EUROPEAN SEMINARS in VIROLOGY

2nd European Seminar in Virology (EuSeV)

University of Bologna Residential Center in **Bertinoro, Italy**

June 13-15, 2014

Vaccines and antivirals

Abstract book

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Vaccines and antivirals

Organizers:

Gabriella Campadelli-Fiume

Dana Wolf

Thomas Mertens (President of the GfV)

on behalf of the European Society

for Virology (ESV)

President Giorgio Palù

and

participation of Società Italiana di Virologia (S.I.V.)

President Franco Maria Ruggeri

FRIDAY, 13th June 2013	
16:00-16:15	**Welcome** **Giorgio Palù, President, European Society for Virology** **Franco Ruggeri, President, Italian Society for Virology** **Dana Wolf, Gabriella Campadelli-Fiume, Thomas Mertens**
Chairs: Bernhard Fleckenstein and Johan Neyts	
16:15-16:45	Rational drug design: a state of the art look for next generation antivirals. *Andrea Brancale (Cardiff University, Cardiff, United Kingdom)*
16:45-17:15	Peptide-based antivirals. *Jan Münch (Ulm University Medical Center, Ulm, Germany)*
17:15-17:45	From screening of novel antivirals to deciphering the mechanism of action. *Mark Prichard (University of Alabama, Birmingham, United States)*
17:45-18:00	Rationale design of terphenyl molecules that inhibit HIV-1 RRE-REV ribonucleinprotein function. *Jose Gallego (Universidad Católica de Valencia, Spain)*
18:00-18:15	The allosteric HIV-1 integrase inhibitor BI-D affects virion maturation but does not influence packaging of a functional RNA genome. *Nikki van Bel (University of Amsterdam, Amsterdam, Netherlands)*
DISCUSSIONS IN FRONT OF POSTERS	
20:00	**Dinner at outside Restaurant**

SATURDAY, 14th June 2014	
Chairs: Jose Este and Jan Münch	
8:30-9:00	New antiviral drugs in development- overview. *Johan Neyts (Rega Institute KU Leuven, Leuven, Belgium)*
9:00-9:30	Advances in HCV and HBV antiviral therapy. *Raffaele de Francesco (IRBM/Merck Res Laboratories, Rome, Italy)*
9:30-10:00	Influenza and emerging respiratory viruses –antiviral drugs and antiviral drug resistance. *Maria Zambon (National Influenza Center, London, United Kingdom)*
10:00-10:15	Correlating Intracellular viral levels of drug resistant and drug sensitive HIV-1 infection to disease progression among drug naïve Africans. *Yvonne Affram (University of Heidelberg, Germany)*
10:15-10:30	Effectiveness of CMX001 for the treatment of digestive diseases and disseminated adenovirus infections in recipients of pediatric hematopoietic stem cells. *Linda Feghoul (University of Paris Diderot, Paris, France)*
BREAK	
Chairs: Dana Wolf and Maria Zambon	
11:00-11:30	Evidence based recommendations for vaccination programs *Thomas Mertens (Ulm University Medical Center, Ulm, Germany)*
11:30-12:00	The polio plot: eradication dreams vs. reality. *Itamar Grotto (Public Health Service, Ministry of Health, Tel Aviv, Israel)*
12:00-12:15	Molecular analysis of group A Rotaviruses detected in adults and adolescents with acute gastroenteritis hospitalized in Italy in 2012. *Giovanni Ianiro (National Center of Immunobiologicals, Research and Evaluation, Rome, Italy)*
12:15-12:30	UL5, a newly identified HCMV protein. *Giulia Anselmi (University of Padua, Padua, Italy)*
12:30-12:45	Human cytomegalovirus (HCMV) pX binds a leucocytes surface receptor and inhibits T-cell proliferation. *Luca Bruno (Novartis Vaccines and Diagnostics, Siena, Italy)*
12:45-13:00	Human Cytomegalovirus Infection Triggers the Interferon Response via the DNA sensor IFI16 in Human Keratinocytes. *Matteo Biolatti (University of Turin, Turin, Italy)*
LUNCH BREAK	
Chairs: Thomas Mertens and Mark Prichard	
14:45-15:15	CMV antiviral treatment: current challenges and prospects. *Dana Wolf (Tel Aviv University, Israel)*
15:15-15:45	The era after nucleos/tides: Novel drugs against herpes viruses. *Helga Rübsamen-Schaeff (AiCuris GmbH & Co KG, Wuppertal, Germa*ny)
15:45-16:15	HCMV infection in specialized cells of the human placenta and suppression by monoclonal antibodies (mAbs) to glycoprotein B and proteins UL130/131A of the viral pentamer.*Lenore Pereira (University of California, San Francisco, United States)*
16:15-16:45	CMV vaccine research- coupling the innate and adaptive immunity arms. *Stipan Jonjic (University of Rijeka, Rijeka,Croatia)*
BREAK	

Chairs: Xavier Saelens and Gabriella Campadelli-Fiume	
17:00-17:30	Adenovirus vector based T-cell response eliciting HCV vaccines in clinical trial. *Antonella Folgori (Okairos, Rome, Italy)*
17:30-18:00	Therapeutic vaccines for hepatitis-related cancers. *Luigi Buonaguro (National Cancer Institute, Naples, Italy)*
18:00-18:15	Signal molecules of the innate immune system as genetic adjuvants in DNA immunizations against Influenza A viruses. *Dennis Lapuente (Ruhr Universität, Bochum, Germany)*
18:15-18:30	West Nile virus candidate vaccines based on recombinant envelope proteins and DNA plasmids. *Sebastian Ulbert (Fraunhofer Institute for Cell Therapy and Immunology, Leipzig, Germany)*
18:30-18:45	Improved MVA vaccine Immunogenicity achieved by using endogenous early promoters. *Naif Alharbi (King Abdullah International Medical Res Center, Riyadh, Saudi Arabia)*
18:45-19:00	Vaccination against Influenza H5N1 and Newcastle Disease Viruses using the antiviral cinnamon fraction. *Michael Ovadia (Tel Aviv University, Tel Aviv, Israel)*
DISCUSSIONS IN FRONT OF POSTERS	
20:00	**Dinner at outside Restaurant**

SUNDAY, 15th June 2014	
Chairs: Gabriella Campadelli-Fiume and Stipan Jonjic	
8:30-9:00	HIV cure: latency and reservoirs. *Guido Poli (San Raffaele Scientific Institute, Milano, Italy)*
9:00-9:30	Controlling virus replication through regulation of host restriction factors. *Jose Este (AIDS Res Institute Irsi Caixa, Barcelona, Spain)*
9:30-9:45	Establishment of Myotis myotis cell lines for investigation of immune responses under lyssavirus infection. *Xiaocui He (Federal Res Institute for Animal Health, Greifswald-Insel Riems, Germany)*
BREAK	
10:00-10:30	The unmet quest for antiviral vaccines / new vaccines in the pipe line. *Sylvie Bertholet (Novartis Vaccines and Diagnostics, Siena, Italy)*
10:30-11:00	Novel influenza Vaccines. *Xavier Saelens (University of Ghent, Ghent, Belgium)*
BRUNCH	
12:00	**Bus leaves for Bologna Airport**

Rational drug design: a state of the art look for next generation antivirals

Andrea Brancale

Peptide-based antivirals

Jan Münch

From screening of novel antivirals to deciphering the mechanism of action

Mark Prichard

Rational design of synthetic terphenyl molecules that inhibit HIV-1 RRE-Rev ribonucleoprotein function

Luis González-Bulnes1, Ignacio Ibáñez2,3, Luis M. Bedoya4, Manuela Beltrán4, Silvia Catalán2,3, José Alcamí4, Santos Fustero2,3 and José Gallego1
1Universidad Católica de Valencia, C/Quevedo 2, 46001 Valencia, Spain
2Universidad de Valencia, Avda. V. A. Estellés s/n, 46100 Burjassot Valencia, Spain
3Centro de Investigación Príncipe Felipe, Avda. Autopista Saler 16, 46012 Valencia, Spain
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Viral RNA structures are not easily targeted by antisense agents and have the advantage of their high sequence and/or three-dimensional structure conservation. However, the development of small RNA-binding agents has been hampered by the difficulties posed by these structures, which have limited physicochemical diversity and are often flexible. The Rev Response Element (RRE) is a strongly conserved 350 -nucleotide structure located in the *env* gene of human immunodeficiency virus type-1 (HIV-1) RNA. Within subdomain IIB of the RRE, an internal loop forms a high-affinity complex with the arginine-rich α-helix of the virally-encoded protein Rev, Rev34-50. This interaction is essential for virus viability, as it triggers a cascade of events allowing the transport of unspliced or incompletely spliced viral RNA molecules to the cytoplasm of the infected cell. Making use of structure-based methods, we have designed, synthesized and evaluated organic mimics of the RNA-binding α-helix of Rev. These compounds contain a novel bilaterally-substituted *p*-terphenylene scaffold that reproduces the interactions of the protein when wrapped by RNA. Cellular assays indicated that the terphenyl compounds blocked Rev function and inhibited HIV-1 replication at post-transcriptional steps of the virus infectious cycle. SPR, ITC and NMR spectroscopy analyses revealed that the terphenyls occupied the binding site of Rev34-50 in the major groove of loop IIB, inducing conformational changes in RNA nucleotides strikingly similar to those brought about by the protein. Most of the small RNA-binding agents described so far are related to peptides or antibiotics, or were discovered by screening. The terphenylene scaffold may provide a new lead for HIV chemotherapy, as well as new avenues for modulating RNA function with small molecules.

Reference: González-Bulnes, L., Ibáñez, I., Bedoya, L.M., Beltrán, M., Catalán, S., Alcamí, J., Fustero, S., Gallego, J. Structure-based design of an RNA-binding p-terphenylene scaffold that inhibits HIV-1 Rev protein function. *Angew. Chem. Int. Ed.* 52, 13405 –13409 (2013).

The allosteric HIV-1 integrase inhibitor BI-D affects virion maturation but does not influence packaging of a functional RNA genome

Nikki van Bel[1], Yme van der Velden[1], Damien Bonnard[2], Erwann Le Rouzic[2], Atze T. Das[1], Richard Benarous[2] and Ben Berkhout[1#]

[1] Laboratory of Experimental Virology, Department of Medical Microbiology, Center for Infection and Immunity Amsterdam, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands. [2] Biodim Mutabilis, Romainville, France

Current antiretroviral therapies against HIV are highly successful in reducing the viral load in infected individuals. However, due to the error-prone viral reverse transcriptase enzyme, HIV evolves rapidly and drug-resistance can emerge quickly when a single antiviral is used. Four classes of inhibitors exist that target key steps of the HIV replication cycle: membrane fusion leading to cell entry and reactions executed by the viral enzymes reverse transcriptase, integrase and protease. The most recently discovered class of integrase inhibitors are the allosteric integrase inhibitors (ALLINIs). For integration, integrase binds the viral cDNA and the complex is tethered by the cellular co-factor LEDGF/p75 to a host chromosome, where integration takes place. ALLINIs were designed to prevent the interaction between integrase and LEDGF/p75 and thus should block integration. Unexpectedly however, the major effect of ALLINIs seems to be at the level of virion assembly and maturation, resulting in a grossly distorted virion morphology. Virion assembly is a highly orchestrated and regulated process in which several viral proteins and RNA molecules closely interact. It is therefore of interest to study whether ALLINIs have additionalRNA effects, such as HIV-1 RNA packaging and annealing of the tRNA primer for reverse transcription. Combining such potential RNA effects with the already reported effects on integration and virion maturation, this could be the first drug with three intrinsic modes of action, thus providing a combinational therapy in a single drug. We show that the ALLINI BI-D inhibits virus replication and that virus produced in the presence of BI-D is non-infectious. Furthermore, we show that the wild-type level of HIV-1 genomic RNA is packaged in virions and that all RNA and enzyme components for reverse transcription are properly present in virions produced in the presence of BI-D. The inhibition of reverse transcription in target cells is thus likely to reflect mislocalization in the virus particle of the components involved.

New antiviral drugs in development - overview

Johan Neyts

Advances in HCV and HBV antiviral therapy

Raffaele de Francesco

Influenza and emerging respiratory viruses – antiviral drugs and antiviral drug resistance

Maria Zambon

Correlating Intracellular Viral Levels of Drug Resistant and Drug Sensitive HIV-1 Infection to Disease Progression Among Drug Naïve Africans

Yvonne Affram[1]; Hans-Georg Kraeusslich[1]

[1]University of Heidelberg, Heidelberg Germany

There is emerging evidence that Intracellular (IC) HIV-1 DNA Load in peripheral blood affect disease progression irrespective of plasma RNA load and CD4 counts. This study aimed to determine how IC HIV-1 DNA load affect disease progression in a cohort of drug naive Africans.

Eighty-six drug naïve patients attending the Nouna district hospital in Burkina Faso were recruited for this study. Plasma viral load and CD4 counts were determined by Real-Time HIV-1 RNA assays and flow cytometry respectively. In-house PCR and sequencing techniques were used for HIV-1 subtyping and determination of drug-resistance mutations in the HIV-1 pol region. HIV-1 DNA load and CCR5 gene copies were measured with a molecular-beacon-based real-time PCR assay.

Patients habouring drug susceptible virus strains were 73(88%) and those with drug resistant virus strains were 10(12%). Three patients were excluded from the analysis because their viral sequences were heterogeneous and could not be read. Among the patients habouring drug resistance mutations, the predominant resistance mutations were minor drug resistance mutations to Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) 8(80%). The main HIV-1 subtype was the recombinant CRF02_AG strain 60(67.8 %). RNA- and DNA-associated pol sequence similarity was high 74(89%). There was a very weak correlation between IC HIV-1 DNA load and plasma RNA levels (co-efficient of correlation (R^2), 0.0086) and between IC HIV-1 DNA load and CD4 counts (R^2, 0.2797). There was no significant difference between IC HIV-1 DNA load of drug sensitive and drug resistant strains (p value 0.6991).

A high similarity existed between RNA and DNA templates therefore genotypic resistance mutations can be determined on either template. Furthermore, there was no correlation between IC HIV-1 DNA load and transmitted drug resistance mutations indicating that transmitted drug resistance does not influence HIV disease progression among drug naïve Africans.

Effectiveness ofCMX001for the treatment ofdigestivediseases anddisseminatedadenovirus infectionsin recipients ofpediatrichematopoietic stem cells

Linda Feghoul[1], Mony Fahd[2], Marie Ouachée[2], Jean-Hugues Dalle[2], André Baruchel[2], François Simon[1], Jérôme Le-Goff[1]
1. Univ Paris Diderot, Inserm U941, Microbiology laboratory, Hôpital Saint-Louis, Paris, France
2. Hematology Department, Hôpital Robert Debré, Paris, France

Background: Adenoviruses (Adv) may cause disseminated infection inallogenichematopoietic stem cell transplant (HSCT) recipients especiallyin pediatrics patients.Disseminated infections usually occur after digestive reactivations and the risk of dissemination is associated with a higher Adv load in stool. Thereis noFDA-approved treatment for Adv disseminated infection or related disease. CMX001 (1-O-hexadecyloxypropyl-cidofovir) is an orally bioavailable lipid conjugate of CDV. The lipid conjugate offers new pharmacokinetic properties with good tissue distribution and high intracellular concentrations, thus increasing antiviral efficacy. The high concentration in the digestive tract of CMX001 might allowthe control of digestive infectionsand might prevent blood dissemination. Here, we report the clinical experience of compassionate use of CMX001 in digestive and disseminated infections in seven HSCT paediatrics recipients at Robert Debré hospital, Paris, France. **Findings:** Four patients (#1, #2, #3 and #4), 7, 16, 12 and 2 years old respectively, received CMX001 for Adv disseminated infection. Patient #1 received unrelated bone marrow and patients #2, #3 and #4 received allogeneic related peripheral blood stem cells. Patient #1 received a first treatment of cidofovir for one week. Upon CDV, Adv load decreased from 5.48 to 3.60 $\log_{10}$ copies/ml in plasma and from 6.60 to 6.17 $\log_{10}$ copies/ml in stool. He received CMX001 9 days after the injection of CDV, at this moment Adv load was at 2.82 $\log_{10}$ copies/ml in plasma and 6.69 $\log_{10}$ copies/ml in stool. For patients #2, #3 and #4 when CMX001 treatment started, Adv loads were 7.10, 5.67 and 2.78 $\log_{10}$ copies/ml in plasma and 3.49, 9.58 and 5.74 $\log_{10}$ copies/ml in stool respectively. Patients #1 and #2 received only 1 dose (4mg/kg) and died within 7 days without any decrease of Adv load, both deaths were attributed to Adv. Patients #3 became undetectable in plasma after 8 doses of CMX001 and remained positive during one month in stool with Adv loads from 2.68 $\log_{10}$ copies/ml to 3.63 $\log_{10}$ copies/ml (2 mg/kg/twice a week). Patients #4 became undetectable in plasma and stool after 4 doses of CMX001.Three patients (#5, #6 and #7), 13, 6, and 6 years old respectively, received antiviral treatment for Adv infection limited to the digestive tract because all received a cord blood transplant and were considered at risk of Adv disseminated infection. They received 3 to 4 doses of cidofovir (5 mg/kg/week) without any significant decrease of Adv viral load in stool. When CMX001 treatment was initiated, Adv loads in stool were 7.09, 9.10, 5.54 in patients #5, #6 and #7 respectively. Patients #5 and #6 cleared the digestive Adv infection in 14 and 42 days after 6 to 10 doses of CMX001 (2 to 4 mg/kg/twice a week). In patient #7, Adv load decreased in stool from 5.54 to 3.58 $\log_{10}$ copies/ml in 13 days after 4 doses of CMX001 (2 mg/kg/ twice a week) but remained positive for 6 months after CMX001 treatment cessation. In these 3 patients, Adv remained negative in plasma. **Conclusion:** In this limited series, CMX001 was effective to control Adv digestive infection and had some impact in patients with Adv disseminated infection. Whether the use of CMX001 in allogenic HSCT pediatric patients with Adv digestive infection could prevent Adv disseminated infection is an area for further studies.

Evidenced based recommendations for vaccination programs

Thomas Mertens

The polio plot: eradication dreams vs. reality

Itamar Grotto

MOLECULAR ANALYSIS OF GROUP A ROTAVIRUSES DETECTED IN ADULTS AND ADOLESCENTS WITH ACUTE GASTROENTERITIS HOSPITALIZED IN ITALY IN 2012

Ianiro G[1], Delogu R[1], Fiore L[1], and Ruggeri FM[2]

[1]National Center for Immunobiologicals Research and Evaluation, and [2]Department of Veterinary Public Health and Food Safety, IstitutoSuperiore di Sanità, Rome, Italy.

Group A rotaviruses (RVA) are the leading cause of acute gastroenteritis (AGE) in young children worldwide, and it is estimated that 455.000 deaths occur every year among children aged 0-5 years, mostly in developing countries of the Sub-Saharan Africa and South-East Asia. For this reason, vaccination is recommended.

Within the national surveillance program of rotavirus (RVA) gastroenteritis in Italy, a total of 1595 samples were collected from patients hospitalized in 2012. Forty-two samples (2.6%) were collected from young adolescents or adults, including elderly. These patients were admitted to hospitals with AGE, and presented several combinations of symptoms.

All 42 RVA strains detected in adolescent and adult patients in Italy in 2012 were genotyped by RT-PCR and nested-PCR, according to the EuroRotaNet protocols. Twelve strains from patients born before 1999 (>13 years-old) were also subjected to nucleotide sequencing of the VP7 and/or the VP4 (VP8*) genes.

Molecular analysis of the genes sequenced from the 12 strains selected revealed that they mostly belonged to RVA lineages reported worldwide, for all five major human genotypes (G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8]), showing high conservation with respect to pediatric RVA strains belonging to the same lineage. Moreover, a rare G3P[19] RVA strain was detected in the stools of a 35 year-old woman who developed acute diarrhea. This study presents the complete genomic characterization performed on this rare genotype detected for the first time in humans, and nucleotide sequencing of the 11 genome segments revealed a P[19] specificity for the VP4 and a complete AU-1 like genomic constellation, (G3-P[19]-I3-R3-C3-M3-A3-N3-T3-E3-H3). The phylogenetic analysis showed possible reassortment events for VP1, VP4 and NSP5 genes.

The circulation of the same RVA strains in both children and adults, together with the detection of a rare, animal-derived strain, suggests that adults may contribute to the spread and persistence of rotaviruses in the population.The constant surveillance, along with genotyping, phylogenetic analysis, and study of viral epitopes of rotaviruses is critical to establish both the status of the immunity in the population and the information useful for the vaccine composition.

UL5, a newly identified HCMV protein

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Human cytomegalovirus is a ubiquitous, highly specific herpes virus, infecting as many as 85% of adults. It establishes latent infection adapting numerous immune evasion strategies. The HCMV genome consists of a linear 230-kbp dsDNA, surrounded by an icosahedral capsid, a proteinaceous and amorphous tegument and a cellular lipid layer containing viral glycoproteins. This work aims to identify and characterize unknown proteins and complexes in HCMV. A reverse vaccinology approach has been carried out to identify, among the over 150 ORFs, the sequences that potentially encode for membrane proteins. A selection has been done based on the expression level and on the positivity to Cytogam® and human sera. Among the candidates, UL5 is a completely uncharacterized ORF. Two isoforms of UL5 of about 14 and 22 kDa were identified in transfection, only in the insoluble fraction, and confocal experiments localize UL5 to the ER and to the cis-Golgi. FACS analysis on transfected cells have shown that the C-terminal region of UL5, tagged with the myc epitope, is not exposed outside the plasma membrane of intact cells, while being detected in permeabilized cells. In order to investigate its structure and role in the environment of infection, recombinant viral particles have been produced with different tags added at the C-termini of the antigen (Flag, V5-SBP, 2xStrepII-2xFlag). Furthermore, recombinant UL5 has been produced in *E. coli*, purified and used to immunize mice. The sera obtained are being implemented to characterize the protein in infection. Our findings suggest that UL5 is transcribed and expressed with an early kinetics and UL5 protein product localizes with markers of the TGN in the so-called ‘assembly complex’ during infection but is not incorporated in the virion. The recombinant tagged viruses are being implemented to set up pull-down experiments followed by MS analysis in order to identify possible interactors.

HUMAN CYTOMEGALOVIRUS (HCMV) pX BINDS A LEUKOCYTES SURFACE RECEPTOR AND INHIBITS T-CELL PROLIFERATION

Luca Bruno1-3, Mirko Cortese1, Giuseppina Monda1, Stefano Calo'1, Erika Bartolini1, Stefano Bonacci1, Mary Schaefer2, Diego Piccioli1, Domenico Maione1, Andrea Carfi2, Marcello Merola1, Yasushi Uematsu1

AFFILIATION: 1Novartis Vaccines and Diagnostics, Siena, Italy

2Novartis Vaccines and Diagnostics, Cambridge, MA, USA

3Università di Roma "La Sapienza", Roma Italy

Human Cytomegalovirus (HCMV) exerts complex effects on the host immune system through expression of several interfering functions, not all of which have been attributed to viral genes.

In a search for new immunomodulatory proteins, using a combined approach of Reverse Vaccinology, recombinant mammalian protein expression, immunoblotting and protein microarrays, we identified a total of 25 viral proteins recognized by immunoglobulin of HCMV infected patients. Among them, the majority were known to be involved in the control of host immune responses.

X is a member of the HCMV RL11 family. The characteristics of the X protein (pX), a predicted heavily glycosylated Ig-like membrane protein known to be dispensable for viral replication in cultured cells, suggested a possible role in host-cell interaction. We showed that pX is cleaved from the cell surface of fibroblasts and epithelial cells. Through ex-vivo cell based assays and flow cytometry experiments on both lymphoid cell lines and primary blood cells, we observed pX interaction with a cellular receptor ubiquitously expressed on the surface of human lymphocytes. Furthermore, pre-incubation of PBMC with purified pX ectodomain results in a significantly impaired T-cell proliferation and pro-inflammatory cytokines production upon different cell stimuli. The induction of tyrosine phosphorylation of multiple signaling proteins upon TCR stimulation is reduced as well.

We therefore concluded that pX might have immunosuppressive properties. Uncovering pX cellular receptor and its role during HCMV infection could allow new insights into the modulation of the immune response by HCMV and ultimately the development of new therapies.

Human Cytomegalovirus Infection Triggers the Interferon Response via the DNA sensor IFI16 in Human Keratinocytes

M. Biolatti[1], I. Lo Cigno[1,2], V. Dell'Oste[1], M. De Andrea[1,2], F. Gugliesi[1], D. Gatti[1], A. Giorgio[1], S. Landolfo[1], M. Gariglio[2]

Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy[1]; Department of Translational Medicine, University of Piemonte Orientale "A. Avogadro", Novara, Italy[2].

Human cytomegalovirus (HCMV), a member of the β-herpesvirus family, infects hosts for life despite a consistent multi-prolonged antiviral immune response that targets the infection. HCMV productively replicates in a broad range of cell types, including epithelial cells. Keratinocytes are the predominant*epithelial cells of* the epidermis, well equipped to recognize bacterial and viral pathogens. We investigated the capability of spontaneously immortalized human keratinocytes(*NIKS)* to support HCMV infection and replication. Growth kinetics and immunofluorescence analysis revealed that NIKS supported productive replication of HCMV clinical isolates (TR and VR1814), albeit with a retarded kinetics compared to that observed in cell lines used for HCMV production, such as Human Foreskin Fibroblasts (HFF). Inflammasome activation and Interferon production are key players in the innate immune response against HCMV infection. Whether such mechanisms are activated in keratinocytes by HCMV infection is poorly defined. In this study we demonstrate that Interferon type III (IFN-λ1), but not IFN type I production is stimulated during HCMV infection. Conversely, we did not observe the activated form of caspase-1, indicating that the inflammasome system is not significantly involved in keratinocytes immunity against HCMV. Moreover, IFI16 knockdown experiments revealed that IFI16 is responsible for inducing IFN-λ1 production in HCMV-infected keratinocytes. We are currently investigating the mechanisms IFI16 rely on to activate IFN-λ1 pathway and the transcription factors triggered by IFI6 to activate IFN-λ1 promoter in keratinocytes.

CMV antiviral treatment: current challenges and prospects

Dana Wolf

The era after nucleos/tides: Novel drugs against herpes viruses

H. Rübsamen-Schaeff, AiCuris GmbH&Co KG, Wuppertal, Germany

For decades, treatment ofherpesvirus infections such as human cytomegalovirus (HCMV) or herpes simplex virus 1 or 2 (HSV-1 and -2) has mainly relied on nucleoside analogues, which target the viral polymerases. However, while efficacious, aciclovir or valaciclovirdo not suppress HSV well enough to avoid outbreaks and sexual transmission, ganciclovir or valganciclovirhave significant tolerability issues and similarly do not achieve a 100% control of HCMV in transplant recipients.

All nucleoside analoguesare prodrugs which require activation and phosphorylation inside the cell by a viral enzyme. Hence, they are not protective to uninfected cells. In addition, for a full and tight antiviral control, a longer halflife would be required.One way to overcome the need for the first phosphorylation step is to generate nucleotides,which can be phosphorylated further by cellular enzymesas has been the case for cidofovir against HCMV. However, there are significant tolerability issues with this HCMV compound as well.

An approachto overcome the need for the phosphorylation step by a viral enzyme and at the same time to avoid side effects due to inhibition of human polymerases is to address a different target than the viral polymerase. We developed two compounds, which inhibit different viral targets and which also cover the EC_{90} in vivo over long periods of time due to long inherent halflives: Letermoviraddresses the terminase complex of HCMV and pritelivirinhibits the helicase-primaseof HSV.Both drugs are highly active in uninfected cells and have long halflives of 10 and 80 h, respectively. In phase II studies when compared with placebo, both compounds demonstrated ahighly efficient and dose-dependent suppression of their respective target virus. In addition, letermovir was very well tolerated in treatment as well as in prophylactic use as opposed to the polymerase inhibitors against HCMV. Data demonstrating the superiority of pritelivir over valaciclovirwill be discussed.

HCMV infection in specialized cells of the human placenta and suppression by monoclonal antibodies (mAbs) to glycoprotein B and proteins UL130/131A of the viral pentamer.

Lenore Pereira1, Takako Tabata1, Martin Zydek1, Matthew Petitt1, June Fang-Hoover1, and Lawrence Kauvar2. 1Department Cell and Tissue Biology, University of California San Francisco, 2Trellis Bioscience, San Francisco, CA, USA

The mechanisms of transplacental transmission of human cytomegalovirus (HCMV), theleading viral cause of congenital infection and birth defects, affecting 1-3% of births in the U.S., arelargely unknown. Transmission is high (30-40%) in primary maternal infection, and symptomaticbabies have permanent birth defects. Recurrent infection is infrequently transmitted (2%) andlargely asymptomatic. We recently reported underlying HCMV infection in cases of idiopathicintrauterine growth restriction (IUGR), analyzing biopsy specimens for viral proteins and pathology.Among 7 IUGR cases, we identified 2 primary and 3 recurrent infections. Virus replicated inglandular epithelium and lymphatic endothelium in the decidua, cytotrophoblasts and smoothmuscle cells in blood vessels of floating villi and the chorion. Large fibrinoids with avascular villi,edema and inflammation were significantly increased. Detection of viral proteins in the amnioticepithelium indicated transmission in IUGR with primary infection and asymptomatic recurrentinfections.

Detailed analysis of the chorion and amnion of placentas from symptomatic congenitalinfection revealed HCMV infected-cell proteins in trophoblast progenitor cells (TBPCs) andamniotic epithelial cells (AECs), which are self-renewing, pluripotent cells. TBPCs give rise to themature cell types of chorionic villi, invasive cytotrophoblasts (iCTBs) and multi-nucleatedsyncytiotrophoblasts (STBs). AECs are bathed in amniotic fluid, face the fetus and express anti-inflammatory properties that prevent pre-term labor and enhance tissue regeneration. We foundthat TBPCs are fully permissive for pathogenic and attenuated HCMV strains. A mutant of TB40/Ethat lacks UL131A, a component of the pentamer complex, replicated in TBPCs, indicating virionentry is pentamer independent. In addition, a human neutralizing mAb to glycoprotein B (gB)blocked infection, whereas a UL131A-specific mAb did not. Functional studies revealed thatneutralization of infection restored the capacity of TBPCs to differentiate and assemble intotrophospheres composed of iCTBs and STBs. In dramatic contrast, only pathogenic HCMV strainswere capable of infecting AECs. Accordingly, mAbs to UL130-131A had the most potent virusneutralizing activity, followed by mAbs to gB and hyperimmune globulin. The results suggest that acombination of anti-gB and anti-pentamer mAbs could suppress HCMV replication in specializedhuman progenitor cells of the placenta, reduce transmission and prevent congenital disease.

CMV vaccine research – coupling the innate and adaptive immunity arms

Stipan Jonjic

A highly immunogenic novel genetic vaccine against HCV in clinical trial

Antonella Folgori

Hepatitis C virus may be spontaneously cleared in a proportion of infected individuals. Many studies of the host genetics and immunology suggest a key role for T cells in protective immunity. Thus, the induction of robust T cell response with a prophylactic vaccine could provide effective immune control of acute HCV infection.

We previously showed that adenoviral vectored vaccines encoding non-structural (NS) HCV proteins induce potent T-cell response. Efficacy studies in chimpanzees demonstrate that this response can be protective against challenge. Our previous vaccine trial in humans with a human adenovirus (Ad6) and a novel simian adenovirus (ChAd3)encoding NS proteins,demonstrate that both vaccines are safe and immunogenic. However, although responses to priming are strong, boosting with heterologous adenoviral vectors doesn’tsignificantly increase responses. Thus, we evaluated a novel vaccine based on Modified Vaccinia Ankara (MVA) as a vector to boost functional HCV specific responses after ChAd3-NSmut prime.

Vaccination was very well tolerated with mild/moderate local and systemic reactions. AdCh3NS prime was highly immunogenic and multi-specific in all individuals; MVA-NS boostwas particularly potent. The induced T cells comprise both CD4+ and CD8+ subsets, secrete multiple cytokines, demonstrate cross-reactivity between HCV genotypes and have strong proliferative capacity. Vaccine induced T cells were also able to cross-recognize different strains of HCV. This vaccine regime is progressing to a phase II efficacy study in IVDU populations.

Therapeutic vaccines for hepatitis-related cancers

Luigi Buonaguro

Signal molecules of the innate immune system as genetic adjuvants in DNA immunizations against Influenza A viruses

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Introduction:

Seasonal trivalent inactivated Influenza vaccines (TIVs) induce only strain specific humoral responses against the highly variable Influenza surface proteins. Continuous protection against new evolving Influenza A (IAV) strains requires therefore a yearly administration of a recent antigen composition. Individuals which had overcome an IAV infection show a slight heterologous immunity most probably due to cellular responses against conserved epitopes. The sensing of IAV components by pattern recognition receptors (PRR) plays a key role in the initiation of these broad acting immune responses and thereby is pivotal in the induction of cell-mediated heterologous immunity.

Objectives:

DNA-based vaccines are promising alternatives to TIVs. The intracellular production of viral antigens induces both humoral and cellular responses. We hypothesized that genetic adjuvants expressing signal molecules which are involved in the PRR-activated pathways can mimic an IAV infection and thereby further enhance heterologous immunity.

Methods:

Balb/c mice were immunized once via intramuscular injection of 30 µg DNA followed by electroporation. The vaccine included same amounts of plasmids coding for Hemagglutinin and Nucleoprotein as well as one of the adjuvant candidates RIG-I, IPS1, IL-1β or IL-18.Humoral and cellular responses were measured at different time points. The protection was characterized by homologous and heterologous IAV infections five weeks after immunization.

Results:

Our studies showed that immunizations with the antigen coding plasmids alone resulted in robust humoral and cellular responses.These led to a complete protection against a lethal dose of homologous IAV and a modest heterologous immunity.

The co-administration of IL-18 increased the quality and quantity of antigen-specific cytotoxic T cells, whereasIL-1β had a significant influence on humoral responses mostly apparent as a shift of specific antibodies towards IgG1 consistent with an increased IL-4 secretion of isolated splenocytes. In challenge experiments with heterologous A(H1N1)pdm09 both adjuvants showed a trend to reduce the weight loss during infection.

Conclusion:

Thus, our data indicate that IL-1β and IL-18 are promising candidates as genetic adjuvants to enhance humoral and cellular immune responses after the immunization with DNA-based IAV vaccines.

West Nile virus candidate vaccines based on recombinant envelope proteins and DNA plasmids

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West Nile virus (WNV), an emerging mosquito-borne and zoonotic flavivirus, continues to spread worldwide and represents a major problem for human and veterinary medicine. In recent years severe outbreaks were observed in the USA and in Europe with neighboring countries, and the virus is considered to be endemic in an increasing number of areas. Although most infections remain asymptomatic, WNV can cause severe, sometimes fatal, neurological disease which affects mostly the elderly and immunocompromised individuals. Several vaccines have been licensed in the veterinary sector, but no human vaccine is available today. Human vaccination strategies against WNV should focus on safe and non-replicating technologies in order to efficiently target the population group at highest risk. Here, we describe a novel WNV candidate vaccine, which has been developed in the context of the EU-funded research consortium WINGS (West Nile Integrated Shield Project), and which is based on a bacterially-expressed WNV envelope (E) protein, in combination with the saponin-based adjuvant Matrix-M. Immunization experiments in mice and non-human primates resulted in strong vaccine-induced immune responses and complete protection after challenge with European WNV strains from the genetic lineages 1 and 2. In addition to a protein-only strategy, we also tested a heterologous prime-boost immunization by first priming the animals with a DNA plasmid encoding the WNV E protein and then boosting them with recombinant protein. The plasmid DNA was applied using a novel nanoparticle-based approach was well-tolerated and led to synergistic immunological effects in combination with the protein boost. These results have implications for the development of immunogenic, safe and cost-effective WNV vaccines for future human use.

IMPROVED MVA VACCINE IMMUNOGENICITY ACHIEVED BY USING ENDOGENOUS EARLY PROMOTERS

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Introduction: Modified *vaccinia* virus Ankara has been extensively used as a vaccine vector to elicit strong $CD8^+$ T cell responses, typically using an early promoter to drive transgene expression. Our previous published work showed that the early promoter of *F11L* or *B8R* ORF, utilised at their natural loci to drive the expression of a $CD8^+$ T cell-specific malaria epitope from *Plasmodium berghei* (pb9), elicited improved cellular immunogenicity in BALB/c mice, compared to that achieved by recombinant MVAs (rMVAs) with the conventional p7.5 or mH5 promoters. In the latter case, those conventional promoters were inserted at the thymidine kinase (TK) locus. Here, we extend our investigation to determine the mechanisms underlying this observed enhancement in immunogenicity, which could be a result of one or many possibilities. These possibilities include the effect of deleting the natural *F11L* or *B8R* ORFs, and the use of these ORFs as insertion sites instead of the conventional TK insertion locus. The presence of intact TK gene in rMVAs that have the endogenous promoters as opposed to the disrupted TK locus in rMVA with the conventional p7.5 or mH5 promoters could also impact on the improved immunogenicity. Finally, the strong activity of the F11L or B8R endogenous promoter was initially, in our previous publication, thought to be the driving mechanism behind the enhanced immunogenicity. We addressed these four possibilities by deriving a range of recombinant MVAs. **Our results** suggested that the improved immunogenicity achieved by utilising the F11L and B8R endogenous promoters in rMVAs is mainly due to the strong activity of those promoters. However, the inactivation of the TK gene in the rMVAs with the endogenous promoters slightly reduced the cellular immunogenicity. Moreover, our results also showed that the F11L or B8R promoter could drive the same level of immunogenicity when they were taken out of their natural context and inserted at the TK locus. This also ruled out the effect of *F11L* or *B8R* insertion sites on the improved immunogenicity. Finally, the deletion of the original *F11L* or *B8R* ORF did not appear to affect the immune responses. **In conclusion**, the F11L or B8R endogenous promoters seems strong promoters that could be used either at their authentic loci or at the TK locus and enhance superior immunogenicity compared to the use of conventional p7.5 or mH5 promoters.

VACCINATION AGAINST INFLUENZA H5N1 AND NEWCASTLE DISEASE VIRUSES USING THE ANTIVIRAL CINNAMON FRACTION

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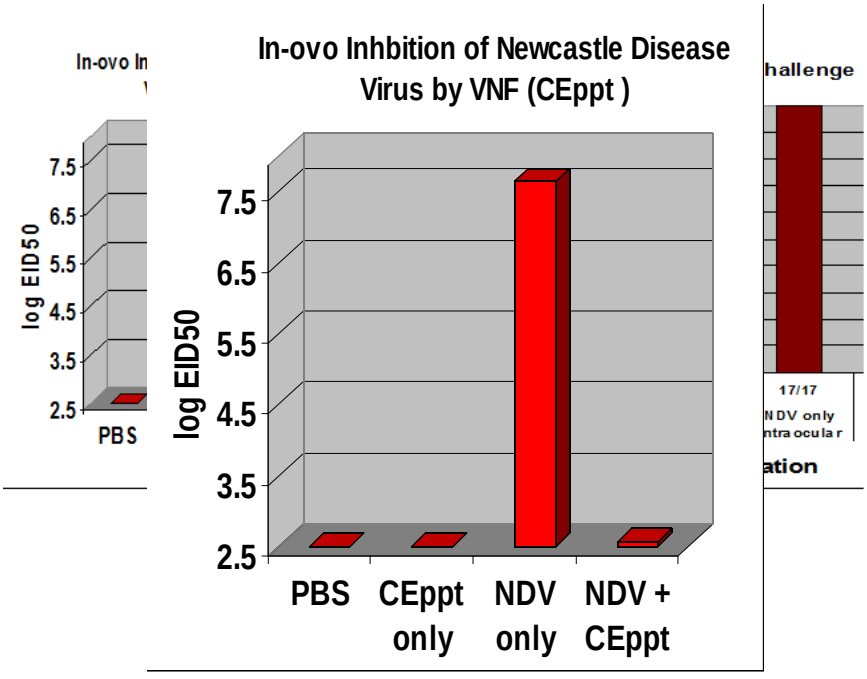
Newcastle disease virus (NDV) and Bird Flu are avian pathogens that cause contagious and fatal viral diseases affecting most species of birds. They are so virulent that many birds die before showing any clinical signs. H5N1 is also virulent in mammals.Previous studies have already demonstrated the antiviral activity of cinnamon extract against human influenza H1N1 (Barak and Ovadia, 2005), avian influenza H9N2 (Sevillia et al, 2007), Sendai virus (Gueta and Ovadia, 2005) and human herpes virus (Bernstein-Golan et al, 2008).The aims of the present research were: 1) to examine the ability of the Viral Neutralizing Fraction (VNF = CEppt), which was precipitated from the crudeCinnamon Extract, toinhibit the avian NDV and the Bird Flu. 2)to develop immunization against NDV by in-ovovaccination, as an alternative approach to post-hatching vaccination of chicks; and 3)to use cinnamon fractionCEppt for vaccination against the Bird FluH5N1 in mammalian animals (mice).

Injection of NDV preincubated with CEppt into 11-day old SPF chicken embryos resulted in a 5 logs decrease of the viral hemagglutinating activity and infectivity,and a significant increase in vitality – these embryos resisted 10^8 EID_{50} of the virus, whereas 10^2-10^3 EID_{50} of the virusalonekilled the embryos.

In-ovo vaccination by NDV preincubated with CEppt into 18-day old SPF chicken embryos induced high titers of antibodies during the following month, as high as with the tedious customary intraocular vaccination of 1-2-day old chicks. The challenge with the violent NDV (Vollogenic strain) was given 35 days past immunization. All 19 chickens, that were immunized in-ovo, resisted the violent virus while all 20 non-vaccinated chickens died (Figures below).

Similar results were obtained with the Bird Flu in mice. Mice were vaccinated twice by a mixture of CEppt and H5N1 virus intranasally at day 0 and day 14. Two weeks past second vaccination the mice were challenged with a lethal dose of the naïve virus alone. The mice were weighed every 2-3 days through the experiment. All vaccinated mice survived the challenge and continued to gain weight, whereas only 17% of the control non- vaccinated group survived.



HIV cure: latency and reservoirs

Guido Poli

Controlling virus replication through regulation of host restriction factors

Jose Este

Establishment of *Myotis myotis* cell lines for investigation of immune responses under lyssavirus infection

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Bats are natural reservoirs for many neurotropic viruses like lyssaviruses. In contrast to the lethal encephalitis in other animals caused by lyssavirus infection, clinical symptoms in bats are normally not seen. This indicates differences in the lyssavirus-host interactions and underlines the necessity to develop natural host related models to study these phenomena. Due to the strict protection of European bat species, immortalized cell lines are the only alternative to investigate the innate anti-lyssavirus immune mechanisms. Here, we report about the establishment of*Myotis myotis* derived cell lines from neural and immune relative tissues: brain (*Mm*Br), tonsil (*Mm*To), peritoneal cavity (*Mm*Pca), nasal epithelium(*Mm*Nep) and nervus olfactorius(*Mm*Nol) after immortalization by SV 40 large T antigen. The usefulness of these cell lines to study antiviral responses has been confirmed by analysis of their susceptibility to lyssavirus infection and the mRNA patterns of immune-relevant genes after poly I:C stimulation. Performed experiments indicated varying susceptibility to lyssavirus infection with *Mm*Br being considerably less susceptible than the other cell lines. Further investigation demonstrated a strong activation of interferon-mediated antiviral response in *Mm*Br contributing to its resistance. The pattern recognition receptors: RIG-I and MDA5 were highly up-regulated during rabies virus infection in *Mm*Br, suggesting their involvement in promotion of antiviral responses. The presence of CD14 and CD68 in *Mm*Br suggested *Mm*Br cells are microglia-like cells which play a key role in host defense against infections in the central nervous system (CNS). Thus the expression pattern of *Mm*Br combined with the observed limitation of lyssavirus replication underpins a protective mechanism of the CNS controlling the lyssavirus infection. Overall, the established cell lines are important tools to analyze antiviral innate immunity in *M. myotis* against neurotropic virus infections and represent a valuable tool for a broad spectrum of future investigations in cellular biology of *M. myotis*.

The unmet quest for antiviral vaccines / new vaccines in the pipe line

Sylvie Bertholet

Novel influenza Vaccines

Xavier Saelens