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vaccines

ABSTRACT BOOK

RIGA, 2022

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- **Maria Issagouliantis**, *Institute of Microbiology and Virology, Rīga Stradiņš University, Latvia / Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden*

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First prize – Massimiliano Bissa (Animal Models and Retroviral Vaccines Section, National Cancer Institute, Bethesda, Maryland, USA) – 300 euro.

Second prize – Andrea Cerasuolo (Istituto Nazionale Tumori IRCCS Fond. Pascale, Naples, Italy) – 150 euro.

Shared third prize – Zane Lucāne and Yuliya Raukach, Rīga Stradiņš University, Riga – 75 euro each.

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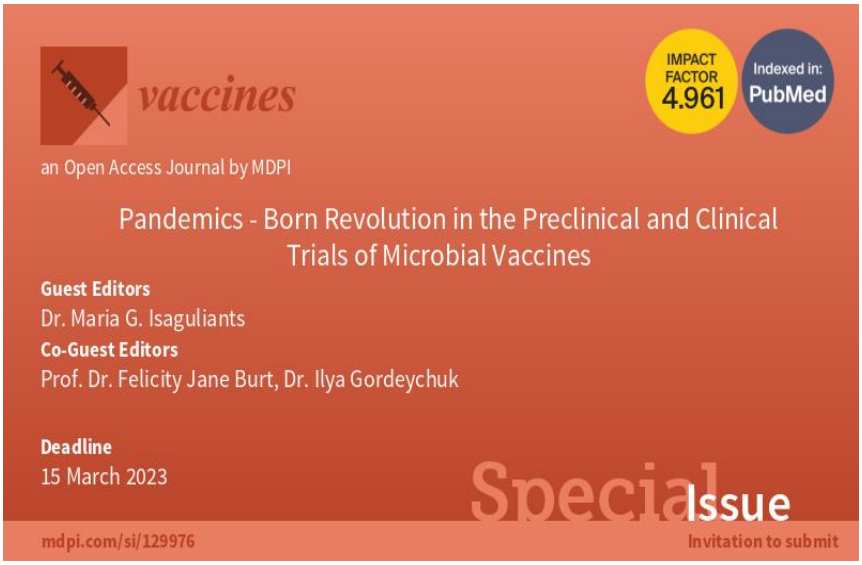

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Winners of the early career researcher contest Massimiliano Bissa (Animal Models and Retroviral Vaccines Section, National Cancer Institute, Bethesda, Maryland, USA) – 1st prize, and Andrea Cerasuolo (Istituto Nazionale Tumori IRCCS Fond. Pascale, Naples, Italy) – 2nd prize, received vouchers for free publication.

Session I: COVID-19 Pandemics & Vaccines

Chairs: Prof. Franco M. Buonaguro and Dr Maria Issagouliantis

Inflammatory Response and Pathogen Consequences from SARS-CoV-2 Infection

Ranjit Ray, *Doisy Research Center, Division of Infectious Diseases, Allergy & Immunology, Saint Louis University, USA*; ranjit.ray@health.slu.edu

Development of DNA Vaccines Against SARS-COV-2: An Overview and Potential for Future Pandemics

Joel N Maslow, *GeneOne Life Science, Seoul, South Korea*; jmaslow@genels.us

Development of Viral Vaccines on Vesicular Stomatitis Virus (Vsv) Platform, Progress for Sars-Cov-2

Manki Song, *International Vaccine Institute, Seoul, Republic of Korea*; mksong@ivi.int

The Potential of Plants as Rapid-Response Expression Platforms for Pandemic Response.

Ed Rybicki, *Biopharming Research Unit, Department of Molecular & Cell Biology, University of Cape Town, South Africa*; ed.rybicki@uct.ac.za

Long-Term Humoral and Cellular Sars-Cov-2 Vaccine-Specific Immune Response in Patients With Primary Antibody Deficiencies

Zane Lucāne, *Rīga Stradiņš University, Latvia*; Zane.Lucane@rsu.lv

Hesitant Bodies: Phenomenological Analysis of the Embodied Experience of Covid-19 Vaccine Hesitancy

Uldis Vēgners, *Rīga Stradiņš University, Latvia*; Uldis.Vegners@rsu.lv

Parents' Attitudes Towards Routine Children's Immunization in the Republic of Belarus in Covid-19 Pandemic

Yuliya Raukach, *Rīga Stradiņš University, Latvia*; 046571@rsu.lv

INFLAMMATORY RESPONSE AND PATHOGENIC CONSEQUENCES FROM SARS-COV-2 INFECTION

Ranjit Ray

Doisy Research Center, Division of Infectious Diseases, Allergy & Immunology, Saint Louis University, USA;

ranjit.ray@health.slu.edu

Background

SARS-CoV-2 infection of the lung may initiate and promote a systemic disease called COVID-19 that involves an aggravated immune response. Growing clinical evidence suggests that thrombotic and microvascular complications may contribute to multiple organ failure and death of COVID-19 patients. Moreover, some patients who recover from COVID-19 develop chronic complications known as Post-Acute Sequelae of COVID.

Topics overviewed

We found that SARS-CoV-2 or spike protein promotes interleukin-6 (IL-6) receptor-mediated pro-inflammatory trans-signaling that is disease aggravating. We identified that human endothelial cells exposed to cell culture supernatant derived from SARS-CoV-2 spike protein expressed on cells display cellular senescence markers, leading to enhanced leukocyte adhesion. We also found that SARS-CoV-2 spike generates monocytic thrombo-inflammatory conditions. SARS-CoV-2 spike protein modulates monocyte responses in a paracrine manner for prothrombogenic stimulus by the generation of C5a complement component. We further observed that exosomes from plasma of COVID -19 patients carry pro-inflammatory molecules like fibrinogen-β and tenascin-C, along with the viral Spike protein. Exosomes appear to play important roles in multiorgan pathogenesis during virus infection. Thus, SARS-CoV-2 infection may induce molecular signals in the form of viral components and/or exosomes that rapidly trigger inflammatory signals in distant organs for adverse complications.

DEVELOPMENT OF DNA VACCINES AGAINST SARS-COV-2: AN OVERVIEW AND POTENTIAL FOR FUTURE PANDEMICS

Joel N Maslow

GeneOne Life Science, Seoul, South Korea;

jmaslow@genels.us

In response to the ongoing SARS-CoV-2 pandemic, numerous vaccines were developed and tested. Here we review DNA vaccines against SARS-CoV-2 and the potential role of DNA vaccines in future pandemics. Three DNA vaccines (ZyCoV-D, INO-4800, and GLS-5310) advanced to at least Phase 2 with ZyCoV-D approved in India with a reported efficacy of 66.6% 1-3.

The vaccines were administered with the PharmaJet needle-free injection system (NFIS), electroporation (EP), and the GeneDerm suction device 4, respectively. Each of the three vaccines induced similar end point titers for binding and neutralizing antibody responses. T cell immune responses by ELISpot were reported as approximately 50 SFU/106 cells for the ZyCoV-D and INO-4800 vaccines and approximately 1200 for GLS-5310. Notably, for GLS-5310 in Phase 1 study binding antibody and T cell responses were stable over the 1 year follow-up period. Post-vaccination immune responses were dose-dependent for ZyCoV-D and INO-4800 whereas immune responses for GLS-5310 were dose-independent.

There are both advantages and challenges to DNA vaccine use for current and future pandemics. Advantages include thermal stability, resistance to degradation in vivo, minimal innate immune activation, and rapid scale up from design to clinic. Post-vaccination immune responses demonstrated longevity for a year with high T-cell responses, although this may be device-dependent. That DNA requires a delivery device for in vivo transfection is a well-known disadvantage. The three devices used to deliver SARS-CoV-2 vaccines differ in training requirements, cost, and ease of scale-up for the device and device disposables with GeneDerm being the least costly and with the least training requirements. Comparative studies of the devices in animals demonstrated similar B cell responses, but superior T cell responses with the GeneDerm suction device relative to DNA vaccine administration using NFIS or EP 5. The neutralizing antibody response of DNA vaccines, while generally consistent to each other, were approximately 2-logs lower than mRNA vaccines.

For future pandemics, the advantages of DNA vaccines with regard to minimal logistic challenges, rapid scale-up, and proven efficacy thus provide clear benefits especially for resource limited and remote regions .

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A VESICULAR STOMATITIS VIRUS-BASED PRIME-BOOST VACCINATION STRATEGY INDUCES POTENT AND PROTECTIVE NEUTRALIZING ANTIBODIES AGAINST THE SARS-COV-2 SPIKE PROTEIN

Manki Song

International Vaccine Institute, Seoul, Republic of Korea;

mksong@ivi.int

Background
The development of safe and effective vaccines to prevent SARS-CoV-2 infections remains an urgent priority worldwide. We have used a recombinant vesicular stomatitis virus (rVSV)-based prime-boost immunization strategy to develop an effective COVID-19 vaccine candidate.

Topics overviewed
We have constructed VSV genomes carrying exogenous genes resulting in the production of avirulent rVSV carrying the full-length spike protein (SF), the S1 subunit, or the receptor binding domain (RBD) plus envelope (E) protein of SARS-CoV-2. Adding the honeybee melittin signal peptide (msp) to the N-terminus enhanced the protein expression, and adding the VSV G protein transmembrane domain and the cytoplasmic tail (Gtc) enhanced protein incorporation into pseudotype VSV. All rVSVs expressed three different forms of SARS-CoV-2 spike proteins, but chimeras with VSV-Gtc demonstrated the highest rVSV-associated expression. In immunized mice, rVSV with chimeric S protein-Gtc derivatives induced the highest level of potent neutralizing antibodies and T cell responses, and rVSV harboring the full-length msp-SF-Gtc proved to be the superior immunogen. More importantly, rVSV-msp-SF-Gtc vaccinated animals were completely protected from a subsequent SARS-CoV-2 challenge.

Conclusions
Overall, we have developed an efficient strategy to induce a protective response in SARS-CoV-2 challenged immunized mice. Vaccination with our rVSV-based vector may be an effective solution in the global fight against emerging infectious disease.

THE POTENTIAL OF PLANTS AS RAPID-RESPONSE EXPRESSION PLATFORMS FOR PANDEMIC RESPONSE

Edward P. Rybicki

Biopharming Research Unit, Department of Molecular & Cell Biology, University of Cape Town, South Africa;

ed.rybicki@uct.ac.za

Background

The practice of "molecular farming", or the production of high-value, complex biologics such as candidate subunit vaccines in plants, is one of the most important developments in the production of biologics in the last thirty years. It allows effectively infinite scaling of production, in hosts that cannot support the multiplication of agents that are harmful to humans or their animals, at a cost for biomass production that is far lower than for any other production system. The advent of transient expression – the use of preproduced non-engineered biomass, and either bacteria or adapted plant viruses to introduce foreign genes into it - has further revolutionised the technology, given that production at any scale is limited only by the availability of plants, and of suitably-engineered DNA vectors. The response time is also very fast, from obtaining particular sequences derived from a pathogen, to expressing many grams of purified material.

Materials & Methods

The technology employed by our laboratory and many others involves the use of recombinant *Rhizobium radiobacter* bacteria to somatically transfer genes of interest into whole *Nicotiana benthamiana* plants by a natural DNA transfer mechanism. Transferred single-stranded DNA is converted to dsDNA in plant cell nuclei, and maintained episomally for many days, during which time it can express mRNA and then proteins. Proteins may be localised in the cytoplasm, or imported into the chloroplasts, the vacuole, the nucleus or the ER. Virus-like particles can assemble, and proteins may be glycosylated or otherwise processed post-translationally.

Results

Our laboratory has successfully made a wide variety of candidate vaccines, including bit not limited to HIV-1 Gag VLPs and soluble trimeric Env gp140, a variety of HPV VLPs, and complex VLPs similar to bluetongue and African horse sickness virions. Most important, however, was our production in 2006 of a H5N1 HPAI influenza A virus haemagglutinin as a candidate pandemic response vaccine, and our production in 2020 of a soluble trimeric SARS-CoV-2 S protein within a few weeks of the release of the sequence.

Conclusions

Our results have demonstrated that even a middle-income developing country could respond rapidly with candidate vaccines to emerging human and animal pathogens – even if there were no cGMP facilities to manufacture them at scale. The success of established vaccine companies in very rapidly producing vaccines based on the same technology cements the conclusion that the technology is here to stay, and has a place in rapid responses to rapidly-emerging pathogens. The challenge ahead lies in expanding the cGMP production capacity to enable even LMICs to do the same.

LONG-TERM HUMORAL AND CELLULAR SARS-COV-2 VACCINE-SPECIFIC IMMUNE RESPONSE IN PATIENTS WITH PRIMARY ANTIBODY DEFICIENCIES

Zane Lucāne [1], B. Slisere [1,2], L. Ozola [1,3], D. Straupmane [2], I. Nartisa [1,3], D. Rots [1,3], S. papirte [1], L. Gailite [1], B. Vilne [1], N. Kurjane [1,2,3]

1 - Riga Stradins University, Riga, Latvia
2 - Pauls Stradins Clinical University Hospital, Riga, Latvia
3 - Children's Clinical University Hospital, Riga, Latvia

zane.lucane@rsu.lv

Background

Inborn errors of immunity are a group of rare diseases characterized by increased susceptibility to infection and multiple non-infectious complications. A higher morbidity and mortality from SARS-CoV-2 have been described and vaccination is the most effective form of prophylaxis in these patients (1). However, the durability of humoral and especially cellular immune responses in this group remains unknown (2).

Materials & Methods

The cross-sectional study included 28 vaccinated patients with primary antibody deficiencies (15 with common variable immunodeficiency (CVID) and 13 with selective IgA deficiency) and 15 healthy controls. The humor response was evaluated using a semi-quantitative enzyme-linked immunosorbent assay that detects specific SARS-CoV-2 IgG antibodies against S1 domain of spike protein (Euroimmun, Germany). T cell immune responses were detected by an interferon-gamma release assay QuantiFERON SARS-CoV-2 (Qiagen, Germany), which measures CD4+ and CD8+ T cell immune responses to S1 and S2 antigen pools. In addition, T and B-cell subclass phenotyping by flow cytometry was performed to identify immunological correlates with the magnitude of SARS-CoV-2 specific immune response.
Differences in categorical variables were examined by using Chi-square and Fisher exact tests. Mann-Whitney U test was used to compare continuous variables by one factor, and Kruskal Wallis test - to compare continuous variables between more than two groups. Spearman's rank test was used to assess the correlation between continuous variables.

Results

The median time after vaccination was 173 (IQR=142) days, ranging from 25 to 345 days in the patient group. Most patients with primary antibody deficiency were able to show a positive humoral (27/28) and cellular (26/28) SARS-CoV-2-specific immune response. Statistically significantly lower levels of anti-spike IgG were observed in CVID patients compared to healthy controls. There were no statistically significant differences in CD4+ and CD8+ T-cell SARS-CoV-2 specific immune responses between the study groups. In the patient group, central memory CD8+ T-cells and, only in the selective IgA group, central memory CD4+ T-cells correlated positively with anti-spike IgG levels, but not SARS-CoV-2 specific T cell responses. No statistically significant differences were detected between SARS-CoV-2 specific humoral or cellular immune responses and B lymphocyte subtypes.

Conclusions

The results of this study indicate that markers of the sustained SARS-CoV-2 spike-specific humoral and cellular immune response are detectable almost up to a year after vaccination either in patients with primary antibody deficiencies as well as in healthy controls. Despite on the significantly lower median levels of anti-spike IgG response in CVID patients than in healthy controls, the T cell response was unchanged in all studied groups.

Acknowledgements: Latvian Council of Science project Nr.lzp-2020/1–0269

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HESITANT BODIES: PHENOMENOLOGICAL ANALYSIS OF THE EMBODIED EXPERIENCE OF COVID-19 VACCINEHESITANCY

Uldis Vēgners [1,2]

1 - *University of Latvia, Riga, Latvia*
2 - Riga Stradins University, Riga, Latvia

uldis.vegners@rsu.lv

Background

One of the major challenges of the global vaccination efforts and progress against infectious diseases is what World Health Organization calls vaccine hesitancy, defining it as delay in acceptance or refusal of vaccines despite availability of vaccination services. The issue has only been exacerbated during the recent years of the global COVID-19 pandemic, making it crucial to understand what lies behind it. In our ongoing research we focus on the embodied context of vaccine hesitancy in relation to COVID-19 in Latvia, by carrying out a phenomenologically informed qualitative research study, that is qualitative research informed by a philosophical movement called phenomenology.

Materials & Methods

To gain insights into the embodied experience of vaccine hesitancy in relation to COVID-19 in Latvia we are conducting a phenomenologically informed empirical research study, based on the methodological framework called 'Phenomenological interview' (Høffding & Martiny, 2016) which integrates the qualitative interview with phenomenological philosophy. In Phenomenological interview phenomenology provides a conceptual framework (concepts like embodiment, lived body and object body, body schema and body image) for data collection and analysis.

Results

We have concluded our data collection phase, during which we performed 16 semi-structured interviews, and, although we have just begun our data analysis phase (and, therefore, all the results presented here, are only tentative) it is possible to discern a number of important embodied aspects of vaccine hesitancy, one among which is the split between the world as presented by science and the embodied life-world.

Conclusions

Based on our provisional results I will argue that COVID-19 vaccine hesitancy is formed in one's own embodied lived context. Vaccine hesitancy should be viewed not only in terms of distrust in safety and efficacy of a specific vaccine, but also more widely in terms of an embodied relationship with the world.

This research is funded by the Latvian Council of Science, project *Hesitant bodies: phenomenological analysis of the embodied experience of vaccine hesitancy*, project No. lzp-2021/1-0360.

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PARENTS' ATTITUDES TOWARDS ROUTINE CHILDREN'S IMMUNIZATION IN THE REPUBLIC OF BELARUS IN COVID-19 PANDEMIC

Yuliya Raukach

Riga Stradins University, Riga, Latvia

046571@rsu.edu.lv

Introduction

UNICEF and WHO have presented data proving that the COVID-19 pandemic has caused a serious decline in childhood vaccinations. The pandemic and associated disruptions have strained health systems, with 25 million children missing out on vaccination in 2021, 5.9 million more than in 2019 and the highest number since 2009. Such a strong shock to the healthcare in the world could not but affect the parent's opinion and their attitude towards routine children's vaccinations. At the same time Infodemic during the COVID-19 pandemic posed a threat to health care and public health that required action plans to manage it. Media platforms and social media (one of available sources of information for parents) have become the source and tools of spreading false rumors and misinformation. The Republic of Belarus, like other countries, faced the same healthcare issues during the pandemic.

Materials & Methods

The aim of this study is to analyze parents' attitudes towards routine vaccination and to identify their sources of information about vaccination in Republic of Belarus in the COVID-19 pandemic. Data were collected from July to October 2022 using a survey (Google form) by social networks (Instagram, Telegram, Viber, WhatsApp). In total, 459 questionnaires were received, but only 427 questionnaires were analyzed after applying the inclusion criterion by Jamovi 2.2.5.

Results and Discussion

Overall sentiment towards routine children's vaccinations in Republic of Belarus is positive – 85.2% respondents believe that children should be vaccinated, 4% respondents found it difficult to answer that can indicate vaccine hesitancy. Vaccination is a voluntary procedure, and a person has the right to refuse it in accordance with the legislation of the Republic of Belarus. Refusal to vaccinate does not result in fines or denial of admission to kindergartens and schools in the future. At the same time only 83.4% respondents agree that the vaccines are effective. 69.8 % parents indicated that their attitude towards routine children's vaccinations did not change by COVID-19 pandemic and 19.2% respondents found it difficult to answer. Parents indicate that the most available sources of information for them are health organization website and social media; the most trusted sources are information received at the appointment from a doctor or nurse and health organization website; and the most used sources are information received at the appointment from a doctor or nurse and social media. However, only 47.5% respondents agree that they can indicate that information about the vaccination of children in the media or on the Internet site is unreliable (false).

Conclusions

Parents who took part in the survey have a positive attitude towards routine vaccination of children and believe that vaccines are effective. According to the research results in the Republic of Belarus, the COVID-19 pandemic has not caused significant changes in their attitudes. However, vaccine hesitancy among parents and their sources of information requires attention and action.

Session II: Development of HIV Vaccines

Chair: Prof. Jean Loius Excler

Current Approaches to HIV Vaccine Development – Do We Get a Boost?

Jean Louis Excler, International Vaccine Institute, Seoul, Republic of Korea; JeanLouis.Excler@ivi.int

Therapeutic HIV-1 Vaccines for HIV-1 Infected Children: Update from the Hurrican Study

Paolo Palma, Academic Department of Pediatrics, Children Hospital / Ospedale-Pediatrico-Bambino-Gesu, Roma, Italy; paolo.palma@opbg.net / paolo.palma@uniroma2.it

HIV-1 Vaccine Trials in Sub-Saharan Africa

Frank Msafiri, Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania; frankmbulinyingi@yahoo.com

Dissecting Immune Correlates of Risk of SIV Acquisition in Macaques Vaccinated with a Promising HIV Vaccine Candidate

Massimiliano Bissa, Animal Models and Retroviral Vaccines Section, National Cancer Institute, Bethesda, Maryland, USA; Massimiliano.Bissa@nih.gov

CURRENT APPROACHES TO HIV VACCINE DEVELOPMENT – DO WE GET A BOOST?

Jean Louis Excler

International Vaccine Institute, Seoul, Republic of Korea

JeanLouis.Excler@ivi.int

Despite tremendous advances made in the global HIV response, overall progress in HIV prevention efforts remains too slow to reach the 2030 targets. In 2021, 1.5 million people became newly infected with HIV. In 2021, around 650 000 people died from AIDS-related illnesses worldwide, compared to 2.0 million in 2004 and 1.4 million in 2010. About 62% of the new infections are among, key populations: men who have sex with men, sex workers, transgender people and people who inject drugs, and their sexual partners. In sub-Saharan Africa women and girls accounted for 63% of all new HIV infections. Ending HIV as a global health threat will require rapid scale-up to near universal coverage of high-impact HIV prevention, and treatment interventions.

The heterologous prime-boost RV144 is the only HIV vaccine trial that has demonstrated efficacy, with 31% efficacy at 3.5 years and in a post hoc analysis, 60% at 12 months. The identification of immune correlates of risk has shed light for the assessments of other vaccines. However, all other HIV vaccine approaches tested since including the 'promising' mosaic antigen-based Ad26 trial HVTN 702 failed to show efficacy. One could make the argument that a vaccine could be licensed if it reaches 60%.

The National Institute of Allergy and Infectious Diseases (NIAID) has launched a Phase 1 clinical trial (HVTN 302) for three mRNA HIV vaccines. Moderna and the International AIDS Vaccine Initiative (IAVI) are partnering. Scientists at IAVI and Scripps Research developed the vaccine antigens as proteins. They previously tested the prime antigen in an adjuvanted protein-based vaccine, which induced the desired B-cell response among 97% of trial participants. Based on these findings, IAVI administered the first doses of an investigational mRNA HIV vaccine to volunteers in a phase 1 clinical trial. The vaccine candidate uses prime and boost antigens to induce specific B-cell responses that ideally will lead to the development of broadly neutralizing HIV antibodies. With Moderna's mRNA platform, the prime-boost combination tested in the current trial could be the first in a vaccine series that induces a range of broadly neutralizing antibodies. IAVI is sponsoring the first mRNA HIV vaccine in Rwanda and South Africa.

The current approved COVID-19 vaccines have set the efficacy bar very high. Whether communities will accept an HIV prevention vaccine with a lower (e.g., efficacy 60%) is unknown. Combination of HIV vaccine and PrEP (e.g., PrEPVacc) is an interesting approach that deserves close attention in particular with the development of long-lasting injectable PrEP. Community preparedness for HIV vaccine trial results will be key to be prepared to accept whatever results we might end up getting.

HIV remains a global pandemic that kills more than 700,000 every year. Finding an HIV vaccine has proven to be a daunting scientific challenge. With the success of safe and highly effective COVID-19 vaccines, we have an exciting opportunity to learn whether mRNA technology can achieve similar results against HIV infection. Finding an effective HIV vaccine is not an option. It is a must.

THERAPEUTIC HIV-1 VACCINES FOR HIV-1 INFECTED CHILDREN, UPDATE FROM THE HURRICAN STUDY

Paolo Palma[1,2], Nicola Cotugno[1,2], Chiara Pighi[1], Paolo Rossi[1,2], Britta Wahren[3], Mark F Cotton[4], Shaun Barnabas[4] & Merlin Robb[5]

1 - Academic Department of Pediatrics (DPUO), Research Unit of Clinical Immunology and Vaccinology, Bambino Gesù Children's Hospital, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), Rome, Italy.

2 - Chair of Pediatrics, Department of Systems Medicine, University of Rome "Tor Vergata", Rome, Italy.

3 - Department of Microbiology, Tumor and Cell Biology, Karolinska Institute, Stockholm, Sweden

4 - Family Center for Research with Ubuntu, Department of Paediatrics and Child Health, Stellenbosch University, Tygerberg, South Africa.

5 - U.S. Military HIV Research Program, Walter Reed Army Institute of Research, Silver SpringbHenry M. Jackson Foundation for The Advancement of Military Medicine, Bethesda, Maryland, USA

paolo.palma@opbg.net / paolo.palma@uniroma2.it

Background

The HVRRICANE project is a phase I, proof of concept, open-label, randomized clinical trial to evaluate the safety and effects of a prime-boost HIVIS DNA and MVA-CMDR vaccine regimen with or without Toll-like Receptor 4 agonist on HIV reservoirs in adolescents with perinatally acquired HIV. This study represents the first combined therapeutic HIV vaccine study in pediatrics.

Aims

The overall aims of HVRRICANE are 1) to quantitate and characterize the HIV reservoirs before and after HIVIS DNA ± TLR4 agonist and MVA-CMDR vaccination 2) to characterize HIV-specific cellular and humoral immune responses before and after vaccination and assess their relationship to the HIV reservoir endpoints. Our goal is to develop therapeutic HIV vaccines to reduce the HIV reservoir in children and youth. Our scientific premise is that the prime-boost HIVIS DNA and MVA-CMDR vaccines induce cellular and humoral immune responses important for clearing infected cells. We firstly included early treated children because of their healthy immunity and small HIV reservoirs. Other novel components include giving a licensed human papilloma virus vaccine that contains the toll-like receptor (TLR) 4 agonist adjuvant to boost immune responses to HIVIS DNA. In addition, we will administer MVA-CMDR to the only 10 HIV+ children worldwide after an HIVIS DNA vaccine as this late boost strategy enhances immunity in adults. We will only immunize South African children but may extend the study, depending on results. Twenty-five participants between 9 and 18 years of age were enrolled in this 48-week study. All started HIV medications prior to 6 months of age and are virally suppressed. They have been randomly assigned to HIV vaccines (n=10) vs. HIV vaccines+TLR4 agonist (n=10) vs. control (no interventions) (n=5). Vaccines were administered through a needle free device (PharmaJet) at weeks 0,4 and 12 for HIVIS DNA with or without TLR4 agonist. No dose limiting adverse events were observed or reported by patients and their families. MVA-CMDR boost are being administered at weeks 24 and 36.

Topics overviewed

Persistence of long-lived latent reservoirs is a major barrier to viral remission and cure. Seeding of the HIV reservoir occurs early during acute HIV infection and sets the stage for establishment of latent reservoirs, particularly in long-lived CD4+ T cells present throughout the immune system. Early ART limits the size of the HIV reservoir and its evolution in the peripheral blood and other organs. Early treated children are an ideal population to investigate therapeutic HIV vaccines. The goal of therapeutic HIV vaccines is to augment virus-specific immune responses and either accelerate the decay of the reservoir during ART or improved control of viral rebound after interruption of ART. In next year we will measure the changes of HIV reservoirs and immune responses post interventions.

Conclusions

This project, funded by NIH, was initially fueled and supported by the EPIICAL consortium (www.epiical.org) whose major goal is to elevate the understanding of strategies towards HIV remission in children. The knowledge generated will contribute to the optimization of therapeutic HIV vaccine strategies and exert sustained influence on HIV cure research for children and youth.

Acknowledgments

We thank patients and their families for joining with enthusiasm the HVRRICANE study. We acknowledge NIH for funding this project through the U01AI135941 grant and the EPIICAL consortium (www.epiical.org) for supporting this initiative.

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HIV VACCINE TESTING IN SUB-SAHARAN AFRICA

Frank Msafiri

Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania

frankmbulinyingi@yahoo.com

Background
Despite the remarkable success of antiretroviral therapy and other HIV prevention methods in reducing HIV transmission and AIDS associated mortality, sub-Saharan Africa continues to be hard hit by HIV. Of the 1.5 million people worldwide who acquired HIV in 2021, half (51%) of them were in sub-Saharan Africa, demonstrating the need for an effective vaccine to curb transmission of HIV-1 subtype C. We reviewed HIV vaccine trials in sub-Saharan Africa to highlight the progress in the search for a sustained control of the HIV epidemic in the region.

Materials & Methods
A comprehensive list of HIV vaccine clinical trials was obtained from the ClinicalTrials.gov database. We searched for trials using "HIV" as the condition of interest and further selected interventional trials using the study type identifier field. From the generated list, we kept only clinical trials that evaluated the safety, immunogenicity and efficacy of HIV vaccine candidates in healthy and HIV infected individuals. A total of 383 HIV vaccine trials were retrieved.

Results
Of the 383 HIV vaccine clinical trials conducted globally, only 46 (12%) took place in sub-Saharan Africa. About two-thirds of them were phase I trials (29/46, 69.1%), while a quarter were phase IIa studies (12/46, 26.1%). The phase I and IIa trials in Africa constituted 9.6% (29/302) and 16.9% (12/71) of the global phase 1 and II studies. Five vaccine concepts have been evaluated for efficacy, four of which were unsuccessful, while one phase IIb trial (PrEPVacc) is ongoing. The HIV vaccine trials in sub-Saharan Africa investigated nine viral vectors, eight DNA plasmids, five envelope proteins, five adjuvants, three monoclonal antibodies, and one Tat protein. The viral vectors expressed gag, protease, pol, env, nef, reverse transcriptase and tat genes from HIV-1 subtypes A, B, C, E and mosaic sequences. The DNA plasmids encoded genes from subtypes A, B, C and E. Half of all HIV vaccine trials in sub-Saharan Africa took place in South Africa (23/46, 50%). Only five (5/46. 10.9%) were conducted in children. Two phase I mRNA HIV vaccine trials are ongoing.

Conclusions
Increasing the number of phase I and IIa trials evaluating HIV-1 subtype C immunogens in sub-Saharan Africa is essential for the control of epidemic in the region. Clinical trials investigating the effectiveness of broadly neutralizing monoclonal antibodies in preventing HIV infection should be increased.

DISSECTING IMMUNE CORRELATES OF RISK OF SIV ACQUISITION IN MACAQUES VACCINATED WITH A PROMISING HIV VACCINE CANDIDATE

Massimiliano Bissa [1], S. Kim [2], V. Galli [1], S. Fourati [3,4], S. Sarkis [1], I.S. Castro [1], M.A. Rahman [1], S. Fujiwara [2], M. Vaccari [1], J.A. Tomalka [3,4], J.D. Stamos [1], L. Schifanella [1], R. Moles [1], A. Gutowska [1], M.N. Doster [1], D.J. Venzon [5], H. Choo-Wosoba [5], T. Cardozo [6], R.P. Sekaly [3,4], G.L. Hager [2], G. Franchini [1]

1 - Animal Models and Retroviral Vaccines Section, National Cancer Institute, Bethesda, Maryland, USA
2 - Laboratory of Receptor Biology and Gene Expression, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA
3 - Department of Pathology, Case Western Reserve University, Cleveland, Ohio, USA
4 - Department of Pathology, Emory University, Atlanta, Georgia, USA
5 - Biostatistics and Data Management Section, Center for Cancer Research, National Cancer Institute, Bethesda, Maryland, USA
6 - New York University School of Medicine, NYU Langone Health, New York, NY, USA

massimiliano.bissa@nih.gov

Introduction

UNAIDS reports that 1.5 million people became newly infected in 2020. A highly effective vaccine will be key to preventing HIV infection. Vaccination with the HIV clades B/A/E recombinant canarypox–derived vector (ALVAC) and bivalent clade AE/B gp120-envelope proteins in alum significantly reduced the risk of HIV acquisition in the RV144 HIV Phase III vaccine trial in Thailand. Both RV144 and pre-clinical studies in macaques identified antibodies to SIV/HIV variable region 2 (V2), CD4 cells, and Antibody Dependent Cell Cytotoxicity (ADCC) as correlates of risk of HIV/SIV acquisition, highlighting this animal model's relevance to HIV in humans. During the years we have improved in macaques the ALVAC-poxvirus based prime-boost approach by substituting the initial prime with DNA encoding Viral Like Particles bearing an envelope protein with deleted V1, and boosting with monomeric V1-deleted gp120 protein, formulated in alum. Additionally, at the aim of further improving the vaccine efficacy we conducted in-depth characterization of the innate and adaptive responses to vaccination.

Aims

Due to the reproducible identification of CD14+ monocytes as a correlate of reduced risk of infection of the DNA/ALVAC/gp120/alum vaccine, and its durability, we hypothesized that the vaccine efficacy could be linked to epigenetic reprogramming of these innate cells. Additionally, since in our studies RAS activation was identified as a mechanism mediating responses correlated with reduced acquisition, we hypothesized that Insulin-like Growth Factor 1 (IGF-1), being a RAS activator, could affect the efficacy of the ALVAC-SIV/gp120 platform by modulating the immune responses.

Material & Methods

To test these hypotheses, we conducted two studies in macaques immunized with DNA/ALVAC/gp120/alum regimen, with or without IGF-1, and exposed to intravaginal challenges with low doses of SIVmac251. Samples collected prior to and following vaccination were analyzed with different canonical (ADCC, flowcytometry, luminex and CD14+ efferocytosis of apoptotic neutrophils) and multiomics (RNA-, and ATAC-sequencing) analyses.

Results

The analyses confirmed the central role of ADCC, and particularly the V2-specific one, in decreasing the risk of acquisition. In addition, we found that, efferocytosis, a cyclic AMP (cAMP)-dependent process of CD14+ monocytes that clear engulfed apoptotic cells, is a novel correlate of reduced risk of SIVmac251 acquisition that complements V2-ADCC. The vaccine-induced modification of the chromatin accessibility to cAMP response element-binding protein 1 (CREB1) within CD14+ monocytes was linked to the level of the V2-

specific ADCC. Moreover, the analyses showed that the engagement of the CCL2/CCR2 axis and tolerogenic dendritic cells producing IL-10 (DC-10) is central to vaccine efficacy.

Following vaccination combined with IGF-1 administration, the antibody response to V2 region and the frequency of CD14+ cells increased; however, the vaccine efficacy was not affected.

Conclusion

These data posit that epigenetic reprogramming in CD14+ cells and its effect on efferocytosis, through the prompt and effective removal of apoptotic infected cells, contributes to vaccine efficacy by decreasing inflammation and maintaining tissue homeostasis. Additionally, IGF-1 administration seems to synergize with the ALVAC-SIV/gp120/alum regimen but is not sufficient to improve significantly vaccine efficacy.

Sesion III: HPV Infection & HPV Vaccination

Chair: Prof. Gunta Lazdāne

The Population-Level Impact and Herd Effects of the Introduction of HPV Vaccination in Sweden

Tina Dalianis, Institute for Oncology Pathology, Karolinska Institutet, Stockholm, Sweden; tina.dalianis@ki.se

Knowledge and Attitude to HPV Vaccination among Women in Latvia

Anda Ķīvīte-Urtāne, Rīga Stradiņš University, Latvia; anda.kivite-urtane@rsu.lv

HPV and HPV Vaccination in People Living with HIV-1

Anna-Lise Williamson, University of Cape Town, South Africa, anna-lise.williamson@uct.ac.za

HPV Genotype Distribution in PWLH for Anal Screening and Early Detection of Anal Cancers

Sona Chowdhury, University of California, San Francisco, USA; sona.chowdhury@ucsf.edu

Role of E6* Isoform Expression as a Marker of Active HPV Infection in Head and Neck Cancers

Andrea Cerasuolo, Istituto Nazionale Tumori IRCCS Fond. Pascale, Naples, Italy; a.cerasuolo@istitutotumori.na.it

THE POPULATION-LEVEL IMPACT AND HERD EFFECTS OF THE INTRODUCTION OF HPV VACCINATION IN SWEDEN

A. Ährlund-Richter[1], N. Grün[1], C. Nordfors[1], L. Chen [2], L. Haeggblom [1], A. Näsman [1], J. Du [1], **Tina Dalianis** [1]

1 - Department of Oncology and Pathology, Karolinska Institutet, Stockholm, Sweden
2 - Department of Microbiology, Tumor and Cell Biology, Centre for Translational Microbiome Research (CTMR), Karolinska Institutet, Stockholm, Sweden

tina.dalianis@ki.se

Background
Human papillomavirus (HPV) infection is one of the most common sexually transmitted diseases. Furthermore, in addition to its causative effect on anogenital cancer it has now been shown and acknowledged that HPV also is involved in the cause and increase of oropharyngeal squamous cell carcinoma, especially tonsillar and base of tongue squamous cell carcinoma. The incidences of these two diseases have increased epidemically in Sweden the past decades. However, in parallel, HPV vaccination has been gradually introduced in Sweden.

Topics overviewed
This presentation will include early data on the prevalence of HPV in tonsillar and base of tongue cancer. In addition, in parallel it presents data of oral and cervical HPV type prevalence at a youth clinic in Stockholm Sweden over one decade, before and after the HPV vaccine was introduced into the school based vaccination program.

Conclusions
Our data suggest that the HPV vaccine has had effects, however, although the HPV vaccine has contributed to reduce the prevalence of HPV infections and HPV-related cancers, not all HPV subtypes are covered.

KNOWLEDGE AND ATTITUDE TO HPV VACCINATION AMONG WOMEN IN LATVIA

Anda Kivite-Urtane[1,2], Jana Zodzika[1], Natalija Berza[1], Anneli Uuskula[3]

1 - Institute of Public Health, Riga Stradins University, Riga, Latvia
2 - Department of Public Health and Epidemiology, Riga Stradins University, Riga, Latvia
3 - Institute of Family Medicine and Public Health, Department of Epidemiology and Biostatistics, University of Tartu, Tartu, Estonia

Anda.Kivite-Urtane@rsu.lv

Background
There is an organized population-based HPV vaccination programme in Latvia (introduced in 2010). Vaccination of the target population is free of charge and includes 12-18-year-old girls in Latvia (since 2022 the vaccine is sex neutral). Vaccination is provided by general practitioners. Despite the fact that the vaccination coverage in Latvia is higher than, for example, in neighboring country Estonia, it could be increased (in 2019 it was 69%), considering the relatively high incidence and mortality rates of cervical cancer in Latvia. Awareness and attitudes towards vaccination play a vital role in promoting coverage. Therefore, it would be useful to find out the population groups in which these indicators are low.

Aim
To find out the awareness about and attitude towards the HPV vaccine, as well as the factors associated with knowledge and attitude in Latvia.

Materials & Methods
From February 2021 to February 2022 there a cross-sectional study was carried out in Latvia. Data on 1313 women aged 25-70 years was collected via self-filled questionnaire and self-collected vaginal sample for HPV testing. Women were recruited via the colposcopy specialists of the Outpatient Department of the Riga East Clinical University Hospital (RAKUS) and via ten general practitioners' practices (2 in each of the Regions of Latvia). The Ethics Committee of Riga Stradins University approved the study. The EEA and Norwegian financial instrument for the 2014-2021 financed the study (project No EMP416).

SPSS 26.0 software was used for data processing. Multivariate logistic regression analysis was performed to find out the factors associated with the knowledge about and attitude towards the HPV vaccine. Results were considered as statistically significant if $p<0.05$.

Results
71% of women have heard that there is a vaccine against HPV. Only 52% of women considered that it is necessary to vaccinate 12-year-old girls against HPV in Latvia. And only 3% reported that they are vaccinated against HPV.

Better awareness about HPV vaccine was significantly associated with Latvian nationality, higher levels of education and income, presence of chronic health conditions or STI anamnesis and non-usage of alcohol. Sexual behaviour or reproductive anamnesis as well as usage of healthcare services were not significantly associated with awareness of HPV vaccine.

Supportive attitude towards vaccination was significantly associated with higher levels of income, nonsmoking status, visiting gynaecologist within the last year and presence of STI risk (self-assessed).

Conclusion
Awareness about and attitude towards the HPV vaccine in Latvia is associated with a range of socio-demographic and economic, health behavioral and health care factors.

HUMAN PAPILLOMAVIRUS (HPV) AND HPV VACCINATION IN PEOPLE LIVING WITH HIV

Anna-Lise Williamson

UCT/SAMRC Gynaecological Cancer Research Centre, University of Cape Town, South Africa

anna-lise.williamson@uct.ac.za

Cervical cancer and a number of other cancers are causally associated with human papillomavirus (HPV). The World Health Organisation Global Strategy for the Elimination of Cervical Cancer has the following targets: 90% of girls fully vaccinated with the HPV vaccine by the age of 15; 70% of women screened with a high-performance test by 35 and 45 years; treatment of 90% of women with precancer to eliminate cancer within the next century. The best intervention, particularly in low-income countries with inadequate cervical screening programs, is to vaccinate girls against HPV. The efficacy of the vaccination could be increased if boys were also vaccinated which would increase herd immunity.

Cervical cancer is a disease of poverty and 90% of cervical cancer deaths occur in low- and middle-income countries. Sub-Saharan Africa has the highest incidence of human papillomavirus (HPV) and cervical cancer in the world, which is further aggravated by the burden of human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) disease, with invasive cervical cancer being an AIDS-defining cancer. HIV-infected people have a very high prevalence of HPV which puts them at elevated risk of persistent infection and of developing HPV associated cancers. The complex interaction between HIV and HPV increases the risk of HIV infection in HPV infected people.

While there is evidence that HIV positive individuals have an immune response to HPV vaccines and that the vaccines are safe, there are still knowledge gaps as far as efficacy and long term follow up is concerned. There is little published data on the nine-valent vaccines (GARDASIL 9) and in HIV positive individuals. As trials are being done to determine the efficacy of one immunisation vs two immunisation of HPV vaccine, it is important that HIV positive people are not excluded from these trials as they would provide useful information on the vaccine regimens for this vulnerable group. The nine-valent vaccines would give better coverage than the two or four valent HPV vaccines, as HIV positive individuals are often infected with multiple types of HPV. It is important to determine efficacy of vaccination in HIV positive individuals by tracking HPV infection and associated disease. This would determine if catch-up campaigns vaccinating older individual had benefit, and if booster vaccinations were needed.

Besides the national programmes to vaccinate girls against HPV and screen women for cervical cancer, there should be targeted cervical cancer screening, treatment and prevention programmes introduced into HIV treatment centres.

HPV GENOTYPE DISTRIBUTION IN PLWH FOR ANAL SCREENING AND EARLY DETECTION OF ANAL CANCERS

Sona Chowdhury [1], T.M. Darragh [2], J.M. Berry-Lawhorn [3], M.G. Isaguliants [4,5], M. Vonsky [6,7], J.F. Hilton [8], A.A. Lazar [9]

1 - Division of Infectious Diseases, Department of Medicine, University of California, San Francisco, CA, USA
2 - Department of Pathology, University of California, San Francisco, CA, USA
3 - Department of Medicine, University of California, San Francisco, CA, USA
4 - Karolinska Institutet, Stockholm, Sweden
5 - Institute of Microbiology and Virology, Riga Stradins University, Latvia
6 - D.I. Mendeleyev Institute for Metrology, St.Petersburg, Russia
7 - Almazov National Medical Research Center, St.Petersburg, Russia
8 - Department of Epidemiology and Biostatistics, University of California, San Francisco, CA, USA
9 - Division of Oral Epidemiology, Division of Biostatistics, University of California, San Francisco, CA, USA

sona.chowdhury@ucsf.edu

Background
The incidence of anal cancer is increasing especially in high-risk groups such as people living with HIV (PLWH). Human papillomavirus (HPV)-16, a high-risk (HR) HPV genotype, is the most common genotype in anal high-grade squamous intraepithelial lesions (HSIL) and squamous cell carcinoma (SCC) in the general population. However, few studies have described the distribution of HR HPV genotypes other than HPV 16 in the anus of PLWH.

Materials & Methods
HPV-genotyping was performed by DNA amplification followed by dot-blot hybridization; to identify the HR and low-risk (LR) genotypes in benign anal lesions, HSIL, SCC of PLWH and HIV-negative individuals.

Results
HPV 16 was the most prominent HR HPV identified, but it was less common in HSIL and SCC from PLWH compared with HIV-negative individuals, and other non-HPV 16 HR HPV (non-16oncHPV) types, were more prevalent in samples from PLWH. A higher proportion of clinically normal tissues from PLWH were positive for one or more HPV genotypes. Multiple HPV infection was a hallmark feature for all tissues (benign, HSIL, SCC) of PLWH.

Conclusions
These results indicate that development of anal screening approaches based on HPV DNA testing need to include non-16oncHPVs along with HPV 16 especially for PLWH. Along with anal cytology these updated screening approaches may help to identify and prevent anal disease progression in PLWH.

ROLE OF E6* ISOFORM EXPRESSION AS A MARKER OF ACTIVE HPV INFECTION IN HEAD AND NECK CANCERS

Andrea Cerasuolo[1], N. Starita[1], A.L. Tornesello[1], G. Salzano[2], F. Ionna[2], R. Rega [3], R. De Cecio [3], G. Ferrara [3], F.M. Buonaguro [1], M.L. Tornesello [1]

1 - Molecular Biology and Viral Oncology Unit, Istituto Nazionale Tumori IRCCS Fond. Pascale, Napoli, Italy
2 - Maxillofacial and ENT Surgery Unit, Istituto Nazionale Tumori IRCCS Fond. Pascale, Napoli, Italy
3 - Pathology Unit, Istituto Nazionale Tumori IRCCS Fond. Pascale, Napoli, Italy

a.cerasuolo@istitutotumori.na.it

Background
Human papillomavirus (HPV) has emerged as a novel etiological agent for a sub-group of head and neck squamous cell carcinomas (HNSCCs). The HPV-related HNSCCs have distinct molecular and prognostic characteristics compared to HPV-unrelated HNSCCs, thus it is important to properly identify all cases associated with viral infection. Since HPV DNA detection does not differentiate transient infections from transforming infections new molecular markers of active HPV are needed for a more accurate diagnosis of HPV-related HNSCC. The aim of this study was to evaluate the role of HPV16 E6* isoform expression as a marker of transforming viral infections.

Materials & Methods
HPV DNA was searched in 31 HNSCC cases, including 14 oropharyngeal carcinoma (OPC), 9 oral carcinoma (OC); 5 laryngeal carcinoma (LC), 1 nasopharyngeal carcinoma (NPC), 1 hypopharyngeal carcinoma (HPC), 1 salivary gland carcinoma (SGC) and 9 head and neck dysplasia (HND) by broad spectrum PCR. HPV16 viral load and E6/E6*I RNA levels were analysed by real time PCR in the samples positive for this viral genotype. In particular, the E6/TP53 copy number ratio was calculated for the viral load analysis. Then, the correlation between the viral load and levels of viral transcripts was statistically evaluated.

Results
HPV was found in 45% of HNSCCs (36% of OPC, 37% of OC, 20% of LC, 100% of NPC and SGC) and 33% of HND. The most frequent viral genotype was HPV16 detected in 86% HPV-positive samples. The HPV16 viral load was higher in HNSCCs (<1 to 115 copies/genome equivalent) than in HND (<1 copy/genome equivalent). The E6 and E6*I transcripts were expressed in 40% and 50% of HNSCC, respectively, with E6*I levels being higher than E6 levels. Notably, no viral transcripts were detected in a fraction of HPV-16 positive OC as well as in LC and SGC. Neither E6 nor E6*I expression was detected in HND. Finally, a statistically significant correlation was found between the viral copy number and E6*I expression levels (p=0.047).

Conclusions
In conclusion, we confirm that HPV16 is the most frequent genotype detected in HPV-related HNSCCs and that E6*I is a sensitive molecular marker of HPV transforming infection in HNSCC, irrespective of the anatomic site.

Acknowledgments
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Session IV: Cancer Vaccines

Chair: Prof. Franco M. Buonaguro

Therapeutic Vaccines to Treat Chronic HPV Infection and Associated Cancer

Joel Palefsky, University of California, San Francisco, USA; joel.palefsky@ucsf.edu

Development of cancer vaccine for HCC

Luigi Buonaguro, National Cancer Institute 'Fondazione Pascale', Naples, Italy; l.buonaguro@istitutotumori.na.it

DNA and mRNA Vaccines for Cancer: Rationale, Mechanisms and Progress

Margaret Liu, Protherimmune, Karolinska Institutet, and University of California, San Francisco, USA; liu@protherimmune.com

An All-in-One Adjuvanted Therapeutic Cancer Vaccine Approach Targeting Dendritic Cells to Indeuce Potent Tumor Killing Cellular Immune Responses

Department of Microbiology, Clinical Vaccine R&D Center, and Combinatorial Tumor Immunotherapy MRC, Chonnam National University Medical School, Gwangju, Republic of Korea; happypa1101@gmail.com

Modelling HPV-Associated Carcinogenesis in Murine Adenocarcinoma 4T1 Cell Line Expressing HPV 16 Oncoproteins E6 and E7

Alesja Dudorova, Pauls Stradiņš Clinical University Hospital, and Rīga Stradiņš University, Latvia; alesjadudorova@gmail.com

DNA Immunization with HPV 16 E6, but not E7, Induces Specific CD4+ T Cell Response and Hinders Growth and Metastatic Activity in Mice Of Adenocarcinoma Cells Made to Express E6 and E7

Juris Jansons, Latvian Biomedical Research and Study Centre, and Rīga Stradiņš University, Latvia; jansons@biomed.lu.lv

THERAPEUTIC VACCINES TO TREAT CHRONIC HPV INFECTION AND ASSOCIATED CANCER

Joel Palefsky

University of California, San Francisco, USA

Joel.Palefsky@ucsf.edu

Background

Approximately 5% of all cancers are caused by human papillomavirus (HPV), predominantly HPV 16. The most common of these is cervical cancer. Cervical cancer is largely concentrated in those parts of the world that struggle to provide routine primary prevention (HPV vaccination) or secondary prevention (detection and removal of the cancer precursor lesion, high-grade squamous intraepithelial lesions (HSIL) before it can progress to cancer). The World Health Organization is currently leading a global campaign to eliminate cervical cancer through promotion of primary and secondary prevention as well as effective treatment of HSIL and cancer.

Current treatments for cervical HSIL rely primarily on ablative or excisional measures to remove the lesions, and treatment of cervical cancer relies on varying combinations of chemotherapy, radiation therapy or surgical excision. Currently there are no therapies targeted specifically at the etiologic agent, HPV. Effective HPV-specific therapies would be ideal to achieve clinical cure with minimal adverse effects on normal tissues.

While HPV is the most commonly sexually transmitted agent, only a small proportion of HPV-infected individuals develop a clinically-detectable HSIL or cancer, presumably due to induction and maintenance of an effective and durable cell-mediated immune (CMI) response which controls viral replication and/or transformation. Consistent with this, conditions that lead to attenuated CMOI response, such as HIV infection, are associated with increased risk of HPV-associated cancer. Boosting HPV-targeted CMI response in the form of a therapeutic vaccine represents an attractive approach to restoring effective CMI leading to immune-mediated clearance of the lesion.

Several attempts to treat lesions with therapeutic vaccines has been made in the past, and success has been modest. Some of the most successful have been long peptide vaccines against HPV oncoproteins E6 and E7, and full-length peptide vaccines delivered through a variety of vectors, adjuvants and delivery methods, such as electroporation. There are many challenges to optimizing the design of a HPV therapeutic vaccine. These include: 1) lack of a clear understanding of the physiologic immune response controlling HPV infection, and therefore lack of knowledge of the most relevant immunologic pathways to target; 2) lack of clear understanding of the most important HPV antigens or combination of antigens to target; 3) lack of understanding of the ideal adjuvant and delivery system to achieve the high level of mucosal immune response that would be needed; 3) lack of understanding of the stage of HPV infection that would be most amenable to therapeutic vaccination, e.g., clinically latent HPV infection vs. HSIL vs cancer; 4) how to interpret animal model data to best predict success in human trials; 5) lack of understanding of how vaccines will perform for treatment of HPV-related disease at different mucosal sites; and 6) incomplete understanding of how to optimize the efficacy of a vaccine through combination with other immunomodulatory approaches, such as checkpoint inhibitors.

Topics overviewed

This presentation will review some of these challenges and describe current efforts by our group to develop therapeutic vaccines to prevent cervical and anal cancer in people living with HIV.

DEVELOPMENT OF CANCER VACCINE FOR HCC

Luigi Buonaguro

National Cancer Institute 'Fondazione Pascale', Naples, Italy

l.buonaguro@istitutotumori.na.it

Background
Hepatocellular carcinoma (HCC) is the most common liver malignancy, representing the third and the fifth leading cause of death from cancer worldwide in men and women, respectively. HCC prognosis is generally poor because of the low effectiveness of available treatments and the overall 5-year survival rate is approximately 5-6%. In this framework, immunotherapeutic interventions, including cancer vaccines, may represent a novel and effective therapeutic tool. However, only few immunotherapy trials for HCC have been conducted so far with contrasting results, suggesting that improvements in several aspects of the immunotherapy approaches need to be implemented. In particular, identification of novel specific tumor antigens and evaluation of most advanced combinatorial strategies could result in unprecedented clinical outcomes with great beneficial effect for HCC patients.

Aim(s)
The state of the art in immunotherapy strategies for HCC and future perspectives are reported in the present review.

Topics overviewed
The different immunotherapy strategies developed in the last years are described. The experimental approaches developed by the Author's group are described in great details, spanning from the discovery to pre-clinical and clinical application.

Conclusions
New target antigens and cancer vaccine formulation for HCC are generated and tested. New early stage clinical trials are about to be initiated. Improved clinical efficacy in HCC patients is at the horizon.

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DNA AND MRNA VACCINES FOR CANCER: RATIONALE, MECHANISMS AND PROGRESS

Margaret A. Liu [1,2]

1 - Karolinska Institutet, Stockholm, Sweden
2 - UCSF, San Francisco, USA

Liu@ProTherImmune.com

Cancer poses unique challenges for the development of therapeutic vaccines. The specific issues for both pathogen-mediated and other cancers will be presented to lay the groundwork for a discussion of the immune responses needed for such therapeutic vaccines. The immune mechanisms of DNA and mRNA vaccines and their attributes relevant to such therapies will be described along with an update of clinical trials.

AN ALL-IN-ONE ADJUVANTED THERAPEUTIC CANCER VACCINE APPROACH TARGETING DENDRITIC CELLS TO INDUCE POTENT TUMOR KILLING CELLULAR IMMUNE RESPONSES

Joon Haeng Rhee

Department of Microbiology, Clinical Vaccine R&D Center, and Combinatorial Tumor Immunotherapy MRC, Chonnam National University Medical School, Gwangju, Republic of Korea

jhrhee@chonnam.ac.kr

Background
Therapeutic cancer vaccines (TCVs) should induce robust tumor-specific T cell responses. To achieve this, TCVs incorporate T cell epitopes and strong adjuvants. Here, we report an all-in-one adjuvanted cancer vaccine platform that targets the intracellular compartment of antigen-presenting cells and subsequently induces effective cytotoxic T cell responses.

Materials & Methods
We screened a novel peptide (DCpep6) that specifically binds and transmits into CD11c+ cells through in vivo phage biopanning. We then engineered a protein-based TCV (DEF) consisting of DCpep6 (D), an optimized HPV E7 tumor antigen (E), and a built-in flagellin adjuvant (F) as a single molecule.

Results
DEF was stably expressed, and each component was functional. In vivo-administered DEF rapidly biodistributed in draining LNs and internalized into CD11c+ cells. DEF immunization elicited strong antitumor T cell responses and provided long-term survival of TC-1 tumor-implanted mice. The DEF-mediated antitumor effect was abolished in NLRC4-/- mice.

Conclusions
Taken together, we propose a protein-based all-in-one TCV platform that intracellularly co-delivers tumor antigen and inflammasome activator to DCs to induce long-lasting antitumor T cell responses.

MODELLING HPV-ASSOCIATED CARCINOGENESIS IN MURINE ADENOCARCINOMA 4T1 CELL LINE MADE TO EXPRESS HPV 16 ONCOPROTEINS E6 AND E7

Alesja Dudorova, J. Jansons [1,3], D. Avdoshina [1,4], D. Skrastina [3], S. Gebrila [1,2], S. Isajevs [5,6], A. Breiksa [2], I. Gordeychuk [1,4*], J. Nazarovs [2], M.L. Tornesello [7], F.M. Buonaguro [7], M. Isaguliants [1]

1 - Riga Stradins University, Riga, Latvia.
2 - Paul Stradins University Hospital, Riga, Latvia
3 - Latvian Biomedical Research and Study Center, Riga, Latvia
4 - Chumakov Federal Scientific Center for Research and Development of Immune-and-Biological Products of Russian Academy of Sciences, Moscow, Russia
5 - Department of Pathology, Faculty of Medicine, University of Latvia, Riga, Latvia
6 - Centre of Pathology, Riga East University Hospital, Riga, Latvia
7 - Molecular Biology and Viral Oncogenesis Unit, Istituto Nazionale Tumori - IRCCS - "Fond G. Pascale", Naples, Italy

alesjadudorova@gmail.com

Introduction

Human papillomaviruses of high oncogenic risk (HR HPV) are the main etiological agents of anogenital, head and neck, and laryngeal cancer. Therapeutic HR HPV vaccines are designed to eradicate or to reduce infected, often transformed, cells, mostly based on HR HPV E6 and E7 oncoproteins. Development of such vaccines requires a reliable, inexpensive and easily accessible small animal models. Mice cannot be infected with HPV, but murine cells, including tumor cells, can be made to express HPV oncoproteins. The question arises to what extent these cells would reproduce ones arising in natural infection and associated carcinogenesis.

Aim

Make murine mammary gland adenocarcinoma 4T1 cells to express HPV16 E6/E7 and study their properties in vitro and in vivo.

Material & Methods

DNA encoding HPV16 E6/E7 was recloned from pHPV16 plasmid (www.atcc.org/products/45113d) into lentiviral vector pLJM1 (Addgene) under the control of EF1a promoter. Resulting lentivirus was used to transduce 4T1luc2 cells (Calipers) generating nine 4T1luc2_E6E7 subclones. Levels of expression of E6/E7, TERT, EF1a mRNA were assessed by real-time RT-PCR. Telomerase activity in cells was assessed using the telomere repeat amplification protocol (TRAP). Cell cycle analysis after propidium iodide staining was performed on BD FACSCantoII cytometer (BD Biosciences). Genomic stability was assessed by counting γ-H2AX foci (anti-γ-H2AX MAb26350). Subclones were ectopically implanted into BALB/c mice; tumor growth was monitored by in vivo, and metastatic activity, by ex vivo organ bioluminescence imaging (BLI) (Lumina, PerkinElmer). Formalin-fixed paraffin-embedded tumors and organs were prepared, sectioned, stained with H&E, scanned on digital scanner (Leica). Images were analysed by ImageScope software (Leica). Data was analysed by nonparametrical statistics (STATISTICA 11; Graphpad).

Results

Subclones demonstrated low levels of expression of E6 and E7 as in naturally infected cells. During long-term cultivation, levels of E6/E7 mRNA expression decreased in all clones, whereas expression of EF1a from the same promoter on contrary increased. Expression of E6 and E7 changed pattern of expression of transcription factors HIF-1α, Twist, Nrf2, and cell cycle progression promoting passage of G2/M checkpoint. Subclones demonstrated a shift towards mesanchymal phenotype, manifested by increased expression of mRNA of Twist and Vimentin; levels of E6 and Vimentin mRNA were correlated ($r(E6/Vimentin)=0.9$; $p<0.0005$). Expression of E6/E7 induced DNA damage and overexpression of TERT mRNA ($r(E6/TERT)=0.8$; $p<0.05$). However, TERT enzymatic activity of subclones was unchanged compared to parental 4T1 cells. Implanted into mice,

4T1luc2_E6E7 cells formed poorly differentiated adenocarcinomas of solid structure not different in size and growth rate from the parental cells. Assessment of organs by ex vivo BLI demonstrated predominant infiltration of tumor cells into liver and lungs. Subclones did not differ in number and size of induced liver metastasis, both were inferior to that of parental cells. E6/E7 mRNA expression had no enhancing effect on either tumorigenic or metastatic activity.

Conclusion

As in HPV-associated squamous cell carcinomas, expression of HPV16 E6/E7 induced DNA damage, changed cell cycle accelerating G2/M passage, increased expression of Twist and Vimentin associated with EMT, and caused overexpression of TERT mRNA, although without any effect of TERT enzymatic activity. Tumorigenic or metastatic activities of 4T1luc2E6E7 subclones correlated with the decrease in proportion of cells in G2/M phase of the cell cycle. All effects were p53-independent (4T1luc2 cells are p53(-)) indicating that some features of HPV-associated carcinogenesis do not rely on its effect on p53. 4T1luc2E6E7 subclones express HPV16 oncoproteins, reproduce some of the features of HPV-associated carcinogenesis, and could be useful for testing HPV vaccines in BALB/c mice.

Acknowledgments

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DNA IMMUNIZATION WITH HPV 16 E6, BUT NOT E7, INDUCES SPECIFIC CD4+ T CELL RESPONSE AND HINDERS GROWTH AND METASTATIC ACTIVITY IN MICE OF ADENOCARCINOMA CELLS MADE TO EXPRESS E6 AND E7

Juris Jansons[1,2], Dace Skrastina[2], Alesja Dudorova[1,3], Elena Royo Rubio[1,2,4], Alina Nikolai[1], Jurijs Nazarovs[3], Maria Isaguliants [1]

1 - Riga Stradins University, Riga, Latvia
2 - Latvian Biomedical Research and Study Centre, Riga, Latvia
3 - Paul Stradins University Hospital, Riga, Latvia
4 - Laboratorio InmunoBiología Molecular, Instituto Investigación Sanitaria Gregorio Marañón (IiSGM), Hospital General Universitario Gregorio Marañón (HGUGM), 28009 Madrid, Spain

juris.jansons@biomed.lu.lv

Introduction

Human papillomaviruses (HPV) productively infect basal epithelial cells of the squamous-columnar junction of the skin, oral cavity and anogenital tract including cervix, causing malignization of infected cells during chronic infection. HPV is responsible for >95% of cervical cancer cases, with the major risk factor being infection with HPV16. HPV infections & associated cancer are preventable by prophylactic HPV vaccines. This leaves a need for therapeutic vaccine(s) to treat chronic infections and HPV-associated neoplasia and tumors. Promising vaccine components are viral oncoproteins E6/E7.

Aims

To evaluate immunogenicity in mice of consensus HPV16 E6 and E7, assess the effects on immunogenicity of single amino acid substitutions in E6 and E7 [1] and evaluate the protective potential of immunization against HPV16 E6/E7 expressing tumors.

Materials & Methods

Sequences of HPV16 E6/E7 derived from 35 HIV, TB or HIV/TB coinfected patients [2] were used to build the amino acid consensus of E6 and E7. Respective expression-optimized synthetic genes modified to exclude p53 and Rb-binding domains were cloned into pVax1 (pVaxE6, pVaxE7) and used in DNA-immunization. Groups of C57bl6 mice (n=5) received 2x20 μg of pVax1, or pVaxE6, or pVaxE7, or separate injections of 20 μg of pVaxE6 and of 20 μg pVaxE7 administered by prime/boost regimen [3]. Injections were done intradermally on days 1 and 21 and were followed by electroporation (CUY21 EditII, BEX). HPV-specific cellular response on day 10 post boost was evaluated by stimulation of murine splenocytes with E6- and E7-derived 25-30-mer oligopeptides covering clusters of epitopes in the regions of amino acid substitutions. E6-1.1 represented R17, E6-1.2 - G17; E6-4.1 - L90 and E6-4.2 - V90 variants of E6. T-cell response was assessed by FACS as percent of IFN-γ/IL-2/TNF-α-producing CD4+ and CD8+ T-cells as described [4]. Groups of BALB/c mice (n=5) immunized as described above were challenged at 11th day post boost, with subcutaneous injections of murine adenocarcinomas 4T1luc2 cells expressing HPV16 E6/E7 (5000 cells per site; two sites). Tumor growth was assessed by in vivo BLI on IVIS Spectrum imager (Perkin Elmer) and morphometrically. On day 21, mice were sacrificed, tumors and organs excised, weighed, and subjected to histochemical analysis by H&E staining using NIS software (Nikon). Infiltration of tumor cells into organs was assessed by ex vivo BLI. Data was analyzed using Statistica 11 (Tibco) and GraphPad Prism software.

Results

E6 and E7 oncoproteins were highly conserved and nearly identical to E6/E7 of the reference HPV16 strain NC_001526. E6 oncoprotein contained several single amino acid (aa) substitutions unique for each of the samples; nearly 50% samples contained substitutions R17G and L90V. To determine their role in E6 immunogenicity, we used peptides encompassing these regions carrying each of aa variants. E6-specific cellular immune response was detected as IFN-γ/IL-2/TNF-α production by CD4+ T-cells in response to E6 peptides, with no response by CD8+ T cells. Of note, E6-1.1 peptide representing R17-variant encoded by

DNA- immunogen was recognized by CD4+ T-cells, while peptide E6-1.2 representing G17-variant was not. C57bl/6 mice receiving pVaxE6+pVaxE7 developed weak anti-E6 response against E6-1.1 not significantly different from the background levels. No response was detected against E7-derived peptides. BALB/c mice DNA-immunized by E6, or E7, or E6 & E7 as separate injections, or empty vector were challenged with 4T1luc2 cells expressing low (4T1luc2eeB2) or high (4T1luc2eeH6) levels of HPV16 E6/E7 mRNA. Immunization caused reduction in the tumor size and weight, restricted infiltration of tumor cells into distal organs, and reduced the number of metastasis in the liver.

Conclusions

HPV16 E6 was immunogenic inducing IFN-γ/IL-2/TNF-α response of CD4+ T-cells against cluster of epitopes incorporating aa 17 of R17-E6 encoded by pVaxE6, but not against G17-E6 variant. Our data indicates that this region harbors T cell epitope recognized in mice and that its recognition depends on aa residue occupying position 17, with no cross-reactivity between R17 and G17 E6 variants. Thus, R17G may represent an immune escape mutation. No epitopes were localized in the region harboring aa 90 of E6. Immune response against HPV16 E7 was not detected. Immunization with HPV16 DNA partly suppressed growth and motility of murine adenocarcinoma cells expressing HPV16 E6E7 in syngeneic mice, and reduced number of liver metastasis. These results are important for the design of therapeutic HPV vaccines.

Acknowledgments

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Session V: Facing New and Re-Emerging Threats

Chair: Prof. Anke L. W. Huckriede

Advances in Vaccine Development for Crimean-Congo Hemorrhagic Fever Virus

Felicity J Burt, Division of Virology, Faculty of Health Sciences, University of the Free State, Bloemfontein, South Africa; burtfj@ufs.ac.za

A Human Blood Cell-Based in Vitro System for Assessing Vaccine Quality and Elucidating Vaccine-Induced Immune Mechanisms

Anke LW Huckriede, Department of Medical Microbiology & Infection Prevention, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands; a.l.w.huckriede@umcg.nl

HIV Vaccine-Induced Antibody Response Impacts the Accuracy of HIV Testing Algorithms in Sub-Saharan Africa

Charlotta Nilsson, The Public Health Agency of Sweden, and Department of Medicine, Karolinska Institutet, Stockholm, Sweden; charlotta.nilsson@folkhalsomyndigheten.se

Do We Need Hepatitis B Vaccine?

Tatjana Tallo, Folkhälsomyndighet, Solna, Sweden; tatjana.tallo@folkhalsomyndigheten.se

Combined Therapeutic/Prophylactic Hepatitis B VLP Vaccine Prototype

Irina Somiskaya, Latvian Biomedical Research and Study Centre, Riga, Latvia; irina@biomed.lu.lv

ADVANCES IN VACCINE DEVELOPMENT FOR CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS

Felicity J. Burt [1,2]

1 - Division of Virology, National Health Laboratory Service, Bloemfontein, South Africa
2 - Pathogen Research Laboratory, Division of Virology, University of the Free State, Bloemfontein, South Africa

burtfj@ufs.ac.za

Background

Crimean-Congo hemorrhagic fever virus (CCHFV) is a tick-borne zoonosis distributed in Africa, Asia, eastern and south western Europe and the Balkans. Humans become infected by tick-bite, or contact with infected tissues of livestock or patients. Disease ranges from mild to severe with fatality rates of up to 30%. The distribution of CCHFV correlates with that of ticks belonging to the genus Hyalomma. The distribution of these ticks has expanded in recent years, hence there is growing concern that this virus has the potential to spread. CCHFV is listed by the World Health Organization as a priority pathogen for research due to the absence of an approved vaccine or specific anti-viral treatment. The discovery of suitable animal models in recent years has enabled progress in vaccine development.

Aim

To summarize recent developments in vaccine research for CCHFV.

Overview

CCHFV vaccine development has been facilitated by the discovery of animal models that are permissive to infection, succumb to disease and share some similarity in disease pathology as described in human disease. Suitable models include interferon deficient mice and a non-human primate, Cynomologous macaque. Various vaccine candidate approaches have been described. Virus–like replicon particles expressing CCHF glycoproteins (GP), nucleoproteins (NP) and/or polymerase protein (L) conferred protection against challenge with survival rates varying from 40% to 100%. Interferon deficient mice immunized with DNA based vaccines expressing GP precursor or NP had 50% to 100% survival rates when challenged. Vectored vaccines have showed similar ranges of survival. The presence of neutralising antibody did not necessarily correlate with protection suggesting that neutralising antibody is not the sole correlate of protection and protection likely requires both B and T cell responses. Protection in mice immunized with NP suggests a role for non-neutralising antibody.

Conclusions

The recent expansion of CCHFV endemic areas is a public health concern and this threat will continue with climate change and expansion of vector populations into new regions. Vaccine development, traditionally hampered by lack of a suitable animal model, has progressed in recent years due to availability of animal models. Further understanding of the immune correlates of protection will contribute towards development of an efficacious vaccine for a virus with potential to cause significant human disease.

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A HUMAN BLOOD CELL-BASED IN VITRO SYSTEM FOR ASSESSING VACCINE QUALITY AND ELUCIDATING VACCINE-INDUCED IMMUNE MECHANISMS

Anke L.W. Huckriede [1], A. Signorazzi [1], E. Marilena [2], G.T. Calle [1], S.H. Gong [1], E. Coccia [2]

1 - Department of Medical Microbiology & Infection Prevention, University Medical Center Groningen and University of Groningen, Groningen, The Netherlands
2 - Department of Infectious Diseases, Istituto Superiore di Sanitá, Rome, Italy

a.l.w.huckriede@umcg.nl

Background
Animal experiments have been a mainstay in the development of vaccines and in the quality assessment of vaccine batches. However, the results from vaccination experiments in mice and even non-human primates are often poorly predictive of the performance of vaccines in humans. Consequently, many vaccine candidates fail during expensive clinical trials. In addition, large numbers of animals are used for mandatory vaccine batch quality assessment although the respective assays often suffer from large variations in outcome. In vitro systems based on human cells can give insight into cell type-specific vaccine effects and vaccine-induced immunological mechanisms.

Topics overviewed
The aim of this presentation is to provide an overview of existing in vitro systems for vaccine evaluation and give examples of how they can be used for the study of different types of vaccines and for vaccine batch quality assessment.

In vitro models capturing human immune reactions range from simple cultures of specific cell types like dendritic cells, via co-culture systems, whole PBMC- or whole blood cell-based systems to artificial lymph nodes to lymphoid tissue-representing organoids [1]. All these systems have their advantages and disadvantages regarding ease of use, costs, and the range of immune reactions that can be studied. Readouts used typically comprise flow cytometry to measure biomarker expression, cytokine determination, and transcriptomics, all assessing the activation of particular cell types and the engagement of certain molecular pathways.

We started some years ago to develop a modular in vitro vaccine evaluation system based on human peripheral blood mononuclear cells (PBMCs) to assess vaccine-driven responses of antigen-presenting cells, T cells, and B cells. PBMCs can be easily derived from buffy coats which can be obtained from a local blood bank and can be cryopreserved for later use and reproducibility testing.

Using PBMC-derived cell systems we could show that whole inactivated virus (WIV) and split virus (SV) influenza vaccines differ significantly in their capacity to activate dendritic cells and antigen-specific CD4+ and CD8+ T cells, as well as follicular T helper cells [2,3]. The superior activity of WIV in these assays correlates well with the fact that in naïve individuals WIV influenza vaccine induces significantly stronger antibody responses than split vaccine.

With respect to vaccine batch quality control, we exposed PBMCs to conforming and non-conforming batches of inactivated tick-borne encephalitis virus (TBEV). Using RNAseq we identified several genes engaged in antiviral defense, eg. ISG56, MxA, and CXCL10, as potential biomarkers of vaccine potency [4,5]. Upregulation of these markers was consistent across different donors and vaccine production batches and strongly correlated with the amount of conforming TBEV in a linear fashion. Thus, a PBMC-based assay can be used to measure vaccine batch potency and confirm batch-to-batch consistency.

Conclusions
Taken together, human blood cell-based systems have great potential in vaccine development and assessment as they can bridge the gap between animal experiments and clinical trials. Next to the elucidation of vaccine-

induced molecular pathways in different cell populations they may help to understand differences in vaccine responsiveness between individuals or between specific target groups.

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HIV VACCINE-INDUCED ANTIBODY RESPONSES IMPACTS THE ACCURACY OF HIV TESTING ALGORITHMS IN SUB-SAHARAN AFRICA

F. Msafiri 1,2, A. Manjate 3,4, S. Lindroth 4, N. Tembe 5, R.M. Chissumba 5, V. Cumbane 5, I. Jani 5, S. Aboud 1, E. Lyamuya 1, S. Andersson 4,6, **Charlotta Nilsson** 2,6

1 - Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania
2 - Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden
3 - Faculdade de Medicina, Universidade Eduardo Mondlane, Maputo, Mozambique
4 - School of Medical Sciences, Örebro University, Sweden
5 - Instituto Nacional de Saúde, Maputo, Mozambique
6 - Public Health Agency of Sweden, Solna, Sweden

charlotta.nilsson@ki.se

Background
Rapid diagnostic tests (RDTs) are the primary tools used for HIV diagnosis in resource-limited settings. Healthy uninfected HIV vaccine recipients will develop antibody responses that result in diagnostic immunoassay reactivity, also known as vaccine-induced seroreactivity (VISR) or vaccine-induced seropositivity (VISP).

Aims
In an exploratory study, we assessed the impact of HIV vaccine-induced immune responses on the performance of HIV diagnostic algorithms used in two African countries.

Materials & Methods
Stored serum/plasma samples were used. The samples had been collected four weeks after the final vaccination from healthy Swedish, Mozambican and Tanzanian vaccinees participating in phase I/IIa HIV vaccine trials evaluating a prime-boost DNA-modified vaccinia virus Ankara-Env protein vaccine strategy. HIV infection was ruled out using HIV DNA or RNA PCR. The HIV testing algorithms used in Tanzania (sequential testing using SD Bioline HIV1/2 for screening and Uni-GoldTM HIV-1/2 for confirmation) and in Mozambique (sequential testing using Alere DetermineTM HIV-1/2 for screening and Uni-GoldTM HIV-1/2 for confirmation) were applied. Reactivity was also assessed using Enzygnost HIV Integral 4 ELISA and HIV western blot (WB, MP Diagnostics HIV Blot 2.2). Antibody titers to subtype C rgp140 were determined using an in-house ELISA.

Results
The VISR was 93% (128/137) by the Enzygnost HIV Integral 4 ELISA, and 66% (91/137) by Western blot assay (WHO interpretation). The Tanzanian HIV diagnostic algorithm would have misdiagnosed 74 (54%) of 137 healthy vaccinees as HIV positive and the Mozambican algorithm would have misdiagnosed 37 (26%) of 137 healthy vaccinees. The difference in seroreactivity depended on the antigens used in the RDTs as well as the magnitude of antibody responses to HIV-1 envelope protein.

Conclusions
In conclusion, the HIV diagnostic algorithms assessed here will potentially misclassify a large proportion of healthy HIV vaccine recipients. Increased efforts are therefore needed to develop differential serological or molecular tools for use at the point-of-care.

DO WE NEED HEPATITIS B VACCINE?

Tatjana Tallo

Department of Microbiology, Public Health Agency of Sweden, Solna, Sweden

tatjana.tallo@folkhalsomyndigheten.se

Background

Hepatitis B virus (HBV) is a worldwide public health problem. HBV is transmitted through parenteral or mucosal exposure to infected blood and body fluids. The mode of transmission is usually vertical or horizontal in highly endemic areas early in life, resulting in a high chronicity rate. In low endemic countries, transmission is usually horizontal in adulthood with self-limiting infection in most cases. HBV infection is estimated to be the cause of 30% of cirrhosis and 53% of liver cancer in the world. Safe and effective hepatitis B vaccines are the best tools to control and prevent hepatitis B. The effective implementation of hepatitis B vaccination programs has resulted in a substantial decrease in the HBV carrier rate and hepatitis B-related morbidity and mortality.

Topics overviewed

In the presentation will be shown the great impact of the hepatitis B vaccine which is the first anticancer vaccine. In addition, existing problems and future perspectives on hepatitis B vaccination required for global prevention of HBV infection will be shortly discussed.

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COMBINED THERAPEUTIC/PROPHYLACTIC HEPATITIS B VLP VACCINE PROTOTYPE

Andris Dishlers, Ivars Petrovskis, Dace Skrastina, Ieva Berza, Ilva Lieknina, Juris Jansons, Inara Akopjana, Larisa Kovalevska, Velta Ose, **Irina Sominskaya**

Latvian Biomedical Research and Study Centre, Ratsupites Str 1, Riga 1067, Latvia

irina@biomed.lu.lv

Introduction

The traditional predominating HBV vaccines are based on the hepatitis B virus surface (HBs) protein and manufactured in yeasts, demonstrating excellent prophylactic but no therapeutic activity. Thus the existing prophylactic HBs vaccines can not be used for the cure of chronic HBV infection, recognition of occult HBV infection, their potential reactivation, and protection against escape mutants and heterologous HBV genotypes. We have tested a set of recombinant HBc-based VLPs bearing surface-exposed HBV preS1 sequences as highly promising candidates for the generation of the universal prophylactic/therapeutic HBV vaccine.

Aims

This study aimed to combine T- and B-cell responses induced by the HBc and preS1 sequences respectively and thus adds to the development of effective new-type therapeutic-prophylactic VLP-based vaccine against HBV.

Materials & Methods

A library of the HBc-preS1 VLPs was created by genetic fusion of HBc-protein, different in length (full-length HBc and C-terminally truncated HBc variants), with the well-known virus-neutralizing preS1 epitopes of the HBV S gene. The possible three-dimensional organization of the preS1 fragments within chimeric HBc-preS1 VLPs was predicted by the molecular protein-modeling program 3D-JIGSAW. PreS1 sequences (preS1phil 12-60+89-119 or 20-47) were inserted within the HBc molecule at position 78 (“MIR”). The production level and VLP formation in E. coli were compared for different HBc-preS1 proteins and the best producers were used to obtain high-quality VLPs suitable for packaging of ODNs. Standard synthetic oligodeoxynucleotide ODN 1668 was used for packaging experiments. For packaging of ODNs osmotic shock and RNase-A treatment of VLPs were used.

Results

Although the VLP formation was found for all studied HBc-preS1 variants, the production level of HBc-preS1 and corresponding VLPs varied significantly. Oligonucleotide packaging ability within modified HBc-preS1 VLPs was performed using chimeric VLPs formed by preS1 (20-47) insertion within full-length HBc. Notably, both preS1 (20–47) and preS1phil containing chimeric VLPs were able to induce a remarkable anti-preS1 response in mice. However, the anti-preS1 (20-47) response was generally higher for constructs with preS1 (20-47) insertion rather than with preS1phil insertion.

Conclusions

In summary, this study shows that HBc-preS1 VLPs can be regarded as prototypes for a long-awaited prophylactic/therapeutic hepatitis B vaccine because they can induce antibodies and package foreign substances.

Acknowledgments

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Session VI: Novel Vaccine Vehicles, Adjuvants and Carriers

Chair: Dr Irina Sominskaya, Dr Juris Jansons

Adjuvants – the Tool to Obtain the Required Immune Responses in Prophylactic and Therapeutic Vaccination

Dennis Christensen, Croda Pharma, Frederikssund, Denmark; Dennis.Christensen@croda.com

Therapeutic Modulation of Tumor Microenvironment with Recombinant Viral Vectors

Anna Zajakina, Latvian Biomedical Research and Study Centre, Riga, Latvia; anna.zajakina@gmail.com

Engineering Pickering Emulsion as Vaccine Delivery System

Yufei Xia, State Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing, People's Republic of China; yfxia@ipe.ac.cn

Gold Nanoparticle-Based Adjuvants for Vaccines against Infectious Diseases

Jorma Hinkula, Linköping University, Linköping, Sweden; jorma.hinkula@lu.se

HBC VLP-Based Platform for the Development of a Recombinant Vaccine Prototype Against SARS-COV-2

Anastasia Nepryakhina, Latvian Biomedical Research and Study Centre, Riga, Latvia; nastjuuuw@gmail.com

ADJUVANTS – THE TOOL TO OBTAIN THE REQUIRED IMMUNE RESPONSES IN PROPHYLACTIC AND THERAPEUTIC VACCINATION

Dennis Christensen

Head of Global Research and Development, Adjuvant Systems, Croda Pharma, Frederikssund, Denmark

Dennis.Christensen@croda.com

Background

Basically vaccination is all about presenting sufficient amounts of the right antigen, in the right conformation, to the right cell populations, while supporting with the right co-stimuli for a sufficient amount of time. Most of the modern vaccines constitute highly purified subunit antigens based on proteins, peptides, RNA or DNA and thus intrinsically contain little ability to facility uptake into and activation of professional antigen presenting cells. Vaccine adjuvants ensuring delivery and immunostimulation thus represent important tool for modern vaccines to induce the required immune responses to obtain protection upon vaccination.

Topics overviewed

This presentation will give an overview on 1) the overall role for vaccine adjuvants, 2) design of SoA adjuvant technologies and 3) their mechanisms of action.

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THERAPEUTIC MODULATION OF TUMOR MICROENVIRONMENT WITH RECOMBINANT VIRAL VECTORS

Anna Zajakina, Kseya Korotkaja, Žhanna Rudeviča, Olga Ņilova, Karina Spunde

Latvian Biomedical Research and Study Centre, Riga, Latvia

anna.zajakina@gmail.com

Background

In the last decade a number of novel anti-tumour immunomodulating approaches showed very good results in preclinical and clinical studies. Enhancement of T-cell mediated anti-tumour immunity by “immune checkpoint” blockade or by T-cell adaptive transfer seems to be particularly promising. However, most patients do not respond to checkpoint inhibition and toxicity remains a problem. It was shown that tumour microenvironment (TME) and tumour-associated immune cells, as the main component of TME, support tumour progression through multiple pathways inducing resistance to treatment and promoting cancer cell escape mechanisms. The anti-tumour responses are not effective in "cold tumours", which are characterized by the lack of T-cell infiltration and tumour oversaturation with immunosuppressive myeloid cell populations. Nevertheless, the immune components of the tumour represent a promising target for therapeutic interventions. Advances in tumour immunology have highlighted a high diversity and plasticity in tumour-infiltrating cell subsets that can play dual functions, depending on their polarization status, e.g. M1 vs M2 macrophages, T-helper 1 (Th1) vs Th17 subsets, type I vs type II NKT cells, and N1 vs N2 neutrophils.

How to induce the anti-tumour reprogramming of the TME? Viruses are able to induce potent immune responses, representing a very promising platform to develop next generation vaccines and immunotherapy modulators for cancer treatment. The vector-based delivery of immune-modulating cytokines and other molecules can reprogramme TME to “hot” state and potentially can help to overcome the limitations of cytokine direct administration, such are the toxicity and the short half-life of cytokines in vivo. Many viruses possess natural tumour tropism to mouse and human cancer cells, which have been documented in preclinical and clinical studies. Moreover, oncolytic viruses mediate strong cytotoxic effects in cancer cells through the induction of immunogenic cell death, and can efficiently overcome immunological tolerance by the activation of innate antiviral pathways and the subsequent triggering of cytotoxic T-lymphocyte responses against the tumour. Therefore, synergistic effects between the expression of pro-inflammatory cytokines and anti-tumour immunity by the vector itself through the attraction of other immune cells to suppress tumour growth including eosinophils, neutrophils, NK and cytotoxic T-cells are expected.

Topics overviewed

The oral presentation considers promising DNA and RNA virus vectors delivering immunomodulatory genes and provides an overview on how these viruses break an immunosuppressive environment.

Acknowledgments

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ENGINEERING PICKERING EMULSION AS VACCINE DELIVERY SYSTEM

Yufei Xia

State Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing, China

yfxia@ipe.ac.cn

Background

To better provoke the responses, it is becoming increasingly important to strengthen the delivery efficacy of the antigenic and immunogenic components. Particles, such as liposomes, polymeric particles, protein aggregates etc, were engineered with multi-scale structures and tunable physicochemical properties to load and deliver the vaccine components. Additionally, these particles can trigger the inherent danger signals and the immune recognitions by mimicking the pathogenic traits. Recent efforts focused on developing particulate carriers to model the sizes, shapes, and compositions of microbes or diseased cells but not antigen fluidity and pliability. How to engineer the softness of the vaccine delivery system may strengthen the lymph-node accumulation, antigen-presenting-cell internalization, and the intracellular transfer of the delivered antigens for the enhanced immune potency and tendency.

Materials & Methods

To address this, we develop a particle-stabilized emulsion (Pickering emulsions), which was densely packed with nanoparticles to offer the high specific surface area for antigen loading and cellular interactions, and processed an oily core to demonstrate the softness to deform on the cellular surface, enlarging the contact area for higher uptake efficacy. Additionally, the soft droplets can also demonstrate the force-dependent deformation, which allow for the droplets to pass through the intercellular gaps with the interstitial flow, evidently increasing the lymph node accumulation of the delivered antigens.

Results

Compared with solid particles and conventional surfactant-stabilized emulsions, the optimized Pickering emulsions enhance the recruitment, antigen uptake and activation of antigen-presenting cells (APCs). Furthermore, the soft adjuvant achieved direct lymph-node delivery of the antigens, which potently enhanced the activations of the lymph-node-residing T cells and B cells, stimulating both humoral and cellular adaptive responses. In H1N1 influenza and SARS-CoV-2 vaccines, Pickering emulsion induced robust neutralizing antibody titer, IFN-r secreting T cells and the increased survival of mice upon lethal challenge, compared with the clinical-relevant adjuvants, including alum, MF59, and AS04.

Conclusions

By engineering the softness, Pickering emulsion were demonstrated with the enhanced lymph node accumulation, cellular uptake and immunogenicity, which may offer an alternative strategy for the efficient vaccine delivery system.

Acknowledgments

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GOLD-NANOPARTICLE ADJUVANTS FOR RESPIRATORY TRACT VIRAL INFECTIOUS DISEASES

A. Sengupta 1, M. Azharuddin 1, H.J.A. Narvaez 1, P.K. Hirak 2, **Jorma Hinkula** 1

1 - Department of Biomedical and Clinical Sciences, Molecular Infection and Inflammation Centre, Linköping University, Linköping, Sweden
2 - Division of Surgery and Interventional Science, University College of London, London, UK

jorma.hinkula@liu.se

Background
Viral proteins, or recombinant subunit proteins can often be weak immunogens if used alone as vaccine antigens. Enhancement of immune reactivity is therefore often required. This is often adjusted, either by significant increase in antigen amount, or by addition of an adjuvant. Suitable mucosal adjuvants are few and highly desirable, and here we suggest AuGP nanoparticle adjuvants as a stable option. The immune-modifying properties of gold-nanoparticles as adjuvants will be exemplified and presented in an in vitro and pre-clinical setting.

Materials & Methods
In this study, we have compared intranasal and subcutaneous vaccine administration in male and female BALB/c mice, and with and without AuGP-nanoparticles (10- or 40 nm size), or cationic N3 lipid-emulsion adjuvants. Influenza rHA and M2e-peptides and Covid-19-S1 antigens were used as gold-coupled vaccine candidates. AuGP-alone was used as comparator adjuvant.

Results
Results show a significantly elevated humoral immune reactivity against especially the HA and S1 antigens if adjuvants were used both as s.c but especially well if given at a dose of 1ug/antigen/mouse twice intranasally (at days 0 and 21). Serum IgG titers were with highest IgG titers among the nasally immunized (median 12 200+/- 6770), followed by the s.c immunized adjuvants (median 3440+/- 1220) if AuGP was used as adjuvant. Lung-wash anti-viral IgA was seen only in the nasally immunized animals. Cell-mediated immune reactivity was seen against all three included antigens in all vaccinated study groups.

When vaccinated and controls were challenged with influenza A virus, vaccinated animals given low-dose vaccine with AuGP-nanoadjuvant full protection from disease was obtained. Control animals or adjuvant alone immunized animals were not protected from disease.

Conclusions
AuGP-nano particles are attractive adjuvant options, especially due to their low to modest cell-toxicity, immune-modulating capacity as small but stable vaccine antigen-carriers and their secretion out of systemic compartments at the small size nanoparticle size of 10 to 40 nm.

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HBC VLP-BASED PLATFORM FOR THE DEVELOPMENT OF A RECOMBINANT VACCINE PROTOTYPE AGAINST SARS-COV-2

Anastasia Nepryakhina, Ivars Petrovskis, Juris Jansons, Ilva Lieknina, Inara Akopjana, Edgars Liepa, Irina Sominskaya

Latvian Biomedical Research and Study Centre, Ratsupites Str 1, Riga 1067, Latvia

nastjuuuw@gmail.com

Introduction

Recently we have described several technological advantages of HBc/G over other HBc proteins: high outcome in E.coli expression system, high stability, and high packaging potential in vitro.

Virus-like particles or VLP are multiprotein structures, which have conformational epitopes that can imitate viruses. VLP are potential vaccines candidates, because it caused strong B-cells respond by cross-licking specific receptors on B cell surface. Nowadays, chimeric VLPs, for instance anti-influenza vaccine based on hepatitis B (HBc), use have significant importance. Different researches show a high immune response against foreign protein epitopes.

SARS-CoV-2 virus caused the pandemic in 2019. Coronaviruses are enveloped virus, which has 30 kb positive-sense single-stranded RNA, and belonging to the subfamily Coronavirinae, which can infect mammals and several other animals. With SARS-CoV-2 mortality rates have been up to 10%.The SARS-CoV-2 virus have nonstructural and structural proteins, which contain spike, nucleocapsid, membrane and envelope proteins. Moreover, it have an accessory protein. To entry into cell, SARS-CoV-2 recognize the angiotensin-converting enzyme 2 (ACE2). In addition, this process is dependent on the host serine protease transmembrane protease serine 2.

As a result of Covid-19 pandemic, the vaccine development has been improving. Vaccine products against SARS-CoV-2 based on different technology platforms. However, the market have huge various of vaccine products, the world is still suffering from the Covid-19 pandemic. The virus mutates fast. The recombinant vaccine cause wide immune cells response and it memory.

Aims

The aim of the study is to create a new type of virus-like particles (VLPs) based on HBV core antigen from genotype G (HBc/G) for the presentation of SARS-Cov-2 specific epitopes from the nucleocapsid and spike protein.

Materials & Methods

- Plasmids – pET 28 and pETDUET T7 promoter.
- Competence cells - Eischerichia colli – XL-1Blue, BL21 DE3, BL21, K802
- Reagents and solutions

Methods:

- Bioinformatic approach
- Transformation
- PCR
- Electrophoresys
- Western Blott
- BigDye Sequence
- Colon purification
- Clonning

- Restriction Digestion
- Ligation
- Preparative gel
- Clonning

Results

The bioinformatics approach was used to design immunogenic epitopes from the nucleocapsid and spike protein of SARS-Cov-2. Selected epitopes were exposed to HBc/G VLPs. The optimization of expression of chimeric VLPs was done. VLP purification was performed by gel filtration chromatography.

Conclusions

It is expected that the creation of the novel virus-like particle platform stimulates design of new vaccine prototype against SARS-Cov-2.

Acknowledgements

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Capacity building Workshops December 8 & December 9

WORKSHOP ON RESEARCH IMPACT AND IMPLEMENTATION

Ways to Achieve High Impact in Academic Research

Dr Charlotta Lindquist (External Engagement Office, Karolinska Institutet, Stockholm, Sweden; charlotta.lindquist@ki.se)

Commercial Implementation of Research Findings

Dr Patrik Blomquist (Karolinska Institutet Innovation AB, Stockholm, Sweden, patrik.blomquist@kiinnovations.se)

Grant Writing, Abc Of Preparing Successful Grant Applications

Dr Ying Zhao (Grants Office, Karolinska Institutet, Stockholm, Sweden; ying.zhao@ki.se)

WORKSHOP ON RESEARCH FUNDING

Health Research in EC Member States Initiatives

Dr Uldis Berkis (Department of Higher Education, Science and Innovation, Ministry of Education and Science of the Republic of Latvia, and Riga Stradins University, Riga, Latvia; uldis.berkis@rsu.lv)

European Community Funding of Vaccine Development

Dr Janis Ancans (Head of National Contact Point for Horizon Europe, Cluster “Health” Expert for Horizon Europe National Contact Point, Latvian Council of Science, Riga, Latvia; janis.ancans@lzp.gov.lv)

COMMERCIAL IMPLEMENTATION OF RESEARCH FINDINGS

Patrik Blomquist
Karolinska Institutet Innovations AB, Solna, Sweden
patrik.blomquist@kiinnovations.se

Background

It's well-known that academic activities, such as teaching and research creates an impact in society. The impact is achieved through more educated workforce, knowledge that can be transferred into policies and that can be used by decision makers. However, it's also clear that academic research give rise to products and services that can improve the quality of life. A study from 2011 in New England journal of Medicine showed that many innovative drugs had an academic origin, *The Role of Public-Sector Research in the Discovery of Drugs and Vaccines*, Stevens AJ et al.

Aim

This presentation will discuss what a researcher in academia need to consider when trying to transfer findings and knowledge into society through commercialisation. I will cover both general things to consider, but also describe how the support is structured at Karolinska Institutet. Several examples that highlights various important parts will be described.

Overview

The impact can be created either by the academics directly or by existing companies that pick up the results and develop those further. In many countries there are support systems and mechanisms in place to increase the transfer of knowledge from academia into society.

FUNDING FOR VACCINE RESEARCH AND DEVELOPMENT IN THE EU – FRAMEWORK PROGRAMMES AND BEYOND

Janis Ancans

Head of Horizon Europe NCP Unit, Latvian Council of Science, Riga, Latvia
janis.ancans@lzp.gov.lv

Funding opportunities for vaccine related research and development activities can be explored via the Funding and Tenders Portal (ec.europa.eu/info/funding-tenders/opportunities/portal/screen/home), which represents convenient single entry point for participants and experts in the funding programmes and tenders managed by the European Commission and other EU bodies.

The European Commission has been at the forefront of supporting research and innovation and coordinating European and global research efforts, including preparedness for pandemics. So far €4.1 billion has been invested from 2007 to 2020 through the 7th Framework Programme (FP) and Horizon 2020 (8th FP) in infectious diseases research. These include 35 specific vaccine related calls, with call budgets reaching €270 million for innovations to accelerate vaccine development and manufacture, funded via Innovative Medicines Initiative. Funding has been intended to tackle scientific bottlenecks in vaccine development and to nurture and expand a vaccines innovation ecosystem by bringing together academics, small and medium-sized enterprises (SMEs) and industry. The following vaccine research areas have been funded extensively: in silico platform for knowledge management and mathematical modelling of the immune system; novel controlled human infection models (CHIMs); next-generation human in vitro systems and assays; mathematical modelling platforms for vaccine substance and product attributes in biomanufacturing, and others. In addition to several past, and ongoing, research actions related to coronaviruses and outbreaks, the Commission also launched several special actions in 2020, as part of a €1 billion for coronavirus research.

Under current 9th Framework Programme, Horizon Europe (2021-2027), the EU's current research and innovation funding programme with over €95.5 billion budget, the main areas of intervention include infectious diseases, including poverty-related and neglected diseases. Another funding programme is the EU4Health programme (2021-2027) which was adopted as a response to the COVID-19 pandemic and to reinforce crisis preparedness in the EU. The pandemic highlighted the fragility of national health systems. With a €5.3 billion budget during the seven-year period, the EU4Health programme is an unparalleled EU financial support in the health area.

To better understand EU health research funding opportunities, including vaccine related calls, network of National Contact Points (NCPs) has been established as the main structure to provide guidance, practical information, and assistance on all aspects of participation in Horizon Europe and related EU funding opportunities. Currently Horizon Europe NCP for are national structures established and financed by governments of the 27 EU member states and the states associated to the framework programme. NCPs provide personalised support on the spot and in applicants' own languages. In general, the following basic services are available in all countries: (1) Guidance on choosing relevant Horizon Europe topics and types of action; (2) Advice on administrative procedures and contractual issues; (3) Training and assistance on proposal writing; (4) Distribution of documentation (forms, guidelines, manuals etc.); (5) Assistance in partner search. NCPs are also established in many non-EU and non-associated countries (so called "third countries"), and EC funding is available to most African, Asian and South American countries.