EUROPEAN SEMINARS in VIROLOGY

Abstract Book and Program

7th European Seminar in Virology (EuSeV)

"Vaccines and antibodies against viral infections"

June 14-16, 2019

Botanical Garden

University of Padova

EUROPEAN SOCIETY for VIROLOGY

7th European Seminar in Virology (EuSeV)

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Organizers:

Gabriella Campadelli-Fiume,

University of Bologna

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Hebrew University Jerusalem

Michael Kann

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MSD

SANOFI

Organizing Secretariat: Arianna Calistri, Ilaria Frasson, Michela Nandi, Marta Trevisan

7th. European Seminars in Virology (EuSeV) 2019 Program	
FRIDAY 14.06.2019	
13:45-14:00	**Welcome** **Dana Wolf, Gabriella Campadelli-Fiume, Thomas Mertens, Michael Kann Giorgio Palù**
Session on Ethical and technological issues about antiviral vaccine and antibodies development Chair: Giorgio Palù	
14:00-14:40	Andrea Grignolio, Cognitive bases for vaccine hesitancy Medical Humanities & Bioethics, Vita-Salute San Raffaele University, Milan, Italy grignolio.andrea@hsr.it
14:40-15:20	Rino Rappuoli, Reverse vaccinology 2.0 GSK, Siena, Italy rino.r.rappuoli@gsk.com
15:20-16:00	Melvin Kohn, MSD's Investigational Ebola Vaccine Regional Director of Medical Affairs Lead for Vaccines, MSD. melvin.kohn@msd.com
16:00-16:30	Discussion
16:30-17:30	**Selected oral presentations**
	Francesco Santoro, Human transcriptomic response to vaccination with recombinant VSV expressing Ebola virus Glycoprotein Laboratory of Molecular Microbiology and Biotechnology (LAMMB), Dept. of Medical Biotechnologies, University of Siena, Italy
	Matteo Castelli, HCV/E2 dynamics controls overall sensitivity to neutralization Laboratory of Microbiology and Virology, Università "Vita-Salute" San Raffaele, Milan, Italy
	Francesco Nicoli, Poor responsiveness of naïve CD8+ T-cells from elderly individuals is associated to their altered basal metabolism Sorbonne Sorbonne Université, INSERM, Centre d'Immunologie et des Maladies Infectieuses (CIMI-Paris), Paris, France - Department of Chemical and Pharmaceutical Sciences, University of Ferrara, Ferrara, Italy
	Dhanik Reshamwala, Novel anti-virals against Enteroviruses Department of Biological and Environmental Science, Nanoscience Center, University of Jyväskylä, Finland
	Alberto Reale, Oncolytic HSV-1 vectors for in situ vaccination against solid tumours Department of Molecular Medicine, University of Padua
17:30-18:00	**DISCUSSIONS IN FRONT OF POSTERS**
20:00 Dinner	

SATURDAY 15.06.2019	
Regulatory and technological issues about antiviral vaccine and antibodies development **Chair: Thomas Mertens & Gabriella Campadelli Fiume**	
09:00-09:40	Guido Silvestri, Towards an HIV vaccine: recent advances from studies in

	non-human primates Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, USA, gsilves@emory.edu
09:40-10:20	Christian Sinzger, CMV-Elite-neutralizing Antibodies Institute of Virology, University of Ulm, Ulm, Germany, christian.sinzger@uniklinik-ulm.de
10:20-11:00	Gabriella Campadelli Fiume, Oncolytic herpes simplex viruses elicit antigen agnostic vaccine-like anticancer protection Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Bologna, Italy, gabriella.campadelli@unibo.it
11:00-11:30	**Break**
11:30-12:10	Thomas Mertens, The art of vaccine recommendations in the real world Institute of Virology, Ulm University Medical Center, Ulm, Germany, thomas.mertens@uni-ulm.de
12:10-13:30	**Selected oral presentations**
	Matti Sallberg,DNA-based prime-boost active immunotherapy to induce a functional cure in patients with chronic hepatitis B Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden, Laboratory Medicine, Karolinska University Hospital Huddinge, Stockholm
	Greta Forlani, HTLV-1 Tax and HBZ oncoproteins expression and subcellular localization in infection and disease progression: implications for immune-based therapeutic approaches Laboratories of General Pathology and Immunology “Giovanna Tosi”, Department of Medicine and Surgery, University of Insubria, Varese, Italy
	Anna Czarnota, Characterization of anti-hepatitis C neutralizing antibody response after immunization with chimeric hepatitis B/hepatitis C virus like particles. Laboratory of Virus Molecular Biology, Intercollegiate Faculty of Biotechnology of the University of Gdańsk and Medical University of Gdańsk
	Nir Paran, Antibody Treatment of Smallpox Vaccine Adverse Events Department of Infectious diseases, Israel Institute for Biological Research (IIBR), Ness-Ziona, Israel
	Carlo De Giuli Morghen, A recombinant avipoxvirus expressing the env gene of Zika virus as a novel putative preventive vaccine Catholic University “Our Lady of Good Counsel” Tirana, Albania, Department of Medical Biotechnologies and Translational Medicine, Univ. of Milan, Italy
	Salomon Ferdinand, An Orf virus-based platform technology for vaccine development using a modulatory brick system University Tuebingen, Interfaculty Institute for Cell Biology, Department for Immunology, Tuebingen, Germany
13:30-15:00	**Lunch break**
Development of vaccines and antibodies against viral infection **Chair: Dana Wolf**	
15:00-15:40	Ali Mirazimi, Development of vaccine against Crimean Congo Hemorrhagic Fever; Past and Future Department of Laboratory Medicine, Karolinska University Hospital and KI, Stockholm, Sweden. Ali.Mirazimi@ki.se
15:40-16:20	Arnaud Marchant, Maternal and neonatal immunization Institute for Medical Immunology, Université Libre de Bruxelles, Brussels, Belgium, arnaud.marchant@ulb.ac.be
16:20-17:00	Frédéric Tangy, Vaccines against Ebolavirus and other emerging viruses

	Viral Genomics and Vaccination Unit, Institut Pasteur, Paris, France, ftangy@pasteur.fr
17:00-17:20	**Break**
17:20-18:00	Florian Klein, HIV-Super-neutralizing Antibodies Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Germany. florian.klein@uk-koeln.de
18:00-19:00	**Selected oral Presentations**
	Eberhard Hildt, Development of a new vaccine platform for induction of a robust B- and T-cell response against viral infections Paul-Ehrlich-Institut, Department of Virology, Langen, Germany; German Center for Infection Research (DZIF), Braunschweig
	Benjamin Gutjahr, Monoclonal antibodies protect mice in Rift Valley fever challenge model Friedrich-Loeffler-Institut, Institute of Novel and Emerging Infectious Diseases (INNT), Greifswald – Isle of Riems, Germany
	Ola Blixt, Efficient identification of immunodominant (glyco-)peptides for type-specific serology and monoclonal antibody developments Department of Chemistry, University of Copenhagen, Frederiksberg C, Denmark
	Sofia Appelberg, Protection Against Crimean-Congo Hemorrhagic Fever Virus Infection in Mice After Immunization with DNA Plasmids Coding for Different Viral Proteins Public Health Agency of Sweden, Stockholm
	Anna Lucia Tornesello, Humoral immune response to HCV peptides as cancer-progression biomarkers of HCV-infections Istituto Nazionale Tumori – IRCCS Fondazione Pascale, Naples, Italy
20:00 Dinner	

SUNDAY 16.06.2019	
Success and failure of antiviral vaccine development **Chair: Michael Kann**	
9:00-9:40	John J. Skehel, Experimental uses and roles in immunity of antibodies that recognize influenza haemagglutinin Francis Crick Institute, London, UK, John.Skehel@crick.ac.uk
09:40-10:20	Emanuele Montomoli, Present and future of anti-influenza vaccinations Department of Molecular and Developmental Medicine, University of Siena, Italy, Emanuele.montomoli@unisi
10:20-11:00	Wolfgang Hammerschmidt, Towards an EBV vaccine Research Unit Gene Vectors, Helmholtz Zentrum München, Germany, hammerschmidt@helmholtz-muenchen.de
11:00-11:40	Sergio Abrignani, Why is it too difficult to develop a vaccine against HCV Istituto Nazionale Genetica Molecolare “Romeo ed Enrica Invernizzi” University of Milan, Department of Clinical Sciences and Community Health, abrignani@ingm.org
11:40-12:20	Giorgio Palù, Clinically and experimentally adopted vaccines against Flavivirus infections and Dengue Department of Molecular Medicine, University of Padova, Italy, giorgio.palu@unipd.it
12:20 Closing Remarks and Brunch	

Abstracts and posters in alphabetical order of authors

Invited Speakers

Why is it so difficult to develop a vaccine against HCV

Sergio Abrignani
INGM, Istituto Nazionale Genetica Molecolare “Romeo ed Enrica Invernizzi”
University of Milan, Department of Clinical Sciences and Community Health

“Why is it so difficult to develop a vaccine against HCV”

With an estimated 2% of the world’s population chronically infected, hepatitis C virus (HCV) represents a health problem for which an efficient vaccination strategy would be highly desirable. Indeed, chronic hepatitis C is recognized as one of the major causes of cirrhosis, hepatocarcinoma and liver failure worldwide and it is the most common indication for liver transplantation, accounting for 40–50% of liver transplants. Much progress has been made in the prevention of HCV transmission and in therapeutic intervention. However, even if directly acting antivirals overcame the problems of relative low efficacy observed with interferon-a and ribavirin, an effective vaccine would be the only means to definitively eradicate infection and to diminish the burden of HCV-related diseases at affordable costs. Although there is evidence that the goal of a prophylactic vaccine could be achieved, there are huge development issues that have impeded reaching this goal and that still have to be addressed. I will address the question of whether an HCV vaccine is needed and whether it could eventually be feasible.

Oncolytic herpes simplex viruses elicit antigen agnostic vaccine-like anticancer protection

Gabriella Campadelli-Fiume, Tatiana Gianni, Andrea Vannini, Valerio Leoni
Department of Experimental Diagnostic and Specialty Medicine, University of Bologna, Italy

Therapeutic cancer vaccines have been a dream in the drawer since ever. They are now at the forefront of novel, targeted approaches to immunotherapy-based cancer treatment. The highly innovative checkpoint inhibitors (CPIs) break the CTL-inhibitory axis mediated mainly by CTL-A4 and its ligands and PD-1 and its ligands PD-L1 and PD-L2. The “pros” are the potential for circumventing drug cross-resistance, and the potential for persistence of the antitumor effect due to immunologic memory. In reality, the “cons” heavily limit the range of efficacy given that only some tumor genotypes can be treated, only a fraction of patients respond, resistance and adverse effects develop.

Oncolytic viruses (OVs) are helping to achieve these goals. They were initially considered to exert their action by inducing death of the cancer cells. Research of the past few years has highlighted that the immunogenic cell death they cause modifies the tumor microenvironment (TME) and derepress the TME immunosuppression. Consequently, they greatly enhance the efficacy of checkpoint inhibitors. All in all, OVs, including oHSV, appear as ideal partners for combination therapy with CPI.

Our laboratory has preclinically developed onco-immunotherapeutic viruses based on herpes simplex virus (o-HSV). These o-HSVs gain their cancer specificity from tropism retargeting to cancer specific receptors of choice, exemplified by HER2. In contrast to the vast majority of o-HSVs approved or under clinical experimentation, the HER2-retargeted oHSV are fully virulent viruses in their target cancer cells.They elicit an innate response to the virus, which may act to limit virus replication, but initiates the innate response to the tumor, which then evolves into a long term durable memory response to the tumor. This immunotherapeutic effect exerts anticancer effects and primes for checkpoint inhibition. When combined with checkpoint inihibitors, the HER2-retargeted o-HSVs induce a protection of approximately 100%.

Cognitive bases for vaccine hesitancy

Andrea Grignolio
Medical Humanities & Bioethics, Vita-Salute San Raffaele University, Milan, Italy

The scientific literature offers unequivocal data that demonstrate how vaccinations today are safe and effective and in the past were the medical intervention that, together with the sanitation of the water, saved more lives than any other medical intervention. Even if they improved life expectancy in advanced countries where herd immunity has been reached, today it is in the very same countries that social resistance to vaccination is putting children at risk of harm. A recent neurocognitive approach is offering an interesting frame to understand the causes and diffusion of anti-vaccine movements. Although novaxxers are heterogeneous social groups, they show some common traits. They are generally educated and affluent people, tending to later parenthood, sensitive to conspiracy theories, unwilling to confront with different ideas, favorable to alternative medicines, and scarce in assessing risk information. Some solutions will be discussed, including the theory of “nudge” and that of “bounded rationality” which have shown some efficacy by offering citizens the right tools to orient themselves in the architecture of health choices.

Towards an EBV vaccine

Wolfgang Hammerschmidt
Research Unit Gene Vectors, Helmholtz Zentrum München, Germany

Epstein-Barr Virus (EBV) is a global threat to human health. There is currently no registered preventative or therapeutic vaccine to EBV infection. EBV infections may manifest acutely as infectious mononucleosis (IM, kissing disease) or as post-transplant lymphoproliferative disorders (PTLD) in immunocompromised patients. EBV is a risk to those immunosuppressed patients who are seronegative and receive a solid organ transplant. Latent EBV infection is also associated with various tumor types such as Hodgkin disease, gastric cancer, nasopharyngeal carcinoma, or Burkitt's lymphoma among others. Our technology enables the production of EBV virus-like particles (EBV-VLP) that resemble native EBV virions in their immunogenicity but are depleted of packaging signals for the virus genome. The thus produced VLPs lack virus DNA and are incapable of propagating the infection. We further designed the vaccine virus genome to be depleted of viral oncogenes and additional factors that may lead to immune evasion. Our technology is about to be transferred to a commercial manufacturer for GMP-compliant process development. The project is supported by public funds from the German Center for Infection Research (DZIF).

MSD's Investigational Ebola Vaccine

Melvin Kohn, MD MPH
Regional Director of Medical Affairs Lead for Vaccines, MSD.

This talk will cover the basic epidemiology, transmission and clinical presentation of Ebola virus disease, and also describe the development of MSD's Investigational Ebola Vaccine, known as V920.

Maternal and neonatal immunization

Arnaud Marchant
Institute for Medical Immnology, Université libre de Bruxelles, Belgium

The immune system of the fetus and of the newborn is programmed to adapt to evolving environmental constraints, from tolerance to maternal tissues and commensal microorganisms to protection against infectious pathogens. This functional plasticity is probably dependent on an efficient balance between regulatory and effector mechanisms. B and T cell responses can already be induced during fetal life by infectious pathogens and at birth by some vaccine formulations. Understanding the rules underlying the induction of effector immune responses in the newborn is helping the development of vaccines protecting against pathogens during the first weeks of life. In the newborn, immunity to pathogens is dependent on maternal antibodies and can be promoted by vaccination during pregnancy. The success of maternal immunization against tetanus toxoid, pertussis and influenza is stimulating the development of vaccines against new targets, including group B streptococcus and RSV. However, increasing maternal antibody transfer to the newborn may reduce the efficacy of infant immunization by interfering with infant B cell responses. Optimization of integrated maternal and infant immunization programs requires a better understanding of the impact of pregnancy on antibody responses, of maternal antibody transfer and of their impact on infant immunity and vaccine responses.

The art of vaccine recommendations in the real world

Thomas Mertens
Institute of Virology, Ulm University Medical Center, Ulm, Germany

The problem of well-known and emerging infectious diseases can only be definitely solved if an efficient vaccine is made available. This remains true in the era of antimicrobial and antiviral therapy which reduce the mortality rate and mortality, but do not provide major effects on the epidemiology of pathogens. So, vaccination and vaccines are (one of) the most important achievement(s) of medical research for the health of individuals and human populations. The story of the success of vaccination is long and impressive. But the enormous success of vaccination has turned into one of its major handicaps in Europe, since diseases are no longer present and the "perception of risk" and the expectations of vaccine safety have changed much.

The strategic aims of vaccination are protection of the vaccine recipient, community-immunity or even regional elimination or eradication, depending on the pathogen. Public interest can be defined based on all mentioned aims. After development of a new vaccine, the efficacy and safety must be thoroughly proven, the vaccine must be approved, and vaccination recommendation must be developed based on the best available evidence and considering the disease burden, the implementation in a vaccination program, and the costs.

In Germany by law the standing committee on vaccination recommendations (NITAG), an independent scientific committee, (STIKO) according to law must develop these recommendations and monitor the effects after implementation. But the tasks of the German NITAG are multiple.

- Achieve confidence in its work
  - Transparency concerning processes and acting people (independency)
  - Recommendations based on the best available evidence for the individuum and the community
- Information of the professional public (and the public)
  - Demand adapted professional communication (comprehensive but correct!)
  - Open communication (problems und risk perception).
    Respond to "fake news" and "fake experts" and invalidate „vaccination myths" by arguments
- Effective monitoring of recommendations (instruments? vaccination registry?)
- International cooperation

Nevertheless, problems remain, which may be subdivided in such that can principally be solved: e.g. to achieve higher vaccination rates, to faster obtain recommendations with equal quality, to enforce mathematical modelling, and re-evaluation of "historical" recommendations (not always necessary, but difficult)

As example for a non solvable problem: we only have mediocre vaccines and a mutating virus, but with enormous clinical and epidemiological impact (=influenza). Finally, the political discussion on mandatory vaccination must be led and instruments for evaluation must be developed and implemented.

Development of vaccine against Crimean Congo Hemorrhagic Fever; Past and Future

Ali Mirazimi,
Karolinska Institute and National veterinary institute

Crimean-Congo hemorrhagic fever virus (CCHFV) is a tick-borne virus found in Africa, Asia, Eastern and Southern Europe. It is the cause of severe disease with 5-30% fatality rate. CCHFV is a neglected emerging pathogen, which causes widespread and fatal epidemics in humans.

The CCHFV constitutes a public health risk in endemic countries as well as neighboring areas. To date, WHO put CCHFV in their blue print and highlight the importance on developing vaccines and treatment for this virus, as there is no good treatment or vaccine against this diseases available. It should however be highlighted that there is a licensed vaccine against CCHFV available in Bulgaria, which is based on CCHFV cultivated in suckling mouse brain and subsequently inactivated and absorbed on Al(OH)3. It should also be noticed that the use of mouse brain derived vaccine unlikely gain widespread international regulatory approval due to several reasons such as possibility to autoimmune responses induced by myelin basic protein and etc. The most obvious problem for development of new vaccine candidates against CCHFV, has been the lack of a good animal models. Nevertheless, to date there are two mice model available which make it possible to assess the protection studies. These models during last years has been used to develop new vaccine candidate for CCHFV. In this lecture, we will review the available vaccine and all the vaccine candidate under development.

Present and future of anti-influenza vaccinations

Emanuele Montomoli
Department of Molecular and Developmental Medicine, University of Siena, Italy

The World Health Organization (WHO) estimates that influenza annually is the cause of one billion infections, of which 3 to 5 million are severe diseases, with between 300.000 and 500.000 deaths worldwide. Among paediatric populations, influenza causes more than 800.000 hospitalizations in children of 5 years old and younger and with more than 300.000 hospitalizations in children of ages <1 year old. Vaccination is the primary strategy for the prevention and control of influenza. Indeed, annual influenza vaccination is currently recommended for groups at high risk of complications from influenza infection such as pregnant women, elderly people, young children, people with chronic diseases, and occupational groups.

The most widely used vaccines are either killed/inactivated or live attenuated influenza vaccines (LAIVs). Current vaccine formulations target three or four seasonal influenza virus strains (H1N1 and H3N2) and one or two (since 2013) influenza B virus strains. These vaccines rely on the induction of neutralizing antibodies targeting the globular head of viral hemagglutinin (HA) and neuraminidase (NA) antigens.

Despite advances in the field, most vaccine formulations continue to be produced by rather antiquated techniques that have been in use for over 60 years and that involve the growth and passaging of the vaccine strains in embryonated chicken eggs. Production in eggs is a relatively slow process and production yields are both unpredictable and highly variable from strain to strain. Although the production of egg-derived vaccines will continue, new technological developments have generated a cell-culture-based influenza vaccine and other more recent platforms, such as synthetic influenza vaccines. The low influenza vaccine effectiveness (VE) observed during the A(H3N2)-dominated 2017–2018 season may be due to vaccine virus adaptation to growth in eggs.

The LAIVs, which are administered intranasally, can stimulate humoral response, inducing both secretory IgA (S-IgA) and serum IgG, as well as cell-mediated immune response, similarly to natural influenza infection. However, they do have some limitations; they are currently not approved or use in high-risk groups, due to the high incidence of allergic reactions as well as the risk of transmission following vaccination.

One approach to improve influenza vaccines is to include adjuvants; substances that boost the immune response. Adjuvants are particularly beneficial for influenza vaccines administered during a pandemic when a rapid response is required or for use in patients with impaired immune responses, such as infants and the elderly. To date, six adjuvants have been used in licensed human vaccines: Alum, MF59, AS03, AF03, virosomes and heat labile enterotoxin (LT).

Approaches to improving immune response, including live, cold-adapted influenza vaccines, new adjuvants, and virosomal vaccines, have met with mixed success. An alternative recent approach could be, the High Dose vaccine contains 4 times the dose of HA (60 mg), which induced significantly higher levels of serum antibody than standard dose influenza vaccine.

The commercialization of the trivalent recombinant hemagglutinin (HA) vaccine (Flublok, Protein Sciences, Meriden, CT) introduced a novel influenza vaccine produced by modern recombinant technology that yields a highly purified protein vaccine and more importantly assures that the antigenic components of the vaccine are exact genetic matches to the HAs of the wild-type strains selected each year for the seasonal vaccine. By avoiding adaptation of infectious virus to growth in eggs, the recombinant technology assures no mutations in the HA gene that can change the antigenic properties of the HA and reduce the protective efficacy of the vaccine.

Although debate and controversies are waging on influenza vaccine efficacy and effectiveness, current influenza vaccines provide beneficial protection against seasonal

influenza virus infection and disease, especially for the elderly . The immune response, and consequently the degree of protection, to influenza vaccine is influenced by several host factors, such as age, genetic differences in immune responsiveness, history of infection and previous vaccination against influenza, gender, medical history, and health status.
Given the relatively limited protection induced by current seasonal influenza vaccines, a more universal influenza vaccine that would protect against more influenza viruses is among the largest unmet medical needs of the 21st century.

Clinically and experimentally adopted vaccines against flavivirus infections …..and dengue

Giorgio Palù
Department of Molecular Medicine, University of Padova, Italy

Different vaccine platforms have been developed and proved efficacious in preclinical studies against infections caused by flaviviruses responsible for large outbreaks worldwide, namely JEV, ZIKV and WNV. Vaccine technologies include PIV particles, VLPs, purified viral subunits, live-attenuated vaccines, chimeric vaccines and viral and non-viral vectors encoding flavivirus structural proteins. Besides immunogenicity and efficacy these vaccines should have a very good safety profile taking into account that children, pregnant women, frail and immunocompromised individuals are relevant target populations. The above vaccine strategies should also minimize the induction of cross-reactive antibodies able to enhance heterotypic flavivirus infection and pathogenicity while maintaining immunogenicity and long-term protective efficacy against the vaccine strain. In this regard, live-attenuated and replication-competent vaccines are expected to provide long-term immunity and to be highly effective with the only constraint that they should not be adopted in immunocompromised individuals and in pregnant women.

A different scenario depicts the case of dengue vaccine. In fact, due to the high burden of disease caused by DENV (about 200 million cases/year, 500,000 DHF and 20,000 deaths/year) and the huge number of individuals exposed (about 2 billion people), design, production and clinical adoption of dengue vaccines are more advanced than in the case of the aforementioned flaviviruses. Two live, genetically modified DENV vaccines have reached phase III, namely TV003/TV005 from US NIH Butantan and DENVax from Takeda. One chimeric YFV/DENV vaccine, namely CYD-TDV Dengvaxia from Sanofi Pasteur has been already registered and extensively employed on tens of thousands of people living in endemic regions (Philippines, Brazil). The efficacy, safety, harmful reactions of Dengvaxia will be described in the context of the serological status of the vaccinees and in light of the mechanistic disease-enhancing effect of heterotypic antibodies. Present recommendations of WHO SAGE group about serological pre-vaccination screening of the susceptible population will be commented. An appraisal of the characteristics of second generation dengue vaccines will be also provided.

Reverse vaccinology 2.0: Human immunology instructs vaccine antigen design

Rino Rappuoli
GSK Vaccines, Siena, Italy

Traditionally, vaccines have been developed by cultivating infectious agents and isolating the inactivated whole pathogen or some of its purified components. 20 years ago, reverse vaccinology enabled vaccine discovery and design based on information deriving from the sequence of microbial genomes rather than via the growth of pathogens. Today, the high throughput discovery of protective human antibodies, sequencing of the B cell repertoire, and the increasing structural characterization of protective antigens and epitopes provide the molecular and mechanistic understanding to drive the discovery of novel vaccines that were previously impossible. We are entering a “reverse vaccinology 2.0” era.

Towards an HIV vaccine: recent advances from studies in non-human primates

Guido Silvestri

Emory University School of Medicine, Emory Vaccine Center, and Yerkes National Primate Research Center; Atlanta, GA, USA.

With more than 1 million new HIV infections per year it is hard to over-estimate the importance of developing a safe and affective vaccine for this deadly virus. The isolation of HIV-1 broadly neutralizing antibodies (bnAbs) from HIV-infected individuals and the finding that passive transfer of bnAbs can protect non-human primates (NHPs) from infection with chimeric simian/human immunodeficiency viruses support the feasibility of an antibody-based HIV vaccine. However, elicitation of broadly neutralizing antibodies (nAbs) against clinically relevant HIV strains (i.e., hard to neutralize tier 2 and tier 3 virus Envelopes, Envs) by immunization has been very difficult difficult. Much of that challenge centers on specific biological features of HIV Env, such as the enormous variability among strains, the extensive level of glycosylation, a remarkable degree of structural flexibility, the "hiding" of neutralization-sensitive epitopes, and a high intrinsic entropy. While not all the immunological implications of these complex biological features are currently known, it is clear that germinal center (GC) B cells and follicular helper T cells (Tfh) play a key role in the host ability (or lack thereof) to generate HIV-Env-specific broadly nAbs. In general, GCs are essential for HIV nAb development, which requires Ab somatic hypermutation (SHM). GCs are sites where B cells compete for antigen availability and undergo repeated rounds of SHM of their immuno-globulins and selection by Tfh to evolve into cells producing high affinity Abs. Of note Tfh help signals to GC B cells and results in proliferation and further SHM. Importantly, the quality of Tfh provided "help" is associated with HIV nAb development in trimer immunized rhesus macaques (RM). In a series of recent studies (Cirelli et al., Cell 2019), we found that slow delivery immunization of RMs resulted in more robust Tfh responses and GC B cells with improved Env-binding ability, as monitored using longitudinal fine needle aspirates. These improved immunological features correlated with the development of > 20-fold higher titers of autologous nAbs. In addition, using a new RM genomic immunoglobulin loci reference, we identified a series of differential Ig variable (V) gene usage associated with slow-delivery immunization. This Ab mapping demonstrated targeting of immuno-dominant non-neutralizing epitopes by conventional bolus immunized animals, while slow delivery immunized animals targeted a more diverse set of epitopes with a relative higher targeting of neutralization-associated HIV-Env epitopes. These advances hold promise to develop a next generation of immunization strategies that may result in more effective candidate HIV vaccines.

HCMV Elite Neutralizing Antibodies

Jessica Julia Falk1, Martina Winkelmann2, Dagmar Stöhr1, Mira Alt4, Hubert Schrezenmeier2,3 Adalbert Krawczyk4, Ramin Lotfi2,3 and **Christian Sinzger**1

1 Institute for Virology, University Hospital Ulm, Germany

2 Institute for Clinical Transfusion Medicine and Immunogenetics Ulm, German Red Cross Blood Transfusion Service Baden-Württemberg – Hessen and University Hospital Ulm Germany

3 Institute for Transfusion Medicine, University of Ulm, Germany

4 Institute for Virology, University Hospital Essen, Germany

The human cytomegalovirus (HCMV) can cause serious medical problems during pregnancy and after transplantation. Antiviral chemotherapeutics are available but their use is limited due to adverse effects and the development of resistance. In principle, the application of antiviral antibodies is a promising alternative, but the respective trials were only partially successful up to now. Possible reasons for this relative failure are: (i) application at insufficient frequency or dose, (ii) low neutralization capacity of donor antibodies, particularly against the infection of fibroblasts, and (iii) almost complete lack of efficacy against cell-to-cell spread of the virus. In order to increase the neutralization capacity of immunoglobulin preparations, we have recently identified 58 “elite neutralizers” out of 9,000 HCMV seropositive blood donors. These „elite neutralizers“ combine an exceptionally high neutralization capacity with a broad efficacy against different HCMV strains in various cell types, and are superior to currently available hyperimmunoglobulins. None of the native plasma samples could inhibit cell-associated spread of HCMV, which underscores the immune-evasive potential of cell-associated spread but does not exclude that cell-to-cell-spread-inhibiting specificities might be contained at low concentration in the complex mixture of various antibodies. When analyzed concerning their effect on individual virus particles during entry, most elite neutralizers preferentially inhibited viral penetration whereas two exceptional plasmas already prevented adsorption of virus to cells. In conclusion, plasma of elite donors can be considered to improve the antibody-based treatment of HCMV infections. It is tempting to speculate that monoclonal antibodies derived from such donors are superior to currently available antibodies.

Experimental uses and roles in immunity of antibodies that recognize influenza haemagglutinin

John J. Skehel
Francis Crick Institute, London, UK

Mechanisms of antibody-mediated neutralisation of Influenza viruses. The two functions of influenza virus haemagglutinins, receptor binding and membrane fusion, are targets for antibodies that block infectivity. The mechanisms that they utilise for their activities, characterised biochemically and structurally, will be described and evaluated in relation to their efficiency in protection from influenza.

Replicating measles vaccine vector to protect from new emerging diseases

Frédéric Tangy
Viral Genomics and Vaccination Unit, Institut Pasteur, Paris, France

During the last decades a number of new viral diseases such as SARS, MERS or Nipah virus infections have emerged at the animal-human interface, and infections such as Ebola, Lassa, or recently Zika virus, have expanded beyond their usual limited territories. To develop effective interventions in a timely manner, we adapted the use of live attenuated measles vaccine, one of the safest and most efficacious vaccines available, as a vaccination platform for the delivery of these new antigens. Measles vaccination has been used for more than 40 years in over 1 billion children and is 95% efficacious after one or two administration. Measles vaccine is genetically stable and reversion to pathogenicity has never been observed. Taking advantage of these characteristics, we developed the attenuated measles Schwarz vaccine virus into a versatile chimeric or recombinant vector. Proof of concept in humans for this technology has been demonstrated for a measles-Chikungunya vaccine. Phase I and phase II clinical trials showed that the vaccine was well tolerated and induced a robust and functional antibody response after 1 or 2 immunizations (100% seroconversion). These trials also demonstrated that pre-existing measles antibodies did not impair the immunogenicity of the heterologous antigen (Ramsauer 2015, Lancet Inf. Dis., Reisinger 2018, Lancet). Thus, pre-immunity to measles due to vaccination or infection does not restrict the use of recombinant measles vector. Many other antigens from HIV, DENV, WNV, SARS, H5N1, Ebola, Zika, and Plasmodium have been expressed in this vector and their strong immunogenicity or protective capacity has been demonstrated in preclinical animal models, also in the presence of pre-existing measles immunity, highlighting the potential of measles vector as a platform for rapid response to new pathogens. These results will be presented and the favorable characteristics of this new vaccine platform will be discussed and compared to other strategies in the context of an Ebola vaccine.

Selected oral presentations

Development of a new vaccine platform for induction of a robust B- and T-cell response against viral infections

S. Akhras[1], T. Zahn[1], and **E. Hildt**[1,2]

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Vaccine platforms that can be flexibly loaded with antigens can contribute to decrease response time to emerging infections. For many pathogens and chronic infections, induction of a robust B-cell response and of a cytotoxic T lymphocytes (CTL) response is desirable. Antigen delivery into cytoplasm of antigen presenting cells favors induction of a CTL-response. By fusion of the cell-permeable translocation motif (TLM)-peptide to the capsid-forming core protein of hepatitis B virus, and by insertion of the strep-tag as adaptor in the spike tip (a domain that protrudes from the surface of the capsid), cell-permeable carrier capsids were generated that can be flexibly loaded with various antigens fused to (monomeric)-streptavidin. Loading with antigens was demonstrated by electron microscopy, density gradient centrifugation and surface plasmon resonance spectroscopy. Confocal immunofluorescence microscopy showed that cell-permeable carrier capsids mediate transfer of cargo antigens into cytoplasm. Ovalbumin and the PreS1PreS2-domain of HBV surface protein were used as cargo antigens. Activation of antigen presenting cells and ovalbumin- or PreS1PreS2- specific $CD8^+$ T-cells, which correlates with enhanced specific killing activity, was found. For PreS1PreS2-loaded carrier capsids immunization of mice induced antibodies able to neutralize HBV.

The membrane permeability of the antigen carrier allows needle free application routes. Ex *vivo* skin resorption models showed migration of TLM-capsids through the skin. *In vivo* after transdermal vaccination an efficient B-cell response inducing neutralizing antibodies was found. Co-culture of lymphocytes of transdermally vaccinated mice with HBV-expressing hepatocytes led to specific killing, high expression of IFN-γ and specific activation reflected by high CD107a presentation.

This demonstrates the capacity of TLM-carrier-capsids to serve as universal antigen carrier to deliver antigens into cytoplasm of APCs, which leads to enhanced MHC class I-mediated presentation and induction of antigen-specific T- and B-cell responses. In addition, this cell-permeable antigen carrier enables non-invasive immunization routes like transdermal or mucosal vaccination.

Protection Against Crimean-Congo Hemorrhagic Fever Virus Infection in Mice After Immunization with DNA Plasmids Coding for Different Viral Proteins.

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Crimean-Congo Hemorrhagic Fever virus (CCHFV) is a RNA virus, which cause severe hemorrhagic fever in humans with a fatality rate as high as 40%. CCHF is the most widespread tick-borne disease in the human population and a serious threat to the global public health due to the severity of the disease and lack of effective treatment or vaccine. Here we used an interferon alpha receptor knockout (IFN-/-) mouse model, which replicate human disease, to investigate protection and antibody response after immunization with plasmids encoding either CCHFV polyprotein M, glycoproteins Gc and Gn or the nucleocapsid protein N. Thirty six female IFN-/- mice were divided into six groups and immunized with different combinations of the plasmids at three separate time points. The control group received a control plasmid. After the last immunization the mice were challenged with CCHFV Hoti and followed for as long as sixteen days post-infection. Increased antibody levels after each immunization were observed in three out of the six groups. All animals, except three, in these groups survived until the end point. Our data indicate that sufficient protection against CCHFV infection can be induced by immunization with DNA coding for CCHFV proteins. Further investigation of samples collected before and after the viral challenge will be completed to examine neutralizing ability of antibodies produced and cellular immune responses to identify necessary immune factors for protection against CCHFV infection.

Efficient identification of immunodominant (glyco-)peptides for type-specific serology and monoclonal antibody developments

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There is a pressing need to identify immunogenic epitopes towards viral envelope glycoproteins for both diagnostic and therapeutic developments. We recently established an efficient peptide antigen synthesis and microarray display screening approach for identification of immunodominant epitopes in patient sera. We also demonstrated that O-glycosylation plays important roles in defining immunogenic epitopes in cancer [1] and viral infections [2, 3]. This platform allows us to generate unique peptidic immunoprofiles for differential diagnostic serology also useful information to for production of monoclonal antibodies. Conventional methods for the production of antibodies, like the hybridoma technique, are elaborative and expensive. The phage display techniques offer rapid, cost-effective and efficient tools for the prokaryotic production of high-affinity mAbs in the scFv or Fab forms, however, the biopanning and scFv screening strategies are somewhat tedious and time consuming. Our microarray technology platform with spot-on-spot printing is suitable for high-throughput and high-content screening of scFv clones obtained from native and recombinant vaccinated mouse libraries [4]. This allows for the generation of a significant number of scFv candidates targeting peptide and glycopeptide epitopes for further assay development.

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HCV/E2 dynamics controls overall sensitivity to neutralization

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Hepatitis C virus glycoprotein E2 variability is a major factor of evasion from the host humoral immune response. E2 displays three hypervariable regions, with the N-terminal hypervariable region 1 (HVR1) acting as an immune decoy and directly shielding the CD81 binding site on E2. Remarkably, a broader protective role of HVR1 involving several non-overlapping neutralizing epitopes was recently identified.

Given HVR1 sequence variability, we tested *in vitro* whether its protective role varies among different HCV strains in a sequence-specific fashion and modeled its structure to establish a structure-function relationship that recapitulates HVR1 protective role. In details, we first grafted HVR1 sequences of several isolates onto the H77 E1E2 sequence and evaluated each HCVcc system sensitivity to monoclonal antibodies neutralization. Several single-point mutations in E2 ectodomain were tested as well. Subsequently, we modeled *in silico* the structures of the region spanning HVR1-AS412 and of E2 ectodomains from the tested mutants and studied their properties in molecular dynamics (MD) simulations.

The results herein presented suggest that several polymorphisms in E2 HVR1, AS412 and front layer regulate HCV overall sensitivity to neutralization. Regulation is sequence-specific and stems from a tight dynamic interplay between these three E2 regions. Indeed, while they are highly plastic, MD simulations highlighted favored conformations shared by resistant or sensitive chimeras. Intriguingly, the same features were identified applying the described computational analysis to the recently published E2 sequences representative of HCV evolution in a patient who spontaneously resolved the infection.

The presented results remark how HVR1-mediated global HCV resistance to neutralization can drastically vary in response to few mutations in different E2 regions, stressing the extremely elevated potential of HCV to evade the host immune response. On the other hand, these results might aid the identification of the ideal E1E2 constructs to be used in HCV vaccinology.

Characterization of anti-hepatitis C neutralizing antibody response after immunization with chimeric hepatitis B/hepatitis C virus like particles.

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Background

Hepatitis C virus (HCV) infection is a major health problem worldwide, affecting an estimated 70 million people worldwide. An HCV vaccine, however, still remains an elusive goal. In contrast, hepatitis B virus (HBV) infection can be prevented with commercially available recombinant vaccine based on small surface antigen of hepatitis B (sHBsAg), which has the ability to form highly immunogenic virus-like particles (VLPs) and represents an attractive antigen carrier for the delivery of foreign sequences.

In our studies, we examined the immunogenic properties of a bivalent HBV/HCV vaccine candidates based on the novel chimeric particles in which highly conserved epitopes of HCV E2 glycoprotein were inserted into the hydrophilic loop of sHBsAg.

Results

The expression of chimeric particles was performed in the *Leishmania tarentolae* expression system, which has the potential to produce high-yields of proteins with the mammalian-like N-glycosylation pattern. Chimeric proteins were next purified using ultracentrifugation and the particles assembly was confirmed using direct transmission electron microscopy. After immunization of mice we confirmed that the mouse sera were able to recognize not only the synthetic peptides covering HCV E2 epitopes, but also yeast-derived sHBsAg. Moreover, we assessed the cross-reactivity and neutralizing antibody response against different HCV genotypes.

Conclusions

In our study we proved that sHBsAg-based VLPs are able to successfully present several HCV-derived regions and to elicit strong and specific anti-HCV antibody response. Although more evaluation of those constructs is still needed, this approach may prove useful in the development of a rationally designed prophylactic vaccine against HCV.

A recombinant avipoxvirus expressing the *env* gene of Zika virus as a novel putative preventive vaccine

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Introduction and Purpose

Zika virus (ZIKV) was first isolated from rhesus macaques in 1947 in Uganda. It belongs to the Flavivirus genus and contains a single-stranded, positive-sense RNA genome of around 11 kb. Although the virus may also be sexually and vertically transmitted, bites by *Aedes aegypti* and *Aedes albopictus* mosquitos represent the main route of infection. ZIKV infections are mainly asymptomatic but, in spite of the generally mild self-limiting symptoms, neurological complications may occur, such as microcephaly in the developing fetus and Guillain-Barré syndrome in adults, as the virus can infect human neural progenitor cells. Since it was introduced in Brazil in 2015, ZIKV has been declared a public health emergency by the World Health Organization (WHO), as it may represent an international threat. Therefore, also considering its easy transmission from asymptomatic patients and the absence of any antiviral therapy, we designed and tested a recombinant immunogen based on the genetic background of the fowlpox virus as a safe and effective vaccine.

Material and methods

Here, we report on the construction of a novel fowlpox-based recombinant (FP*zenv*) that contains part of the capsid (c), the pre-membrane (membrane precursor) (Pr), the membrane (M) and the envelope (E) genes of ZIKV (cPrM-Env), and evaluated its safety and immunogenicity in a mouse model challenged with an epidemic ZIKV$_{MR766}$ strain. For the assessment of transgene expression, the recombinant has been used to infect different cell lines (CEF, MRC-5 and Vero). Protein expression was verified by Western blotting and immunofluorescence.

Results an conclusions

1. the *env* gene of ZIKV is expressed by the FP*zenv* recombinant in replication-restrictive mammalian Vero and MRC-5 cells
2. specific humoral immunity is elicited in mice primed with DNA*zenv* and boosted with the FP*zenv* recombinant
3. interestingly, a significant increase in antibody titers was observed after the T4 boost, corresponding to the FP*zenv* boost by the i.n. route

In conclusion, we demonstrate that DNA*zenv* prime followed by FP*zenv* mucosal boost is safe and immunogenic. In vivo experiments in the mice model are in progress to verify the protective efficacy of this immunization regimen.

HTLV-1 Tax and HBZ oncoproteins expression and subcellular localization in infection and disease progression: implications for immune-based therapeutic approaches.

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Background

HTLV-1 is the etiological agent of Adult T cell Leukemia (ATL) and of *HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP)* neurologic disorder for which no effective therapies are yet available. Two viral proteins, Tax-1 and HBZ play important roles in the pathogenesis of both diseases. Their expression and subcellular localization during infection and disease progression to ATL or HAM/TSP is not yet clarified and may shed light to the distinct genesis of the diseases.

Material and Methods

Subcellular distribution of endogenous HBZ and Tax-1 proteins were assessed by confocal microscopy with the 4D4-F3 and A51-2 mAbs, respectively in PBMC of HTLV-1 infected asymptomatic carriers (AC), ATL and HAM/TSP patients.

Results

By analyzing a large panel of HTLV-1 patients, we demonstrate that HBZ is expressed preferentially in the nucleus of leukemic cells, while Tax-1 is not present in most of them. In contrast in HAM/TSP, HBZ is a cytoplasmic protein, while Tax-1 may localize in both nucleus and cytoplasm. In AC both HBZ and Tax-1 are cytoplasmic. HBZ and Tax-1 were rarely found in the same cell, with only few cases co-expressing the two oncoprotein in a limited number of cells. HBZ and Tax-1 were almost exclusively found in the CD4+ T cell compartment and very rarely in CD8+ T cells.

Conclusions

These results extend our previous observation on the dichotomy of HBZ localization between HAM/TSP and ATL, pointing to either cytoplasmic or nuclear localization in the two disease states, respectively. Moreover, HBZ and Tax-1were selectively expressed in distinct cells. The observation that in AC, HBZ is a cytoplasmic protein, suggests a progressive modification of HBZ localization in the diseases associated to HTLV-1 infection. Specific targeting of HBZ by vaccination procedures focused on activating HBZ peptide–specific CTL may provide a tool to fight infection and preventing disease progression and neoplastic transformation.

Monoclonal antibodies protect mice in Rift Valley fever challenge model

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Introduction:
Rift Valley fever virus (RVFV) is a mosquito-borne arbovirus that causes large outbreaks affecting human and many vertebrate hosts throughout Africa and the Arabian Peninsula. To date, there is no specific therapy to prevent or treat RVF infections in humans. However, monoclonal antibodies (mAb) are one of the most promising treatment options. Therefore, the application of two monoclonal antibodies directed against RVFV glycoprotein Gn were evaluated in a mouse challenge model.
Methods:
A neutralizing monoclonal antibody (mAb Gn3) was used alone or in combination with a non-neutralizing mAb (mAb Gn32) by intravenous administration. BALB/C mice were challenged either 30min before (post-exposure) or after (prophylactic) antibody treatment with virulent RVFV strain 35/74. An additional control group was mock treated with PBS and challenged with virus. Blood samples were taken daily and mice were necropsied after 13 days at the latest or earlier, when reaching a human endpoint. Viral loads of blood and organic tissue were assessed in PCR followed by histological examinations of different tissues. Finally antibody response was analysed by ELISA and serum neutralization test.
Results:
Single mAb Gn3 treatment showed only a moderate protection with a survival ratio of 58,3% in both challenge groups. In contrast, prophylactic treatment of the both mAb showed 83,3% survival and post-exposure treatment even a complete protection. In particular, the combined mAb administration lead to a significant decrease of viral load and virus replication in corresponding tissues of infected mice.
Conclusion:
A neutralizing and non- neutralizing monoclonal antibody were applied in a mouse challenge model. Neutralizing antibody alone showed only a moderate protection. However, a strong cooperative effect was achieved by combined treatment with a non- neutralizing mAb, leading to complete protection against RVF infection.
In conclusion, neutralizing monoclonal antibodies are a promising method in treating RVFV infection.

Antibody Treatment of Smallpox Vaccine Adverse Events

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Smallpox has been formally eradicated from nature by a worldwide vaccination campaign with Vaccinia viruses on 1980. Yet, the virus might reemerge by accidental or intentional release requiring active vaccination of the entire world population. Beside their outstanding and approved efficacy, smallpox vaccines are associated with rare, yet severe adverse events (AE's) including post vaccinal encephalitis, eczema vaccinatum and progressive vaccinia. Despite careful screening to exclude persons with contraindications, there has been a number of instances in which a vaccinee (mostly following primary vaccine) or a household contact, experienced a disease manifested with disseminated vaccinia virus. Adequate modeling of these complications requires simulating the background of the human disease in mice.

The only approved treatment for Progressive vaccinia and Eczema vaccinatum is Vaccinia Immune Globulin (VIG) that has limited availability, is extremely expensive and has marginal potency.

To allow the development and evaluation of additional therapeutic countermeasures we have developed and thoroughly characterized small-animal models for the above smallpox vaccine AE's. Having these established models allowed us to elaborate on the therapeutic potential of antiviral antibodies in treated these AE's.

While repeated administration of high titer neutralizing antibodies or an antiviral nucleoside analog achieved effective treatment, their combination conferred significant protection even when treatment started 8 or 12 days post exposure.

Poor responsiveness of naïve CD8+ T-cells from elderly individuals is associated to their altered basal metabolism

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Quantitative and qualitative alterations of naïve $CD8^+$ T-cells are primary hallmarks of immune aging. The naïve T-cell compartment represents the source of de novo cellular responses, and its impairment an important obstacle to respond to and clear emerging pathogens and tumours in old individuals. Notably, elderly subjects also show severe defects in responding to vaccinations, and this has been associated to both the decline in the absolute numbers of Naïve $CD8^+$ T-lymphocytes as well as to the disruption of their priming and differentiation capacity upon TCR mediated activation.

However, the intrinsic defects associated with their altered functionality remain unclear, which is a critical obstacle for the development of vaccines tailored for the elderly population. Considering the important role of intracellular metabolism in lymphocyte functionality and the systemic bioenergetics dysfunctions characterizing elderly subjects, we aimed here at studying the metabolic features of naïve $CD8^+$ T-cells in elderly humans. Our data indicate that naïve $CD8^+$ T-cells from elderly donors present an active basal state, which is supported by alterations at the level of mitochondrial metabolism and increased fatty acid uptake and storage. IL-7, known to drive T-cell homeostatic proliferation and present at elevated levels with aging, induces lipid uptake and storage, similarly to observations in naïve $CD8^+$ T-cells from elderly donors. This particular bioenergetics profile is associated with increased apoptotic levels and decreased proliferative response of old naïve $CD8^+$ T-cells upon TCR-triggering. Notably, lipid lowering strategies are able to restore normal TCR-induced caspase-3 expression and proliferation. The present work highlights that alterations of the lipid metabolism in naïve $CD8^+$ T-cells with old age may alter their responsiveness. Approaches favoring lipid catabolism may represent interesting therapeutic strategies to improve T-cell immunity and vaccine efficacy in the elderly.

Oncolytic HSV-1 vectors for in situ vaccination against solid tumours

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Introduction

Immunotherapy is achieving impressive results in the treatment of advanced solid tumours. Oncolytic viruses (OVs) are antitumoral therapeutics that can be used as cancer cell killers and gene therapy vectors. OVs are also considered a form of immunotherapy, in which a self-amplifying virus contributes to an antitumoral immune response. A herpes simplex type 1 (HSV-1) based OV (talimogene laherparepvec) has been approved for the treatment of metastatic melanoma following successful clinical trials. However, solid tumours surrounded by an immunosuppressive tumour microenvironment (TME), including triple negative breast cancer (TNBC) are mostly resistant to immunotherapy and OVs, requiring a more effective approach.

Methods

We therefore engineered an attenuated HSV-1 genome to express multiple therapeutic genes that disrupt the immunosuppressive features of the TME. A strain 17+ HSV-1 genome including a double deletion of the “neurovirulence” γ34.5 gene, embedded in a bacterial artificial chromosome (BAC), was further modified by BAC mutagenesis in a strain of *Escherichia coli* expressing heat-inducible recombinases. Viral replication and therapeutic gene expression were evaluated by plaque titration assay and reverse transcriptase PCR (RT-PCR) *in vitro.*

Results

The Us12 gene was deleted to obtain a backbone analogous to the clinically approved virus. Recombinant viruses were generated by insertion of foreign genes in the UL55-UL56 intergenic region, including i)enhanced green fluorescent protein, ii)human and murine IL-12, iii)FMS-like tyrosine kinase ligand 3 (FLT3L), iv) soluble programmed cell death 1 (sPD-1), v)a single chain antibody against the CCR4 receptor on regulatory T cells, vi)a synthetic peptide which blocks the IL4 receptor, important for the activation of myeloid-derived suppressor cells (MDSCs). All viruses efficiently replicated and expressed therapeutic genes in human and murine breast cancer cell lines *in vitro.* They also showed selectivity for murine breast cancer organoids compared to non-tumoral murine breast organoids.

Conclusions

OVs will be tested in an immunocompetent TNBC mouse model to allow evaluation of their efficacy in the presence of an intact immune system, while also providing an important opportunity to study the relative importance of different immunosuppressive mechanisms in the TME *in vivo.*

Novel anti-virals against Enteroviruses

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Introduction - Enteroviruses can cause an array of diseases ranging from common cold to more serious acute and chronic infections. Enteroviruses can lead to inflammations in the myocardium, brain and pancreas resulting in atherosclerosis and myocardial infarction [1]. Recent evidence suggests that many species-B enteroviruses (EV-B) are associated with type I diabetes [2]. In addition, non-enveloped viruses like enteroviruses are resistant to chemical disinfectants. Currently, no antiviral therapy is available for enterovirus infections, making it imperative to explore alternatives other than the traditional approaches for encountering these viruses. Here, we report the use of natural derived compounds (NDC) and biofunctionalized gold nanoparticles (Bf-AuNPs) as a substitute to traditional antivirals agents.

Methods - The Bf-AuNPs were synthesized using green chemistry. NDCs were either extracted from natural origin or purchased from commercial sources. Cytotoxicity and anti-viral efficacy of the compounds were assessed using ATP and cytopathic effect (CPE) inhibition assay respectively. *In-vitro* studies to understand the NDC and Bf-AuNPs interaction with viruses was done using real-time spectroscopy analysis, confocal microscopy and thermal assay.

Results – The compounds tested were not cytotoxic to A549 cells and were effective in protecting the cells from coxsackievirus B1 (CVB1), coxsackievirus B3 (CVB3) and coxsackievirus A9 (CVA9) infection. However, pretreatment of cells with NDC did not safeguard the cells from virus infection, implying a direct virucidal action of NDC on virus particles rather than having targets on host cells. *In-vitro* studies of Bf-AuNPs on the other hand suggested an enhanced stabilization effect of Bf-AuNPs on the virus capsid, thus preventing its genome release.

Conclusion - The studied NDCs showed direct virucidal effect on the virus particles whereas Bf-AuNPs stabilized the virus capsid. Based on our preliminary findings, we introduce NDC and Bf-AuNPs as novel anti-virals against enteroviruses.

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DNA-based prime-boost active immunotherapy to induce a functional cure in patients with chronic hepatitis B

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Chronic hepatitis B and D virus (HBV/HDV) infections cause cancer and are treated either life-long with nucleoside analogues, or with interferon therapy inducing a functional cure in a few patients. There is a consensus that a sustained therapy response most likely involves an activation of the host immune response. A hallmark of the chronic infection is a profound T cell tolerance to HBV. We here describe a new active immunotherapy, designed to circumvent the T cell tolerance, and targeting viral entry to complement existing antivirals that inhibit viral maturation. The strategy consists of a DNA-prime and protein boost immunotherapy that induce receptor blocking antibodies to the HBV PreS1 domain and that induce HBV- and HDV-specic T cells. We use HDAg as a T cell cerrier as >90 of those with chronic HB Vare not co-infected by HDV, thus their HDV-specific T cells are healthy. In addition, these T cells may protect against HDV superinfection. We found that the DNA-protein prime-boost strategy was superior in inducing both antibodies and T cells, as compared to either DNA or protein alone. Importantly, the prime-boost strategy induced superior levels of neutralizing antibodies to HBV. Finally, we have shown that IgG from rabbits immunized DNA vaccine using in vivo electroporation can protect mice with human livers against HBV infection. In conclusion, this is a highly promising active immunotherapy to be used in combination therapies for treating chronic HBV to obtain a functional cure and prevent the need for life-long therapies.

An Orf virus-based platform technology for vaccine development using a modulatory brick system

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The *Orf virus* (ORFV; *Parapoxvirus*) strain D1701 exhibits an attenuated phenotype and excellent immunogenic capacity. It is successfully used for the generation of recombinant vaccines against different viral infections. Adaptation for growth on Vero cells lead to genomic rearrangements resulting in the strain variant D1701-V. In this overview we discuss the several properties that make D1701-V a platform technology particularly suitable for vaccine development using a modulatory brick system.

The attractiveness of D1701-V based viral vectors rely on the following advantages: (i) a very restricted host range, (ii) no evidence for viral systemic spread, (iii) a fast induction of a strong humoral, but also cellular immune response against the inserted antigen in also non-permissive hosts deficient for viral vector replication and (iv) a short-term vector specific immunity allowing multiple re-immunizations. The most recent improvements made on D1701-V included various immunogen design approaches for the induction of adaptive antigen-specific immune responses and demonstrated the suitability of several deletion sites for the simultaneous expression of foreign genes using different synthetic early promotors. On this basis, combination of antigen and encoded modulatory elements such as cytokines, co-stimulatory molecules or antibodies further increased the immunization efficacy also in tumor models. Herewith, we provide evidence that this versatile platform technology allows the production of potent polyvalent vaccines containing several different antigens and/or immunomodulatory elements in a single vectored ORFV vaccine.

Human transcriptomic response to vaccination with recombinant VSV expressing Ebola virus Glycoprotein

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rVSV-ZEBOV is a live-attenuated recombinant vesicular stomatitis virus vaccine expressing Ebolavirus glycoprotein G and is the only Ebola vaccine with demonstrated clinical efficacy. Here we studied the blood transcriptomic response upon injection of a single dose of vaccine. Whole blood RNA from 64 healthy volunteers, 51 injected either with 10^7 or $5x10^7$ PFU of rVSV-ZEBOV and 13 with placebo, collected at different time points after vaccination, was analysed by targeted transcriptome sequencing. At each time point, differentially expressed genes (DEGs) were identified with edgeR, ranked by FDR, and used to find biological signatures assessing the activation of 346 blood transcription modules. Between baseline and day 1 after vaccination, 5,469 DEGs were detected. This number decreased over time: at day 28 no DEGs were detected. Functional analysis identified 145 different modules affected by vaccination. Innate immunity pathways were activated from day 1 to day 14. At days 2 and 3, neutrophil modules were downregulated and complement-related modules upregulated. T-cell and cell-cycle associated modules were upregulated at days 7 and 14, while at day 28 no modules remained activated. Correlation analysis of module activation with ZEBOV glycoprotein-specific antibody titres identified seven significant directly correlated modules at day 14 after vaccination, including two related to B cell activation.

Humoral immune response to HCV peptides as cancer-progression biomarkers of HCV-infections

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HCV infections are the main cause of chronic liver disease and in part of lymphoproliferative disorders. Most HCV infections (>90%) determine chronic hepatitis, 30% of which progress to liver cirrhosis and 3% annually to Hepatocellular Carcinoma (HCC). The progression rate is mainly articulated in low (>40 years) and high (<10 years) speed progressors, with the latter being associated to male gender, <40 years of age, >150ml daily alcohol consumption. Current progression markers are mainly based on biochemical evaluation of liver damage (elevation of alanine and aspartate transaminases) and inflammation (elevation of alpha-fetoprotein). Such markers are not specific and elevated also for other infections (i.e. HBV and HCMV) or metabolic disorders (i.e. steatosis). Specific HCV-related markers would be relevant to identify HCV co-factors and to select high priority people for direct anti-viral treatment.

To identify HCC progression markers, serum samples from 71 HCV-positive patients, including 49 diagnosed with HCC, 9 with cryoglobulinemia and 13 with asymptomatic HCV infection, have been analyzed on a newly developed HCV-derived 15-amino-acid-long peptides microarray which carries 5952 overlapping peptides covering the whole HCV proteome. The currently available data demonstrates that in asymptomatic patients the level of anti-HCV is in general very low (including anti-capsid/core proteins), while high levels of antibodies against non-structural proteins is observed in liver cancer patients. Confirmation of such data would support the anti-non structural response as biomarker of cancer progression in HCV+ patients.

Poster presentations

Production of flavivirus virus-like particles for vaccine purposes

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Emerging epidemics caused by new pathogens or more pathogenic strains of pre-existing viruses call for the development of effective new generation vaccines. Many of these epidemics are caused by viruses belonging to the Flaviviridae family. The family includes, among others, dengue virus, West Nile virus, hepatitis C virus, Zika virus (ZIKV) and tick-borne encephalitis virus (TBEV). Flaviviruses are similar in virion structure, genome organization, replication cycle and function of viral proteins. Human flavivirus infections elicit both virus species-specific neutralization and flavivirus cross-reactive antibody responses leading to antibody-dependent enhancement of infection. Thus, the development of universal technology for the production of recombinant antigens is a rational approach to vaccine design. Virus-like particles (VLPs) may provide an alternative, specific antigens used for vaccination purposes. VLPs are composed of recombinant viral proteins, organized almost identically to the native virions with antigenic properties similar to native virions. Moreover, VLPs do not contain genetic material, which makes them non-infectious. The main aim of the study was to evaluate the universal methodology for efficient production of VLPs in different expression systems (insect cells, mammalian and protozoan) using two flavivirus representatives. Production of flavivirus VLPs was determined by Western blotting, indirect immunofluorescence assay, dynamic light scattering, sucrose gradient sedimentation as well as electron microscopy. Functional and conformational analysis of obtained VLPs was performed by enzyme-linked immunosorbent assay (ELISA) using a panel of conformational antibodies. The study suggests that flavivirus VLPs can be produced in different expression systems however due to the differences in the efficiency levels, the production conditions must be optimized separately for each virus. Although, the methodology needs further optimization, VLPs still offer a promising approach for vaccine purposes.

A therapeutic vaccination to design a rational strategy for prevention and treatment of HIV-related lymphoma.

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Despite the induction of virologic suppression and immune recovery obtained by the combined antiretroviral therapy (cART), lymphoma incidence remains still high in human immunodeficiency virus-infected patients (HIV^+). These findings suggest that HIV may contribute to B-cell lymphomagenesis by promoting a pro-lymphomagenic microenvironment. The HIV matrix protein p17 (p17) accumulates and persists in lymphoid tissues even during cART. Ultra-deep pyrosequencing (UDP) showed that p17 variants (vp17s) are more frequently detected in plasma of HIV^+ subjects with than without Non-Hodgkin Lymphoma (NHL). Differently from wild-type p17, vp17s isolated from NHL specimens of HIV^+ patients promote B cell growth by activating the pro-oncogenic Akt pathway. Antibodies to p17 raised during the natural course of HIV-1 infection do not neutralize the p17 biological activity. To induce p17 neutralizing antibodies, a therapeutic vaccination based on a synthetic peptide (AT20) representative of the p17 functional region, coupled to keyhole limpet hemocyanin (KLH) AT20-KLH was developed and evaluated for its safety and immunogenicity. All vaccinated subjects developed high titers of high-avidity anti-AT20 antibodies in response to vaccination, which resulted to be completely safe and well tolerated. The anti-AT20 antibodies were maintained at more than two years after the last immunization being still able to neutralize the p17 biological activity. This finding supports the hypothesis that the synthetic minimalist therapeutic vaccine approach against AIDS, based on the possibility to target the highly conserved AT20 epitope, may also prove useful to design a rational strategy for prevention and/or treatment of HIV-related lymphoma.

B cell epitope mapping and selection of Toscana virus neutralizing epitopes for a vaccine design

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INTRODUCTION

Toscana virus (TOSV) is an emergent pathogen associated with neurological disease. Our aim was to localize the neutralizing epitopes on Gn glycoprotein using human mAbs obtained by immortalization of B cells from a subject infected with TOSV. The influence of these findings can pave the way for a rational design and optimization of vaccine antigens against TOSV.

METHODS

Human memory B cells immortalization.
Pepscan analysis.
Characterization of antibodies by ELISA and Neutralization test.
In vivo immunization of BALB/c mice.

RESULTS

MAbs were obtained from PBMCs of a man affected with TOSV meningitis, one year post infection. MAbs positively reactive against TOSV glycoproteins and having neutralizing activity were selected. Three regions of the Gn protein were recognized and identified. Two of them were localized in the N-terminal half of the protein (peptide 1 and 2), the third one was close to the transmembrane region (peptide 3), leading us to suppose that they could represent a discontinuous epitope. To confirm this hypothesis, different combinations of the peptides were used to immunize mice; the mixture of peptide 1 and 2, fused with GST and peptide 3, conjugated with KLH, provided the best result, inducing neutralizing antibodies (titre 1/128). The immunization of mice with GST fused with the three peptides is in course. So far, the results suggest that the epitope recognized by neutralizing mAbs could probably represent a discontinuous epitope and the antibodies developed in immunized mice are able to neutralize the virus reacting separately with the peptide 1-2 and peptide 3.

CONCLUSIONS

We isolated a panel of human mAbs with neutralizing activity directed to TOSV Gn. We identified a potential neutralizing epitope including the three peptides likely corresponding to a discontinuous epitope. These results could be used in order to design epitopes that can serve as potential targets for the production of epitope-based diagnostics and vaccines.

The mode of action of Orf virus-based vectors to induce strong cellular immune responses

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The highly attenuated and apathogenic Orf virus (ORFV) strain D1701-V represents several favorable properties and is a promising candidate for viral recombinant vaccines. ORFV-based vectors were tested as prophylactic vaccines and were able to induce strong and long-lasting humoral immune responses and mediated protection against subsequent infections in different species. However, only little is known about the potential of live ORFV to induce cellular immune responses, which can be used for therapeutic vaccinations. Here we describe the capacity and the mode of action of recombinant ORFV to induce strong cellular immunity.

Two immunization of mice with an ORFV vector encoding Ovalbumin resulted in Ovalbumin-specific T cell frequencies of 80 % and subsequent analysis revealed the polyfunctionality of T cells. When tested an ORFV vector encoding for antigens of human cytomegalovirus, we were able to induce strong antigen-specific T cell responses. Both expansion of antigen-specific memory T cells and priming of naïve T cells was demonstrated.

With an ORFV recombinant expressing the fluorescence marker mCherry we were able to identify professional antigen-presenting cells (APCs) that have taken up ORFV. Moreover, we showed that the infection occurs via phagocytosis and macropinocytosis. The uptake of ORFV leads to activation of APCs which was demonstrated by the upregulation of activation markers and cytokine secretion most likely responsible for the strong induction of the antigen-specific immune response.

Identifying key components of the Virus-Cell interaction using a chemical biology approach

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Introduction. Kaposi´s Sarcoma-associated herpesvirus (KSHV or HHV-8) is an oncogenic human γ-herpesvirus and the etiological agent of Kaposi´s Sarcoma KS, the plasma cell variant of multicentric Castelman´s disease (MCD) and primary effusion lymphoma (PEL). Current treatments are unsatisfactory, resulting in an urgent need to develop novel KSHV drugs.
Methods. We screened a library of 18,656 small molecules in order to identify some that are able to abrogate KSHV lytic reactivation. Using classical cell-based assays combined with the medicinal chemistry approach 12 inhibitors of the early KSHV lytic replication phase were selected for future characterization.

Results. The 12 molecules have an IC_{50} < 5µM and are able to inhibit the expression of early lytic genes in KSHV-infected cells. Among the 12 inhibitors PANH_165 was selected for further studies. It has an IC_{50} of 0.9µM and it is able to inhibit the expression of early lytic genes in a dose dependent manner in two KSHV-infected cell lines, BJABrKSHV.219 and iSLKsKSHV-BAC16, representing B- and epithelial cells, respectively. PANH_165 was tested against other members of the Herpesviridae family and it was able to block Varicella Zoster (VZV) lytic cycle.

Conclusions: PANH_165 is a promising molecule able to inhibit KSHV and VZV lytic cycle. PANH_165 derivatives will be tested in order to identify more powerful inhibitors. Using the "*Click chemistry approach"*, we attempt to identify the target of our selected compound.

New preventive/therapeutic vaccines against HPV16 by innovative technologies of plant production and by adjuvanted chimeric genetic formulations.

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The long lasting activities of our group on HPV16 E6/E7 vaccine production in plants have achieved great results. However, to render our preparations more suitable for scale-up and GMP production new vaccines were developed and validated in mouse models. In particular, two new technologies were employed involving hairy root production and chimeric DNA formulations.

"Hairy root" cultures (HRCs) are a robust platform that combines the benefits of cultivating eukaryotic plant cells as bioreactors of molecules, with sterility and ease of scale-up to GMP. Plant expression vectors were used to obtain vaccine-expressing HRC clones from tomato plants. Several clones accumulated the fusion antigen in the soluble fraction of crude extracts. HRC extracts containing the E7GGG-SAPKQ (fusion vaccine of HPV16 E7 and SAPKQ, a non-cytotoxic form of the saporin protein from Saponaria officinalis that we demonstrated to have an intrinsic adjuvant activity) were administered in TC-1 experimental mouse model of HPV-associated tumor via heterologous prime-boost regimens. Vaccine DNA as prime and HRC extracts as boost demonstrated highest efficacy in inducing cell-mediated immune response, and tumor regression.

In a vaccine DNA formulation, the signal sequences (ss) of the Polygalacturonase-inhibiting protein (PGIPss) from Phaseolus vulgaris fused to the N-terminus of a HPV16 antigen enhanced humoral responses, providing a tool for preventive vaccines. To develop a preventive/therapeutic vaccine, a chimeric construct consisting of L2 (first 200 aa.)-E7 (E7GGG harmless version) of HPV16 was fused to PGIPss. Chimeric PGIPss-L2-E7 construct induced a high titre of both anti-L2 and anti-E7 IgGs in mice. In two mouse models of HPV expressing tumors (TC-1-C57BL/6 and orthotopic AT84E7luc-C3H), this chimeric vaccine induced a dramatic reduction of tumor growth as a consequence of specific antibody and cell-mediated immune responses.

These data highlight relevance of this low-cost technologies for plant-derived HPV vaccines and efficacy of PGIPss-chimeric DNA formulations for preventive-therapeutic vaccines.

Development of an *Orf Virus*-based vaccine suitable for the therapeutic treatment of papillomavirus-induced tumors

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During the recent years Orf virus (ORFV) belonging to the genus *Parapoxvirus* of *Poxviridae* has proven to represent a very suitable virus vector for the generation of efficient, prophylactic vaccines in different hosts. The present study investigates for the first time the therapeutic application of ORFV vector-based vaccines against tumors induced by *Cottontail Rabbit Papillomavirus* (CRPV), which is a well-known model for therapeutic vaccine approaches against high-risk *Human Papillomavirus*. Four ORFV-CRPV recombinants were constructed expressing the CRPV gene E1, E2, E7, or LE6, respectively, which are known to elicit anti-tumorigenic immunity.

The skin of rabbits was infected with CRPV DNA using a gene-gun and five weeks after the appearance of skin tumors the rabbits were immunized with a mixture of the 4 ORFV-CRPV recombinants or empty ORFV vector as control and the subsequent growth of the tumors was monitored. We could demonstrate that immunizations with the ORFV-CRPV recombinants resulted in highly significant growth retardation and shrinkage of tumors or even complete remission without tumor reappearance, whereas the control group immunized with empty ORFV vector revealed no effect. Results of delayed-type hypersensitivity skin tests suggest the induction of a cellular immune response mediated by the ORFV-CRPV vaccine. The data presented show for the first time a therapeutic, specific anti-tumoral effectiveness of the ORFV vector platform and encourage further studies for the development of ORFV-based therapeutic vaccine against tumors.

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