microscopically. In addition the content was removed, and cells were stained with 1% crystal violet in 20% ethanol, washed with PBS (Phosphate buffer saline), and the bound color was extracted with the mixture PBS: 70% ethanol (1:1). Photometric reading of the plates was performed at 600 nm. Equations used for the calculation of 50% CPE reduction endpoints are as follows: optical density (OD) for 50% CPE = (OD of cell control + OD of virus control)/2; and IF titer = [(OD above 50% CPE OD – 50% CPE OD) x difference between higher and lower dilutions]/(difference between higher OD and lower OD) + lower dilution. The following results were obtained: (1) CIEB cells are sensitive for HuIFN-α and HuIFN-γ. (2) In the case of HuIFN-α the lowest amount that can be measured was 3-5 I.U./ml (3) The response to HuIFN-α is dose dependent between 5 and 1000 I.U./ml. (4) In the case of HuIFN-γ, the lowest amount that can be measured was 5-10 I.U./ml. (5) The response to HuIFN-γ is dose dependent between 10 and 800 I.U./mL

05-27/P

SYNERGISTIC ACTIVATION OF MONOCYTES BY POLYSACCHARIDES ISOLATED FROM *SALICORNIA HERBACEA* AND INTERFERON-γ

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The water extract of *Salicornia herbacea*, SHE, has recently been shown to have strong immunomodulatory activity. The active component of SHE appeared to be polysaccharides. In the present study, we purified the polysaccharides (termed SHP) and examined the immunomodulatory activity alone and in combination with interferon (IFN)-γ. Treatment of mouse monocytic cell line, RAW 264.7 cell, with a combination of SHP and IFN-γ synergistically activated RAW cells to produce cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β, and nitric oxide (NO). Increased production of TNF-α, IL-1β and NO was correlated with the increased level of respective transcripts. The combination of SHP and IFN-γ also effectively induced the expression of co-stimulatory molecules such as B7-1 and CD40. In addition, the combination of SHP and IFN-γ synergistically inhibited the growth of RAW cell inducing further differentiation to strongly adherent macrophages. The differentiation-inducing activity of SHP alone and in combination with IFN-γ was confirmed by the changes in the expression of differentiation antigens such as CD11b, CD18 and CD24. These results show that immunomodulatory activity of the polysaccharides isolated from *Salicornia herbacea* can be augmented synergistically when combined with small doses of IFN-γ.

05-28/P

EVALUATION OF MACAQUE MONKEYS AS MODEL ORGANISMS FOR HUMAN INTERFERON-ALPHA RESEARCH

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ABSTRACT: IFN-alpha (IFN-α) possesses antiviral and antiproliferative activity. These properties made IFN-α (especially the IFN-α2/αA variants) valuable therapeutically to treat certain cancers and viral infections. However, the properties of IFN-α subtypes are less conserved across diverse species than those of IFN-β. Coupled with the increased species-specificity of IFN-α, the translation of results of IFN-α research from mice to humans is troublesome and the role of specific subtypes of IFN-α in immune function is not well established. Employing chimpanzees alleviates this concern but is expensive, limiting their use to mandatory preclinical studies. Establishing more economical primate model systems for human IFN-α function, such as rhesus monkeys (*Macaca mulatta*) or more diminutive cynomolgus monkeys (*M. fascicularis*) will accelerate our understanding of human IFN-α subtype-specific effects in human immunology and virology. Analysis of the *M. mulatta* genome sequence allows an *in silico* evaluation of macaques as alternatives for human IFN-α research. The IFN-β protein sequences of *M. mulatta* and *M. fascicularis* are identical, suggesting that the IFN sequences of *M. mulatta* may apply to *M. fascicularis*. The type I IFN gene cluster is remarkably well conserved between *M. mulatta* and humans, except for the region between the *IFNA16* and *IFNA21* genes, where the intervening genes in humans (IFN-α17, α4, α10, and α7) are instead a group of IFN-α30 homologs [C.D. Krause and S. Pestka *Pharmacol Ther* 2005; 106: 299-346]. Other human IFN-α subtypes have about 92% identity with homologs in the *M. mulatta* genome. The *M. mulatta* and *M. fascicularis* IFN-α2 genes were cloned and the encoded proteins used to develop antibodies and showed antiviral activity on the respective primate cells. The human IFN-αs tested had antiviral activity on macaque cells. Overall, macaques can likely be used to study the effects of human IFN-αs on immunological and other biological activities.

05-29/O

PRIMATE INTERFERON-ALPHA SPECIES AND THE ORIGIN AND EVOLUTION OF HUMAN INTERFERON-ALPHAS

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IFN-alpha (IFN-α) is a highly duplicated subclass of eutherial mammalian Type I IFNs possessing antiviral and antiproliferative activity. Of particular interest in the treatment of certain cancers and viral infections is the IFN-α2/αA variants. However, the IFN-α subtypes exhibit much less uniformity than IFN-β across various mammalian orders. Consequentially, subtype-specific effects of IFN-α in immune function are less established relative to IFN-β because most human IFN-αs are inactive in mice and because results with mouse IFN-αs used in rodents do not translate to humans as reproducibly as that of IFN-β. Phylogenetic analyses of Type I IFNs suggest that the lack of consistency in function of a particular IFN-α subtype across species arises from independant evolution of distinct sets of IFN-α duplicates. To find a more suitable animal model for human IFN-α function, we determined the origins of various human IFN-α subtypes in primate evolution by analyzing the genome content of several primates. One highly divergent primate (galago, *Otolemur garnetti*) has no homolog to any human IFN-α subtype in its genome. Human IFNs α2, α8 and α5 appear to be the most conserved subtypes as seen in the marmoset (*Callithrix jacchus*) genome. IFN-α17, α4, α10, and α7, the least conserved, are seen in chimpanzees but not in orangutans (*Pongo pygmaeus*) or more distant primates. Other subtypes are seen in macaques and baboons (*Macaca* and *Papio* sp.), primates that may make good model systems for human IFN-α function. A "hot spot" between the *IFNA16* and *IFNA21* genes appeared to generate new type I IFNs rapidly in evolution as the conservation of a particular IFN-α subtype is proportional to its distance from the "hot spot". The first human IFN-α subtypes surviving to modern times originated in the medial primate lineages and may have survived due to their distance from the "hot spot."

05-30/O

IDENTIFICATION AND CHARACTERIZATION OF A POXVIRUS-ENCODED IFN-λ ANTAGONIST

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Three distinct types of interferons (IFNs) are involved in the establishment of multifaceted antiviral response. Multiple functions of type I IFNs (IFN-α/β) and type II IFN (IFN-γ) for the innate and adaptive arms of antiviral response are well recognized. Although it was demonstrated that recently described type III IFNs (IFN-λs) possess intrinsic antiviral activity similar to that of type I IFNs, their functional importance and uniqueness for antiviral resistance remains to be characterized. Viruses explore various strategies to circumvent IFN-induced antiviral protection. For example, vaccinia virus (VACV), which belongs to the poxvirus family, encodes two secreted proteins which function as IFN antagonists: B8R protein is the soluble receptor of IFN-γ, whereas B18R protein serves as IFN-α/β antagonist. We discovered that poxviruses differ in their ability to neutralize IFN-λs. We found that one of the poxviruses encodes a protein which functions as IFN-λ antagonist. This protein binds IFN-λ and suppresses interaction of type III IFNs with their membrane-bound receptor complex resulting in the inhibition of IFN-λ-mediated biological activities including up-regulation of MHC class I antigen expression and induction of antiviral state. These experiments demonstrate that although type I IFNs and type III IFNs share many biological activities including the ability to induce antiviral state in cells, poxviruses developed their unique strategies to counteract IFN-mediated antiviral protection. These results also suggest that IFN-λs may be effective treatment for some poxviral infections.

05-31/O

INTERFERON, INFLUENZA & IMMUNITY

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The 3 major influenza pandemics in the 20th century together killed more people than any other natural or man-made disaster, including WWI and WWII, distinguishing influenza as one of the deadliest acute infectious diseases in human history. An unprecedented epizootic influenza A virus, H5N1, that is highly pathogenic in humans, has crossed the species barrier in Asia and most recently, Turkey, to cause many human fatalities and poses an increasing pandemic threat. Interferons (IFN), because of their direct antiviral properties and effects on innate and adaptive immune responses, present as candidate antivirals against influenza. Virus-responsive dendritic cells (DC) function as the sentinels of virus infection, orchestrating the development of adaptive immunity. Moreover, plasmacytoid (p)DC produce high levels of IFN-α/β upon virus activation. In this study, we have investigated the role of IFN-β in the homeostasis of DCs, in mice null for IFN-β. IFN-$\beta^{-/-}$ mice showed a selective deficiency in pDCs but not conventional DCs in peripheral lymph nodes and spleen. Based on evidence that pDCs play an important role in modulating adaptive immunity against pulmonary viral infections, we investigated the influence of IFN-β deficiency on pulmonary influenza viral infection and pDC-mediated events. Specifically, we evaluated the effects of intra-nasal inoculation of wildtype and IFN-$ß^{-/-}$ mice with influenza A/WSN/33 virus. IFN-$ß^{-/-}$ mice are more susceptible to intranasal infection compared to their wildtype littermates, as determined by severity of disease and viral load in lung tissues. Additionally, we observe differences in migration of pDCs *and* conventional DCs into the lung draining mediastinal lymph nodes and associated differences in virus-specific cytotoxic T cell responses. These data demonstrate the importance of IFN-β in mucosal immunity against pulmonary virus infection, in part mediated by modulation of the homeostasis and function of pDCs, and suggest the potential benefit of IFN therapy against influenza.

05-32/P

BOTH HOST CELL AND NS GENE ORIGIN DETERMINE THE INTERFERON ACTION AND INDUCTION PHENOTYPE OF INFLUENZA VIRUS H5N1(NS)

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We used 3 recombinants of A/PR/8/34 (H1N1) generated by Seo, *et al* (Nature Medicine 8:950-954, 2002): (1) rgPR/8/34-NS (H1N1) contained aspartic acid in position 92 (D92) of the NS1 protein; (2) rgPR/8/34-NS(H5N1) contained glutamic acid in position 92 of NS1(E92); and (3) rgPR/8/34-NS(E92D) (H5N1) had E92 replaced with D92 as in the original PR/8/34. Seo *et al* showed that all H5N1-NS/97 isolates tested were resistant to the action of IFN as measured in a line of pig lung cells (SJPL), and that IFN resistance required E92 in NS1, and was lost upon changing E92D (*op cit*). Since influenza viruses are reported to be intrinsically sensitive to the action of IFN (JICR 20:963, 2000) in avian cells, and the H5N1 strains most likely have evolved in avian species, we compared their sensitivity to IFN in chicken embryonic kidney (CEK) cells treated with rChIFN-α. *Both* PR/8/34 recombinants with the NS genes derived from Hong Kong/97 H5N1-NS were equally sensitive to the action of IFN measured in CEK. Thus, the resistance to IFN reported in SJPL cells challenged with rgPR/8/34-NS(H5N1) was not observed in CEK cells, showing that the host cell plays a critical role in the regulation of IFN action through some product encoded by the NS gene of H5N1/97. As reported previously, PR/8/34 was a weak inducer of IFN in primary chicken embryo cells and functioned phenotypically as an IFN induction-suppressing particle (ISP) (J.Virol. 79:2880,2005). In marked contrast, *both* PR/8/34 constructs with the H5N1/57-derived NS induced plateau yields of IFN ≈ 70-fold higher. Replacing the NS gene of PR/8/34 (H1N1) with that from either of the H5N1/97-NS constructs converted the ISP phenotype into an IFN-inducing particle, (IFP). Thus, the NS gene origin and host cell determine the expression of the IFN system –both its action and induction.

05-33/P

CHICKEN IFN-α IN DRINKING WATER FUNCTIONS AS AN ADJUVANT FOR INFLUENZA VIRUS

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rChIFN-α administered in drinking water has been shown to ameliorate several avian viral diseases. We now report that the continuous peroral exposure of the chicken oromucosal system to rChIFN-α functions as an adjuvant with respect to the HA of A/CK/CT/72/03 (H7N2). Two groups of uninfected 3-week-old SPF chickens (sentinel birds) were comingled with cagemates already infected with AIV/CT to simulate conditions of natural infection by the respiratory/oral-fecal route. One group received plain drinking water and the other water which contained 2,000 U/ml rChIFN-α. Water was provided *ad libitum* and changed daily. Serum and tracheal samples from each bird were obtained periodically for 4 weeks. Seroconversion to the HA Ag, scored by AGP and HI tests, was observed in all overtly-infected birds in both groups. However, 0 of the 25 sentinel birds infected naturally seroconverted when plain water was ingested. In marked contrast, 23 of the 25 sentinel birds seroconverted when given IFN in the drinking water. Oropharyngeal swabs examined by matrix RRT-PCR revealed equivalent amounts of infectious virus in both groups up to the 10 dpi tested thus far, indicating that the absence of seroconversion in chickens on plain water was not due to a lack of AIV Ag. We postulate that natural infection by AIV-CT, a good inducer of IFN, restricted the amount of viral replication and Ag load to levels below those required to stimulate Ab production, and that perorally delivered rChIFN-α functions as an adjuvant to enhance the immune response. We propose the peroral use of rChIFN-α as an economically feasible means of treating large numbers of chickens to stimulate the adaptive immune response. Chickens given IFN-water could serve as super-sentinels to detect LP-AIV early, gaining time to abort its potential to mutate to HP-AIV. Peroral use of IFN might also serve to spare Ag during routine vaccination.

05-34/O

THE EUKARYOTIC TRANSLATION INITIATION FACTOR-2α KINASE PKR IS A NEGATIVE REGULATOR OF STAT1 AND STAT3.

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The signal transducer and activators of transcription (Stat) family of proteins participate in the regulation of many cellular processes including development, cell growth, proliferation and apoptotic cell death. Stat1 plays an important role in the cellular response to interferon (IFN) and virus infection, regulation of proliferation and apoptosis. Stat1 knock-out mice are extremely susceptible to infection with viruses and other pathogens demonstrating the essential role of this transcription factor in innate immunity. Numerous mechanisms exist which regulate Stat1, including dephosphorylation in the nucleus by the tyrosine phosphatase TC-PTP. Stat1 activity is further controlled by the eukaryotic translation initiation factor 2α (eIF2α) kinase PKR, a ubiquitously expressed dual specificity kinase induced by IFN-α/β and activated by double stranded (ds) RNA. PKR physically interacts with Stat1, and inhibits the formation of Stat1 transcriptional complexes. We set out to determine if an indirect pathway exists that controls Stat1 phosphorylation via PKR. Using an inducible PKR cell line, we demonstrated that tyrosine phosphorylation of Stat1, and a related family member Stat3, is

reduced in the presence of active PKR. We show that this regulation occurs through activation of TC-PTP, as targeted reduction of TC-PTP by RNAi in the same cell line resulted in a partial rescue of Stat1 and Stat3 phosphorylation, nuclear localization and transcriptional activity. Interestingly, induction of Stat1 and Stat3 dephosphorylation by TC-PTP is facilitated in part, by the inhibition of protein synthesis as a result of PKR activation and eIF2α phosphorylation. These results describe a previously unknown pathway regulating the activity of Stat1 and Stat3, and also identify a TC-PTP as a novel substrate of PKR. Since both PKR and Stat1 are critical players in the cellular anti-viral response, the inter-relation of these pathways may illustrate a heretofore uncharacterized mechanism employed by viruses to evade the host response.

05-35/P

LOW MOLECULAR IFN INDUCER CYCLOFERON IN TREATMENT OF ATOPIC BRONCHIAL ASTHMA EXACERBATIONS.

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Acute respiratory viral infections facilitate development of IFN-immunodeficiency and are the most common triggers of atopic asthma exacerbations. It is known, that low molecular IFN inducer cycloferon have both antiviral and immunomodulating activities, can be successfully used in acute respiratory viral diseases.
The purpose of this study was appreciated the clinical and immunological effects of the cycloferon in bronchial asthma exacerbations patients with respiratory viral infections.
The 11 basic group patients with light and middle degree BA exacerbations and respiratory viral infections was treated in addition to traditional therapy by 12,5% cycloferon (intramuscular 2 ml x once day x 10 days). The 8 patients in control group used only traditional therapy. The biological method of IFN-status for determinations endogenous serum IFN, spontaneous and inducible production of IFN-α and IFN-γ was used. The levels of serum IL-4 and IFN-γ were measured by ELISA.
All 19 patients had high general and specific IgE levels. The levels of general IgE compared between the two groups. In basic group general IgE was 448,2±122,1 kUn/l ; in control group- 474±150 kUn/l . All patients of basic and control groups had deficiency IFN-producing capacity of leukocytes: IFN-α -110±24,06 Un/ml, IFN-γ- 14,18±2,56 Un/ml and IFN-α -123±34,64 Un/ml, IFN-γ- 6,0±0,84 Un/ml correspondindly. After cycloferon treatment (n = 11) increased IFN-α indices- 206,7±48,7 Un/ml ($p<0,05$) and IFN-γ -30,55±7,3 Un/ml ($p<0,05$). After traditional treatment (n = 8) increased IFN-α indices- 153±42 Un/ml ($p>0,05$) and IFN-γ -12,75±2,2 Un/ml ($p<0,01$). The serum and spontaneous IFN levels of many patients were normal (<2-8 and <2 Un/ml respectively). Before treatment serum indices of IL-4 was 40,95±28,1 pg/ml and IFN-γ -37,85±14,19 pg/ml in basic group; IL-4 was 12,88±0,02 pg/ml and IFN-γ -62,9±0,00 pg/ml in control group. After treatment serum indices of IL-4 was 62,79±18,0 pg/ml and IFN-γ -28,44±10,69 pg/ml in basic group; IL-4 was 205,7±192,5 pg/ml and IFN-γ -44,59±21,6 pg/ml in control group.
It has been shown that the cycloferon treatment in atopic bronchial asthma exacerbations patients with respiratory virus infections resulted increase and correction both IFN-α and IFN-γ production and had a beneficial therapeutic effects. After cycloferon treatment only in 2 patients (18%) preserved attack of suffocation and labored respiration (37,5%- in control group). The patients were in hospital 13,45 days (16 days- in control group). The quicker from outset of acute respiratory viral infection begin using of cycloferon the having more rapid compartme of disease exacerbation.

05-36/O

INTERFERONS LIMIT INFLAMMATORY RESPONSES BY INDUCTION OF TRISTETRAPROLIN

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Interferons (IFNs) are cytokines with pronounced proinflammatory properties. Here we provide evidence that IFNs play a key role also in decline of inflammation by inducing expression of tristetraprolin (TTP). TTP is an RNA-binding protein that destabilizes several AU-rich element-containing mRNAs including TNFα. By promoting mRNA decay TTP significantly contributes to cytokine homeostasis. Now we report that IFNs strongly stimulate expression of TTP if a co-stimulatory stress signal is provided. IFN-induced expression of TTP depends on the IFN-activated transcription factor STAT1, and the co-stimulatory stress signal requires p38 MAPK. Within the TTP promoter we have identified a functional gamma interferon-activated sequence that recruits STAT1. Consistently, STAT1 is required for full expression of TTP in response to LPS that stimulates both p38 MAPK and, indirectly, interferon signaling. We demonstrate that in macrophages IFN-induced TTP protein limits LPS-stimulated expression of several proinflammatory genes such as TNFα, IL-6, Ccl2 and Ccl3. Thus, our findings establish a link between interferon responses and TTP-mediated mRNA decay during inflammation, and propose a novel immunomodulatory role of IFNs.

05-37/P

ANTIVIRAL AND ILLUMINATED A NEW MOUSE LINE TO MONITOR β-INTERFERON INDUCTION

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Type I Interferons have been reported to be involved in a wide variety of diseases like viral and bacterial infections as well as inflammatory, allergic and autoimmune reactions. Within the group of Type I IFNs which consists of more than 10 α-IFNs and a single β-IFN, β-IFN has been shown to play a central role. In fibroblasts the complete Type I IFN response depends on the presence of this “immediate early” interferon. Although this regulatory function has been carefully described, up to now the impact on Type I IFN dependent disease is poorly understood. Since spleen cells in contrast to fibroblasts can produce α-IFNs independent of β-IFN a key question is which cells are the Type I IFN producers in various situations. To adress this questions we generated a new mouse line in which the β-IFN gene can be inactivated tissue specifically. The cre mediated deletion places a luciferase gene under the β-IFN promoter and thus allows the sensitive detection of the β-IFN producing cells. Here we demonstrate the β-IFN monitoring capacity after deletion of the floxed β-IFN allele in the whole mouse. The heterozygote mice have an intact Type I IFN response and at the same time express luciferase if β-IFN is induced. To induce β-IFN we choose two model pathogens, Newcastle Disease Virus and *Listeria monocytogenes*. Detecting the light emission we follow the β-IFN producers in the whole mouse down to the cellular level. These mice provide a powerful tool to pinpoint the β-IFN producing cells in different diseases and to characterise their function in the induction of the Type I IFN response.

05-38/P

NOVEL INTERFERON-β INDUCED GENE EXPRESSION IN HUMAN BLOOD CELLS

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Recombinant interferon-beta (IFN-β) is used for treating Multiple Sclerosis (MS), a chronic inflammatory demyelinating disease of the central nervous system. The protein products synthesized in re-

sponse to IFN-β injections {IFN-stimulated genes (ISGs)} mediate clinical effects of IFN-β in patients with MS. We hypothesized that the induction of ISGs will vary in individual MS patients, and that the individual molecular responses to IFN-β account for individual therapeutic responses. Microarray analysis of human fibrosarcoma cells treated with IFNs revealed the induction of about 500 ISGs. The induction of ISGs in response to IFN-β is selective and cell-type specific. To identify genes selectively induced by IFN-β in leukocytes, we used cDNA Microarrays with about 3000 unique cDNAs including ISGs, dsRNA genes, cancer related genes and AU rich genes. An innovative approach of exposing fresh, whole blood to physiological concentrations of IFN-β for varying times was used. Peripheral blood mononuclear cells were prepared after treatment with IFN-β and RNA isolated. Microarray analysis led to the identification of 38 ISGs and 20 IFN-repressed genes (IRGs). Many of the ISGs were genes previously known to be induced by IFN-β, but 6 ISGs were novel. We have confirmed the induction of two novel ISGs, GTP cyclohydrolase and Hypothetical protein LOC129607 by quantitative PCR. We have also shown the down-regulation of CXCL1 by IFN-β. These studies advance our objectives of identifying molecular biomarkers of the therapeutic response to IFN-β in MS patients.

05-39/P

A NOVEL RIBONUCLEASE PROTECTION ASSAY FOR INTERFERON-β INDUCED GENE EXPRESSION IN HUMAN BLOOD CELLS

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Multiple Sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system. Recombinant interferon-beta (IFN-β) treatment provides benefit in MS, reducing relapses, new MRI lesions, and disability progression. Recent studies suggest that there are responders and nonresponders to IFN-β in MS. There are no established biomarkers of the therapeutic response to IFN-β. Our earlier work revealed that IFN-β-mediated induction of a subset of ISGs required JAK-STAT and accessory signaling pathways. We have separated ISGs into 2 functional categories: majority of ISGs, for which signaling through the JAK-STAT pathway is both necessary and sufficient, are referred to as "JAK-STAT Only" (JSO) ISGs (eg., p56, 6-16, 9-27, and MxA), and a small number of ISGs for which "Accessory Signaling is Required" (ASR) ISGs (eg., β-R1, TRAIL and IP-10). A recent report correlated durable IFN-β-mediated TRAIL induction with therapeutic responses in MS patients. We hypothesize that induction of ASR genes by IFN-β will correlate with beneficial responses to IFN-β treatment in MS patients. We have developed an RNase protection assay to monitor simultaneous and quantitative expression of 11 mRNA species including 4 JSO ISGs, 3 ASR ISGs, 1 housekeeping gene (γ-actin) and 3 leukocyte lineage marker genes i.e., CD3ε for T lymphocytes, CD-14 for monocytes and Defensin α3 for neutrophils (to correct for shifts in leukocyte population on treatment with IFN-β). The assay has been validated using RNA isolated from fibrosarcoma cells, astrocytoma cells and peripheral blood leukocytes (obtained from blood of healthy volunteers) treated with IFN-β *in vitro*. The assay is convenient, rapid and inexpensive and can be used to discriminate MS patients who express both ASR and JSO genes or only JSO genes, following IFN-β injections. It can therefore be used in clinical studies of MS for biological monitoring of MS patients during therapy with IFN-β possibly leading to identification of predictive biomarkers.

05-40/P

INGESTED (ORAL) IFN-A REPRESSES TNF-A IN RRMS

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Background: In a phase II trial in relapsing remitting multiple sclerosis (RRMS), patients ingesting 10000 IU, but not 30000 IU IFN-α, showed fewer gadolinium enhancements at month 5 and 6 along with decreased pro-inflammatory TNF-α protein secretion. *Objectives:* We examined MxA mRNA induction and TNF-α mRNA repression after ingested IFN-α in RRMS patients to determine the optimal dose(s) for future clinical trials in MS. *Methods:* 24 RRMS, ages 18 and 55, EDSS < 6.0, without previous chemoRx or IFN-β/glatiramer acetate within 90 days, or steroids within 30 days were assayed for Mx induction/TNF-α repression after ingesting IFN-α2a (Roferon®) at 100, 300, 1000, 3000, and 10000 IU using a forced titration study. Eligible patients had a pre-ingestion phlebotomy, 100 IU IFN-α2a was swallowed and had a post-ingestion phlebotomy 4 hours later. Each patient returned for 300, 1000, 3000, and 10000 IU dosing. Buffy coat PMNC was obtained, total RNA submitted to RT-PCR and relative numbers of transcripts for β-actin, TNF-α and MxA were measured. The mean measured MxA or TNF-α transcript levels were normalized to the β-actin control (normalized man = MxA mean β-actin x 100) and expressed as % β-actin molecules. *Results:* Dose levels at which maximum TNF-α mRNA repression occur at ≤ 10000 IU in 21/24 patients. Ten of twenty-four subjects (40%) had maximal repression of TNF-α at 100 IU. Large numbers of MS patients also showed maximal repression of TNF-α mRNA at other doses e.g., 5/24 at 1000 IU (21%) and 5/24 at 3000 IU (21%) ($p < 0.05$). These data show that maximum TNF-α repression occurs at doses lower than 10000 IU. Despite a bimodal distribution for overall maximum MxA induction at 300 (n = 7) and 3000 IU (n = 7), there was not a statistical difference for MxA induction overall. *Conclusion:* Maximal TNF-α repression occurs 100, 1000 and 3000 IU. These data provide new optimal doses for additional clinical studies using ingested IFN-α in MS for reduction in MR and clinical activity.

05-41/P

THE ABSENCE OF IFN-GAMMA EXAGGERATES ACUTE PANCREATITIS WITH DECREASED STAT1 ACETYLATION AND A RECIPROCAL NF-κB ACTIVATION

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We explored the pathophysiological roles of IFN-γ in cerulein-induced acute pancreatitis using IFN-γ deficient (KO) mice. BALB/c (WT) mice were i.p. administered hourly for 9 hours (total: 10 injections) with a dose of cerulein (50 μg/kg). At 4 hrs after the 1st cerulein injection, serum amylase level started to elevate, and reached a peak at 12 hrs. Histopathologically, WT mice exhibited acute pancreatitis characterized by interstitial edema, acinar cell necrosis, and cytoplasmic vacuolization with neutrophil recruitment. Moreover, the gene expression of CXC chemokines such as MIP-2 and KC, and that of COX-2 was up-regulated along with the IFN-γ gene, in the pancreas of WT mice injected with cerulein. On the contrary, in KO mice, the elevation of serum amylase levels was significantly enhanced, with markedly exaggerated histopathological changes in the pancreas, including enhanced neutrophil recruitment, compared with WT mice. Moreover, the enhanced gene expression of MIP-2, KC and COX-2 was significantly exaggerated in KO mice, compared with WT mice. The administration of a COX-2 inhibitor, NS-398, suppressed the elevation of serum amylase levels, implying that COX-2 has injurious roles in cerulein-induced acute pancreatitis. MIP-2, KC and COX-2 expression is induced by NF-κB activation. The nuclear amounts of p65 were increased in WT mice transiently at 7 hours after the first cerulein injection. On the contrary, in KO mice, the nuclear translocation of p65 were enhanced and prolonged, compared with WT mice. Acetylation of Stat1 inhibits nuclear translocation of NF-κB based on *in vitro* observations. Indeed, acetylation of Stat1 was suppressed in KO mice, compared with WT mice. Collectively, the absence of IFN-γ may enhance NF-κB activation through suppressing acetylation of Stat1, resulting in the exaggeration of cerulein-induced acute pancreatitis.

05-42/P

NATIVE MULTI-SUBTYPE IFN-α SUPRESSES DEVELOPMENT OF RESITANCE MORE EFFICIENTLY THAN SINGLE SUBTYPE IFN-α2B IN MELANOMA CELLS.

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The malignant melanoma line WM 266-4, derived from a lymph node metastasis, is sensitive to type I IFN. We have studied the difference in effects on growth and survival using equal molar amounts of a multi-subtype IFN-α and a single subtype IFN-α2b. The multi-subtype is the native blend of six major IFN-α which is produced by leucocytes upon challenge with Sendai virus. The native blend contains less than 20% IFN-α2. When the cells were treated with the two IFN-α preparations for six days no difference in reduction of viability was distinguished. Cell-cycle analysis revealed a transient accumulation of cells in S-phase equal for the two types of IFN-α. However, long term treatment of the melanoma cells with 167pM IFN-α during 4-5 weeks displayed outgrowth of cell colonies. The number of colonies was significant higher when the cells were treated with IFN-α2b. In an effort to determine the frequency of the IFN-resistant precursor cells we used analysis by limiting dilution. The frequency of IFN resistant precursors was 10-fold lower in presence of the multi-subtype IFN-α compared to IFN-α2b. There were qualitative differences between the resistant colonies selected with the two IFNs with respect to morphology and growth rate. In contrast the ability to cause growth inhibition in Daudi cells was twofold higher for IFN-α2b and mirrored the difference in specific activity measured in anti-viral units. The results indicate a tissue- and/or specific response upon treatment with different IFN-α.

05-43/P

TYPE III INTERFERONS INHIBIT HANTAAN VIRUS REPLICATION *IN VITRO*

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Hantaviruses are rodent-borne viruses that cause two severe human diseases: hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HCPS). Little is known about the mechanisms causing the pathogenesis and no therapuetic drugs are as yet available.
Previously, others have shown that pre-treatment of cells with Type I IFNs (IFN-α/β) inhibit Hantaan virus (HTNV) growth in a dose-dependent manner. Furthermore, several IFN-stimulated genes (ISGs) have been shown to be upregulated in HTNV infected cells.
The newly discovered type III IFNs (IFN-λ) are induced by viruses and IFNs, and have been shown to display antiviral activity both *in vitro* and *in vivo*.
We aim to examine if type III IFNs have any affect upon HTNV replication *in vitro*. Our preliminary results indicate that pre-treatment of A549 cells with IFN-λ1 and -λ2 does inhibit HTNV replication. However, pre-treatment with IFN-α, -β or -γ gives stronger antiviral responses than with type III IFNs.
Future plans include investigating how the type III ISG expression (e. g. MxA) profile looks like and if combining different IFNs induce any synergistic effetcts upon viral titers and/or ISG expression. We are also interested in examining if treatment with IFNs after infection affects viral titers in supernatant. We hope to present data showing results from these additional experiments.

05-44/P

PLASMACYTOID DENDRITIC CELLS PRODUCE IFN-LAMBDA IN RESPONSE TO VIRUS AND CPG AND RESPOND TO IFN-LAMBDA STIMULATION

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Plasmacytoid dendritic cells (PDC) are rare cells found in peripheral blood and lymphoid tissues of humans and other mammals. PDC are considered to be "professional" type I interferon (IFN) producing cells in that they produce 10-100-fold more IFN-alpha in response to enveloped virus or synthetic TLR-7 and -9 agonists than other cell types. We were interested in determining the ability of human PDC to produce IFN-lambda in response to enveloped viruses and whether these cells respond to IFN-lambda treatment with upregulated production of IFN. Unstimulated, purified PDC were found to express IFN-lambda receptor mRNA. Moreover, purified PDC but not monocytes responded to stimulation by HSV with strong upregulation of IFN-lambda message. We developed an intracellular flow cytometry assay using antibodies to IFN-lambda to assess the expression of IFN-lambda protein by PDC. A subset of human PDC were found to express intracellular IFN-lambda as well as IFN-alpha after stimulation with HSV, influenza virus, Sendai virus as well as HSV-infected fibroblasts and CpG A but not CpG B, although fewer cells responded with IFN-lambda expression than IFN-alpha. The cells that co-expressed IFN-alpha and IFN-lambda tended to be those cells with the highest levels of IFN-alpha expression. Moreover, exogenous IFN-lambda and IFN-alpha treatment of PDC led to upregulation ("priming") of the intracellular expression of both IFN-alpha and IFN-lambda. In addition, exogenous IFN-lambda, like IFN-alpha, was found to inhibit apoptosis of PDC. We conclude that PDC are major producers of IFN-lambda in response to viral stimulation and also express receptors for this cytokine. Moreover, these receptors are functional in that IFN-lambda has autocrine effects that strengthen the antiviral effect of the PDC by increasing IFN-alpha and -lambda production and promoting PDC survival.

05-45/P

FOLLOW-UP OF VIRAL INFECTION AND INTERFERON EFFECT WITH A DIGITAL HOLOGRAPHIC MICROSCOPE.

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The most direct and simplest test to titrate the biological effect of Interferon (IFN) according its basic antiviral definition , has been for years , despite it is tedious and time consuming, the CPE-inhibition assay. As the development of cytopathogenicity is a dynamic process counteracted by the establishment of IFN antiviral state which is also a time dependent property, we used this system as a model to evaluate the feasibility to implement in bioreactors, to follow on line bioproductions, the digital holographic microscope (DHM) designed and constructed in our University (F. Dubois C. et al. in "Imagerie et Photonique pour les Sciences du vivant et la médecine", 2004, pp 287-302), and recently succesfully tested in the International Spatial Station to follow in real time biomolecular processes.
In this study, we showed that either in static monolayers on Tissue Culture plates, or in dynamic conditions on Microcarriers (Cytodex 3), the digital holographic microscope, will enable accurate characterization of a number of cell properties under different conditions. It was indeed possible to visualize on line with DHM, usual cell culture steps as seeding, spreading or detachment by trypsin. It was also possible to observe the evolution during time, of VSV viral cytopathogenicity, as well as the protection provided by IFN treatment.
Furthermore, as the fluorescence mode could also be used, and as mathematical treatment of data could be applied to analyze and quantify 3D properties, our approach paved the way to many important applications of this newly designed and versatile microscope in the biotechnological field, as vaccine and recombinant protein productions, as well as for basic research in cell biology, for which this work demonstrated the feasibility.