EUROPEAN COMMISSION

Community Research

AGENDA

FLUINNATE

Kick off Meeting

January 19 - 20, 2007

ab 13.00 Uhr im Hörsaal, EG, Haus C
Institut für Med. Mikrobiologie und Hygiene

Organised by
Otto Haller
FLUINNATE Project Coordinator

VIROLOGY FREIBURG

UNIVERSITÄTS KLINIKUM FREIBURG

Participants

PARTNER 1 Otto Haller, UKL-FR (Freiburg, Germany)

PARTNER 2 Stefania Crotta, NOVARTIS (Siena, Italy)

PARTNER 3 Kristien Van Reeth, UGent (Merelbeke, Belgium)

PARTNER 4 Mikhail Matrosovich, UNIMAR (Marburg, Germany)

PARTNER 5 Ervin Fodor, UOXF.BV (Oxford, United Kingdom)

PARTNER 6 Nadia Naffakh, IP (Paris, France)

PARTNER 7 Alberto Mantovani, HUMANITAS (Milan, Italy)

PARTNER 8 Bing Sun, IPS (Shanghai, China)

PARTNER 9 Aldo Tagliabue/Paola Cesaroni, ALTA (Siena, Italy)

Invited: Cornelius Schmaltz (European Commission)

Hans-Dieter Klenk (Marburg): Keynote Lecture

Christoph Peters (Dean of the Medical Faculty Freiburg): Welcome

FLUINNATE : A new approach towards the control of influenza

Influenza A viruses are still a major public health problem. They cause a highly contagious respiratory disease in humans and are responsible for periodic epidemics or pandemics, with high mortality rates. The most devastating pandemic occurred in 1918 with millions of deaths worldwide. The avian H5N1 strains currently circulating in birds across Asia and Europe have a high pathogenic potential for humans and are feared to cause the next pandemic if they acquire sufficient human-to-human transmissibility. The molecular mechanisms which determine increased virus virulence in humans are presently not well understood. Influenza viruses enter the human respiratory tract and must replicate in the face of multiple innate immune defence mechanisms to establish infection *in vivo*. Successful viruses must adapt to intrinsic cellular restriction factors and evolve the capacity of counteracting the antiviral interferon response.

FLUINNATE combines the expertise of leading laboratories in the field. The consortium will identify and characterize the essential viral and host factors that determine the outcome of infection. The emphasis is on viral replication fitness, host adaptation processes and host defense mechanisms. Human, avian and porcine influenza A viruses will be studied in animal models and in cell culture systems, such as human airway epithelium. FLUINNATE will provide new information which is important for better understanding emerging influenza viruses and for generating efficient control measures against these devastating pathogens.

I welcome all participants to the Kick-Off Meeting at the Department of Virology in Freiburg. Support of the Meeting by the Medical Faculty of the University of Freiburg is gratefully acknowledged.

Otto Haller
Coordinator, FLUINNATE

Scientific Program

Friday, January 19, 2007

13:00 – 13:15	**Christoph Peters,** Dean of the Medical Faculty Freiburg Welcome
13:15-13:45	**Otto Haller,** FLUINNATE Coordinator Presentation of the FLUINNATE project
13:45-14:15	**Cornelius Schmaltz,** Scientific Officer EU Introductory remarks
14:15- 15:00	**Hans Dieter Klenk,** University of Marburg Keynote Lecture: **Host Range and Pathogenicity Determinants of Influenza Viruses**

15.00 - 18:40 *Focus Presentations of Partners*
(15 min. each and 5 min. discussion)

15:00 – 15:20	**Otto Haller**, UKL-FR (Freiburg, Germany)	Molecular basis of influenza virus virulence in mice with a functional *Mx1* resistance gene
15:20 – 15:40	**Stefania Crotta**, NOVARTIS (Siena, Italy)	Receptor specificity and innate immunity
15:40 – 16:00	**Kristien Van Reeth**, UGent (Merelbeke, Belgium)	Role of interferon (IFN) in the pathogenesis of avian influenza viruses (WP5)
16:00 – 16:20	**Mikhail Matrosovich**, UNIMAR (Marburg, Germany)	Receptor specificity and innate immunity
16:20 – 17:00		*Coffee break*

17:00 – 17:20	**Ervin Fodor**, UOXF.BV (Oxford, U. K.)	The influenza virus RNA polymerase complex – interactions with host factors
17:20 – 17:40	**Nadia Naffakh**, IP (Paris, France)	Identification of cellular factors interacting with the polymerase complex of influenza A viruses
17:40 – 18:00	**Alberto Mantovani**, HUMANITAS (Milan, Italy)	Role of PTX3 and other components of innate immunity in influenza virus infection
18:00 – 18:20	**Bing Sun**, IPS (Shanghai, China)	The pathogenicity of RNA polymerase and NS1 suppression on IFN□/ß production in different strains of avian flu viruses (AIV) from China
18:20 – 18:40	**Aldo Tagliabue and Paola Cesaroni**, ALTA (Siena, Italy)	Project management
19:00	*D i n n e r*	
21:00 – 22:00	Round Table	

Saturday, January 20, 2007

9.00-13.00	FLUINNATE Project Partners only	**General discussion and preparations to start the project**

13.00 End of Kick off Meeting

Keynote Lecture

Avian Influenza:
Host Range and Pathogenicity Determinants of Influenza Viruses

Hans-Dieter Klenk, Institut für Virologie, Philipps-Universität Marburg, Germany

Influenza is a global threat that has 2 dimensions: human and avian influenza. Human influenza viruses cause epidemics with millions of human illness cases and many thousands deaths every year. Avian influenza viruses cause periodically large outbreaks in domestic fowl with high economic loss in the poultry industry. In fact, all influenza viruses – the human ones included – come originally from birds. Their natural hosts are wild aquatic birds – ducks, geese, gulls, and many others. Influenza viruses occur in this reservoir with a very high variety defined by 16 hemagglutinin and 9 neuraminidase subtypes. We are therefore talking of H1N1, H3N2, H5N1 viruses and so forth. Usually influenza viruses are confined to their natural hosts – the birds. Occasionally, however, the viruses are transmitted to other species, such as terrestrial birds (chickens, turkeys), pigs, and horses, where they cause disease without having changed their genetic make-up. This is where we are now with the human cases caused by the H5N1 bird flu virus. On rare occasions, adaptation occurs to the new species. Then, for example, a duck virus is converted into a pig virus. If this occurs in humans, and if a virus with a new hemagglutinin or a new neuraminidase is introduced by this mechanism into humans, we have a pandemic – a worldwide outbreak affecting the entire human population with millions of deaths. In the last century, we had 3 such pandemics: the great Spanish flu of 1918 causing more than 40 Mio deaths (this was probably the most devastating outbreak, ever, of an infections disease in a limited time period) and 2 less severe pandemics in 1957 and 1968.

Because of their importance as disease agents, influenza viruses have been studied in great detail. The structure of the virus particles has been elucidated, their replication strategies are known, and their genome has been sequenced. A big step forward in our knowledge on the biology of influenza viruses has been made, when it became possible to manipulate them by gene technology. Thus, we are beginning now to understand the molecular mechanisms underlying interspecies transmission, host adaptation, and pathogenicity.

H5N1 virus came to our attention first in 1997, when it caused a large outbreak in poultry in Hong Kong with 18 human cases, 6 of which died. The virus disappeared then for a while from the scene and re-emerged in 2002. Since 2004 it is endemic in domestic fowl in South East Asia. On rare occasions however, it spreads to other species (monkeys, cats, tigers, leopards, civets, stone martens, and man) where it causes severe disease. So far, ca 200 human cases are known of which about 100 died. These 200 human cases have to be seen in the context of over 200 Mio dead chickens. So, this is still an animal disease. About a year ago something unusual happened: the virus was re-introduced into wild birds. This became evident, when in May 2005 a large outbreak was observed in a nature reserve at Lake Qinghai in NW China where thousands of wild geese, ducks, and gulls died. Subsequently, the virus rapidly spread to Siberia, Southern Russia, and other countries surrounding the Black Sea. By the end of last year it had arrived in Central Europe. Now it is also in Africa. H5N1 has several features that underline its potential to cause a human pandemic. These include: rapid spread in poultry and wild birds, enhanced exposition of the human population, interspecies transmission, high pathogenicity for man, and the absence of immune protection in the human population. Fortunately, the virus has not acquired yet the ability to be transmitted from man to man, the only circumstance that has saved as so far from a pandemic. Many countries have responded to this threat by pandemic preparedness plans. They include surveillance of influenza viruses in man and animals, development of new strategies for vaccination and vaccine production, stockpiling of antivirals, development of new antivirals, eradication of avian influenza, and patient management.

In conclusion: Avian influenza is still an animal disease and has not yet adapted to humans. It is under control in some of the countries where it originally appeared (Thailand, Vietnam), but still active in other parts of Asia and in Africa. The alert for a pandemic caused by H5N1 or another influenza virus has therefore to continue.

Molecular basis of influenza virus virulence in mice with a functional *Mx1* resistance gene

(Partner 1)

Otto Haller, Georg Kochs & Peter Staeheli
Department of Virology, University of Freiburg, Germany

We propose to identify the essential viral and host factors that determine the early outcome of influenza A virus (FLUAV) infection in immunocompetent $Mx1^{+/+}$ mice. The IFN-inducible MxGTPase is a major effector molecule blocking FLUAV replication in tissue culture and *in vivo* and is conserved in mice and man. The aim is to define the molecular basis of the enhanced pathogenicity of a highly virulent PR8-like FLUAV strain (hvPR8) in $Mx1^{+/+}$ mice. Our analysis will reveal which combinations of viral genes and gene products are responsible for the high virulence phenotype.

Generation and functional analysis of reassortant viruses

Using cloned virus gene segments derived from hvPR8 and a corresponding low virulent PR8 strain (lvPR8), single- and multi-segment reassortant viruses will be produced and the resulting reassortants will be tested for virulence in $Mx1^{+/+}$ mice. Our preliminary findings suggest that the virulence of the hvPR8 strain is polygenic and that the viral hemagglutinin (HA) and neuraminidase (NA) are important. Moreover, the viral polymerase complex seems to be a critical factor.

Role of HA and NA for increased virulence

HA and NA mediate virus entry in cells and release from infected cells. Amino acid substitutions at critical positions in these molecules are expected to greatly influence viral virulence. We will determine relevant amino acid substitutions in both HA and NA by sequence comparisons and mutational analyses. The fine receptor specificity of HA will be determined using a panel of synthetic polyacrylamide-based sialylglycopolymers (which serve as analogues of natural receptors) as well as airway epithelial cell cultures. In addition, cleavability and fusion activity will be determined using established assays. Likewise, the sialidase activity of NA and the effect of mutations at critical NA residues will be investigated.

Reconstitution of the active viral polymerase complex

The growth behaviour of hvPR8 suggests that this exceptional strain is equipped with an unusually active polymerase and our recent results with reasssortant viruses support this assumption. The aim is to find out which particular component(s) or subdomain(s) of the complex are responsible for increased activity. The biochemical approach will include *in vitro* reconstitution of the active viral polymerase complex by expression of subunits from either hvPR8 or lvPR8. Mutational analyses of single components should help to identify crucial interaction domains or single amino acid residues critically involved in activity.

Role of host cell factors

The quality of specific interactions with cellular factors may determine the activity of the viral polymerase complex. We will test candidate interactors for differential association with the polymerase complexes of hvPR8, lvPR8 or the reconstituted mixed complexes by analyzing their colocalization and transcriptional activity in co-transfected cells. Overexpression and siRNA knock-down experiments will serve to assess the functional significance of particular interactors.

Receptor specificity and innate immunity

(Partner 2)

Stefania Crotta, Novartis Vaccines & Diagnostics, Siena, Italy

Influenza viruses attach to target cells via interactions of the viral hemagglutinin protein with sialyloligosaccharide sequences of cellular glycoconjugates. As sialic acids are ubiquitously expressed on the surface of most avian and mammalian cells, in addition to infect susceptible cells influenza viruses can bind to a variety of other cell types leading to significant biological responses.

The goal of WP4 is to test how receptor specificity and sialidase activity can affect immune responses to the virus and thus determine its virulence and pathogenicity.

HAs and NAs from well-characterized viral isolates will be used to generate recombinant viruses which will differ solely by their receptor specificity/ NA activity in an otherwise identical genetic background (Matrosovich).

At Novartis Vaccines, we will be involved in studying innate responses to these recombinant viruses in an in vitro system of human airway epithelium and in purified human immune cells (macrophages, dendritic cells, T- and B- lymphocytes, neutrophils, NK-cells). We will study how viruses with distinct receptor specificity are able to elicit different cytokine responses and cytopathic effects in these cells, performing analyses both at the level of gene regulation (RT-PCR, gene arrays) and gene expression (ELISA, flow cytometry).

Novartis Vaccines & Diagnostics is a new division of Novartis that was formed following the recent acquisition of Chiron Corporation. Novartis Vaccines is the world's fifth-largest vaccines manufacturer and second-largest supplier of flu vaccines in the US and is carrying on Chiron’s long-lasting interest in influenza. Novartis Vaccines supplies adjuvanted and non-adjuvanted flu subunit and split vaccines and is presently developing and manufacturing a cell culture-derived influenza vaccine. The focus is both to supply seasonal influenza vaccine and to respond rapidly in the event of an influenza pandemic.

Role of interferon (IFN) in the pathogenesis of avian influenza viruses (WP5)

(partner 3)

Kristien Van Reeth, Laboratory of Virology,
Faculty of Veterinary Medicine, Ghent University, Belgium

Objectives

We will study the role of IFN in the pathogenesis of influenza in a natural virus host, the pig. We have 4 specific aims:

1) To study the exact role (both beneficial and detrimental effects) of IFN in the pathogenesis of swine influenza.
2) To get insights into the mechanisms of IFN induction by influenza viruses.
3) To examine whether different avian influenza (AI) viruses differ in their IFN inducing capacity in mammalian cells and/or their sensitivity to the antiviral effects of IFNs.
4) To study the exact role of IFN during an infection of pigs with avian influenza (AI) viruses.

Approach

The 4 specific aims will be approached as follows:

(1) We will perform experimental infections of pigs with swine influenza virus (sw/Belgium/1/98, H1N1) with known IFN and pro-inflammatory cytokine profile. The pigs will be inoculated intratracheally with 7.5 log10 EID50 virus, because this method reproduces the typical swine influenza symptoms. The role of IFN-alpha will be studied by intratracheal and intraperitoneal administration of specific anti-porcine IFN-alpha neutralizing antibodies. We will compare clinical signs, lung inflammatory parameters, virus replication and correlates of the innate (pro-inflammatory cytokines, acute phase proteins) and specific (antibodies and cellular immunity) immune response in IFN antibody-treated and control pigs.

(2) Our preliminary in vitro studies have indicated that the mere contact between one of the influenza viral proteins and porcine IFN-producing cells is sufficient to trigger the production of IFN-alpha, because infectious virus is not required. We will use our in vitro model, namely cocultures of SIV infected primary porcine lung epithelial cells and peripheral blood mononuclear cells to determine the (glyco)protein (sequence) responsible for IFN induction. For this purpose we will use polyclonal and monoclonal antibodies to HA, NA and M2 proteins, as well as virus mutants with differences at the receptor binding site of the HA and/or other proteins. Parallel studies will be undertaken in an in vitro system with human airway epithelial cells and conventional human influenza viruses at University of Marburg.

(3) As for aim 2, aim 3 will be studied in vitro in both swine (UGent) and human (UNIMAR) respiratory epithelial cell cultures. The selection of the AI viruses to be used will be based on a) in vivo data on the susceptibility of pigs to different AI virus subtypes and isolates which are available from other projects in our lab; b) genetic analysis of the supposed IFN inducing viral protein. The viruses will be compared for their replication efficiency, IFN inducing capacity and susceptibility to the antiviral effects of recombinant IFNs in swine and human cultures. This will allow us to select AI viruses for final in vivo experiments to test the role of IFN during an AI infection in pigs (aim 4).

(4) The exact role of IFN during an infection of pigs with AI viruses will be studied in vivo. We will use AI viruses with differences in their IFN response, if available, as well as neutralizing anti-IFN antibodies. All of the clinicopathological, virological and immunological parameters studied during infection with swine influenza virus (aim 1) will also be studied.

Receptor specificity and innate immunity

(Partner 4)

Mikhail Matrosovich and Hans-Dieter Klenk
Institute of Virology, Philipps University, Marburg, Germany
MRC National Institute for Medical Research, Mill Hill, London, U.K.

Studies on influenza virus infection in human target tissues are limited due to a lack of suitable experimental models. To address this problem, we employ cultures of differentiated human airway epithelial cells which closely mimic airway epithelium *in vivo* and appear to represent a model of choice for the studies on influenza virus interactions with cellular receptors and inhibitors in humans. Using this system, we found that the receptor–binding specificity of the viral hemagglutinin (HA) determines virus cellular tropism in the epithelium and that the receptor-destroying activity of the viral neuraminidase (NA) is essential for the initiation of infection. These findings suggest that receptor specificity of influenza viruses can affect antiviral responses in the airway epithelium in a cell type-dependent manner.

Sialic acids are ubiquitous on the surface of most avian and mammalian cells. Therefore, in addition to mediating infection in susceptible epithelial cells, influenza viruses can bind to a variety of other cell types leading to important biological effects, such as polyclonal activation of B-lymphocytes and deactivation of neutrophils. Any correlation between the receptor specificity of the viruses and their ability to cause these effects has yet to be investigated.

Our project will test the hypothesis that distinctions in the receptor specificity of influenza viruses can affect antiviral innate responses in airway epithelial cells and in immuno-competent cells.

> To reach our goals, we will use natural and recombinant influenza viruses with distinct receptor specificities. H5N1 viruses and 1918 viruses will be included in the study to test whether distinctive receptor specificity of these viruses contributes to their high pathogenicity.

We will test how infection with live viruses (as well as binding to cells of inactivated viruses) affects expression of genes and cellular responses in cultures of differentiated human airway epithelium and in isolated human immuno-competent cells (macrophages, dendritic cells, T- and B-lymphocytes, neutrophils, NK-cells). Based on these data and using the same virus panels, we will test whether viral receptor specificity affects innate responses in animal models.

We expect that new information on the roles of hemagglutinin and neuraminidase of influenza viruses in antiviral innate immunity will improve surveillance for emerging influenza viruses and will provide the rationale for new approaches to influenza prophylaxis and treatment.

The influenza virus RNA polymerase complex – interactions with host factors

(Partner 5)

E. Fodor & G. G. Brownlee
Sir William Dunn School of Pathology, University of Oxford, South Parks Road,
Oxford OX1 3RE, U.K.

The focus of our research is on the RNA-dependent RNA polymerase of influenza viruses and the molecular mechanisms of transcription and replication of the influenza virus RNA genome. Our current research interests include: (i) identification of functional domains of the viral RNA polymerase; (ii) transport and assembly of the viral RNA polymerase complex; (iii) the role of host factors in transcription and replication of the influenza virus RNA genome; (iv) the role of the viral RNA polymerase in pathogenesis and host range determination.

Transport and assembly of the influenza virus RNA polymerase complex

The influenza A virus RNA-dependent RNA polymerase is a heterotrimeric complex of PB1, PB2, and PA subunits. It performs transcription and replication of the viral RNA genome in the nucleus of infected cells. We have identified a nuclear import factor, Ran Binding Protein 5 (RanBP5), as an interactor of the PB1 subunit. RanBP5 interacted with either PB1 alone or with a PB1-PA dimer, but not with a PB1-PB2 dimer or the trimeric complex. The interaction between RanBP5 and PB1-PA was disrupted by RanGTP in vitro allowing PB2 to bind to the PB1-PA dimer to form a functional trimeric RNA polymerase complex. We propose a model in which RanBP5 acts as an import factor for the newly synthesized polymerase by targeting the PB1-PA dimer to the nucleus. In agreement with this model, siRNA mediated knock down of RanBP5 inhibited the nuclear accumulation of the PB1-PA dimer. Moreover, siRNA knock-down of RanBP5 resulted in the delayed accumulation of viral RNAs in infected cells, confirming that RanBP5 plays a biological role during the influenza life cycle.

Interactions of the influenza virus RNA polymerase complex with the host RNA polymerase II transcriptional machinery

Transcription of the eight segments of the single-stranded negative-sense RNA genome of influenza A virus by the viral RNA polymerase is dependent on cellular RNA processing activities known to be associated with cellular RNA polymerase II (Pol II) transcription, namely capping and splicing. This suggested that transcription by the viral RNA polymerase and Pol II might be functionally linked. Indeed, we were able to demonstrate that the trimeric viral RNA polymerase complex associates with the host Pol II transcriptional machinery via its interaction with the C-terminal domain (CTD) of the large subunit of Pol II. All three subunits of the viral RNA polymerase complex, PB1, PB2, and PA, were required for the interaction indicating that it is the trimeric complex that recognizes the CTD. The viral RNA polymerase interacted with the CTD of Pol II which is serine-5 phosphorylated. Serine-5 phosphorylation is associated with Pol II engaged in transcription initiation at the promoter proximal region where the capping process of pre-mRNAs is taking place. We propose that this interaction allows the influenza RNA polymerase to access host mRNA-derived 5' capped RNA fragments used as primers for the initiation of viral RNA transcription and possibly other host factors involved in viral mRNA processing. Studies are currently under way to determine the significance of this interaction for viral transcription and replication. We are also interested in determining the consequences of this interaction for the functionality of the Pol II transcriptional machinery.

References Deng, T., Engelhardt, O. G., Thomas, B., Akoulitchev, A. V., Brownlee, G. G., and Fodor, E. (2006). Role of Ran Binding Protein 5 (RanBP5) in nuclear import and assembly of the influenza virus RNA polymerase complex. *J. Virol.* **80:** 11911-11919.
Engelhardt, O. G., and Fodor, E. (2006). Functional association between viral and cellular transcription during influenza virus infection. *Rev. Med. Virol.* **16:** 329-345.

Identification of cellular factors interacting with the polymerase complex of influenza A viruses

(Partner 6)

Nadia Naffakh, Sylvie van der Werf. Unité de Génétique Moléculaire des Virus Respiratoires, Institut Pasteur, Paris, France

The three subunits of the polymerase complex (PB1, PB2 and PA) and the nucleoprotein (NP) of Influenza A viruses have been shown to be the minimal set of proteins required for the transcription and replication of the viral genome, and to function in close association with the cellular machinery of transcription. It is a likely hypothesis that host range and virulence are partly determined by patterns of interactions between the viral polymerase complex and host proteins.

Our aim is to identify cellular factors interacting with the polymerase complex of Influenza A viruses, using two distinct approaches : 1) co-purification or co-immunoprecipitation assays using recombinant Influenza viruses expressing a tagged polymerase complex ; and 2) two- and three-hybrid screening in yeast. The importance with respect to host range and virulence will be evaluated for a selection of interactors. In the longer term, understanding of the molecular mechanisms involved could help predicting the potential of Influenza A viruses to cause severe disease in humans, and provide a rationale for new approaches to control influenza by developing antivirals that could interfere with the function of the viral polymerase.

Influenza viruses expressing a PB2 protein fused with various purification tags at the N- or C-terminus have been produced by reverse genetics. Cytoplasmic and nuclear fractions prepared from cells infected with a PB2-tagged recombinant virus or from control cells infected with a wild-type virus have been processed in parallel and analyzed by SDS-PAGE. Preliminary results indicate that molecular interactions involving the polymerase complex during the viral multiplication cycle can indeed be characterized using this approach.

In collaboration with Drs Vidalain, Jacob and Tangy (I-MAP Project, Institut Pasteur),we are setting up a three-hybrid screening system in yeast, were PB1 + PB2 or PB1 + PA complexes are used as a bait to screen available cDNA libraries. Standard two-hybrid screening using each of the PB1, PB2 or PA protein individually expressed are being performed in parallel. Complementarity between this genetic approach and the biochemical approach described above is expected.

Role of PTX3 and other components of innate immunity in influenza virus infection.

(Partner 7)

Alberto Mantovani, HUMANITAS, Milan, Italy

Pentraxins are a superfamily of highly conserved proteins characterized by a multimeric structure and playing essential functions in innate defense against microbes. PTX3 is the prototype of the long pentraxin subfamily: it is rapidly produced by cells of the innate immune system in response to primary inflammatory signals and TLR engagement. PTX3 binds with high affinity the complement component C1q, the extracellular matrix component Tumor necrosis factor a induced protein 6 (TNFAIP6 or TSG-6) and selected micro-organisms, including *Aspergillus fumigatus* and *Pseudomonas aeruginosa*. PTX3 activates the classical pathway of complement activation and facilitates pathogen recognition by macrophages and DC.

Results obtained so far in gene-modified mice demonstrate that PTX3 has several non-redundant functions *in vivo*, ranging from modulating inflammation and innate immunity against selected pathogens to the assembly of a hyaluronic-rich extracellular matrix.

No data are available on the role of PTX3 in viral infections, however preliminar data indicate that PTX3 recognize selected influenza virus strains suggesting a possible role of this protein in the infection. Our contribution to the project will involve the assessment of the spectrum of influenza virus recognition by PTX3 and other pentraxins; the identification of influenza virus component recognized by PTX3; the susceptibility of gene-modified mice to influenza virus infection and the measurement of PTX3 levels in infected mice. The results will be helpful for the evaluation of PTX3 role in influenza virus infections.

The pathogenicity of RNA polymerase and NS1 suppression on IFN-α/ß production in different strains of avian flu viruses (AIV) from China

(Partner 8)

Bing Sun, Institute Pasteur of Shanghai, PR China

Since RNA polymerase (RdRp) plays an important role in the pathogenesis of virus infection and IFN-a/b production is critical for virus replication in host cells, in this study we focus on analysis of RdRp activity from highly pathogenic avian influenza viruses (HPAIV) H5N1 strain from avian isolates and fatal human infection cases in China, and identify new molecules of host factors regulating NS1-induced IFN-a/b signal pathway. In RdRp part, we studied RdRp activity with replicon system and in vitro using purified enzymes and try to reconstitute the activity with cis- and trans-factors including host cellular factors interacting with viral RdRp. In IFN-a/b part, NS1 gene sequences from different strains (H5N1 and H9N2) were compared and their functions in suppressing IFN-a/b production were investigated and confirmed with mutation experiments. In addition, two-yeast hybridization technique was used to screen unknown cellular proteins that can interact with NS1 and influence its function. The MyD88-dependent and TRIF-dependent signal pathways initiated by TLRs were also be measured. The current experiments demonstrated that Influenza virus RdRp consisting of hetero-trimer complex of PB1, PB2 and PA was constructed. A constructed PB2 mutant revealed an enhancement in replicon activity. Although there were some differences between the protein sequences of the H5N1- and H9N2-NS1 genes, no significant differences were found in IFN antagonist ability. Eight genes interacting with NS1 have been selected and their functions modulating NS1 function will be further investigated.

Project Management

(Partner 9)

Aldo Tagliabue, ALTA Srl, Siena, Italy

ALTA S.r.l. (www.altaweb.eu) is service-provider company operating in the field of knowledge engineering. It is specialized in managing the life cycle of publicy funded research and developmnent projects in the area of biomedicine and biotechnology

ALTA provides services employing transaction systems for administering the life cycle of publicly funded research projects. ALTA acts at the level of:

- Preparation of the project proposal
- Proposal Submission
- Contract Negotiation
- Contracting
- Reporting period every year on scientific report and administrative report
- Assisting in co–ordination

Working in close contact with the Project Coordinator, the specific ALTA's contribution to FLUINNATE project will be:

1. To give support for the preparation of the Consortium Agreement;
2. To create public awareness of the Consortium;
3. To develop and implement plans for dissemination and for raising public participation and awareness;
4. To develop a project web page;
5. To give support for meeting organisation;
6. To give general secretarial assistance (i.e. request and collection of technical and financial reports, prepare and send to all participants letters or emails related to any project activities such as meeting participation, communication from EU Commission etc.)