REPORT

Report of the Conference on Immunological Mechanisms of Vaccination; December 13-18, 2012, Ottawa, Canada

✍ Ingrid Rodríguez-Alonso, Darién García-Díaz, Yaimín del C Santisteban, Daymir García, Yordanka Soria, Enma Brown, Enrique Iglesias

Centro de Ingeniería Genética y Biotecnología, CIGB
Ave. 31 e/ 158 y 190, Cubanacan, Playa, CP 11 600, La Habana, Cuba
✍ ingrid.rodriguez@cigb.edu.cu

ABSTRACT

The conference Immunological Mechanisms of Vaccination of the Keystone Symposia was held on December 13-18, 2012, in Ottawa, Canada. There were more than 400 participants from all over the world in the field of immunology and vaccine research. During five days, attendees had the possibility to present and discuss their current work and also to encourage new collaborations. Fifty-five oral presentations were grouped in seven sessions according to the following topics: Innate sensing of pathogens and vaccines, Augmenting immune response to vaccines, T and B cell memory to vaccines, Understanding signatures of vaccine protective efficacy, Translating immunity to vaccines and Vaccines against global threats. Additionally, more than 240 posters were presented and two workshops on Novel adjuvants and Vaccine delivery were organized. The quality of the papers presented at this conference shows that there is a global concern in eradicating chronic and re-emerging infectious diseases. Currently, a special attention is focused to the search for new and potent adjuvants and delivery systems that allows the generation of the immune response at the systemic and mucosal compartments to increase vaccine efficacy.

Keywords: vaccine, infectious disease, delivery system, adjuvant, immune system

Biotecnología Aplicada 2013;30:216-221

RESUMEN

Reporte del congreso Mecanismos inmunológicos de la vacunación; diciembre 13-18 de 2012, Ottawa, Canadá. El Congreso Mecanismos Inmunológicos de la Vacunación reunió más de 400 expertos en el campo de la inmunología y la vacunología. Durante cinco días, tales especialistas tuvieron la oportunidad de presentar y discutir sus resultados de investigación y promover nuevas colaboraciones. En siete sesiones, se agruparon 55 presentaciones orales, cuyos temas fueron: reconocimiento de agentes patógenos y vacunas por el sistema inmune innato; aumento de la respuesta inmune a las vacunas; memoria de células T y B frente a las vacunas; comprensión de los mecanismos de la eficacia protectora de las vacunas; del conocimiento de la inmunidad a las vacunas; y vacunas contra las amenazas mundiales. Además se presentaron más de 240 carteles y se organizaron dos talleres acerca de adyuvantes novedosos y sistemas de administración de vacunas. La calidad de los trabajos presentados en este evento demostró que existe una preocupación mundial por la erradicación de las enfermedades infecciosas crónicas y reemergentes. Actualmente, se dedica una especial atención a la búsqueda de nuevos adyuvantes y sistemas de administración, que permitan generar respuesta inmunitaria en los compartimentos sistémicos y mucosales, para aumentar así la eficacia de la vacunación.

Palabras clave: vacuna, enfermedades infecciosas, sistemas de administración, adyuvantes, sistema inmune

*I*ntroduction

The Keystone Symposia on Molecular and Cellular Biology is a nonprofit organization with the mission to accelerate life sciences discovery. One of the conferences programmed in the year of its 40th anniversary was held at the Fairmont Chateau Laurier in Ottawa, Ontario, Canada. The meeting joined more than 400 participants around the world in the field of immunology and vaccine research. During five days of intense scientific schedule, attendees had the possibility to present and discuss their current work and also to encourage new collaborations. Fifty-five oral presentations and more than 240 posters were presented. Meeting location and the scientific environment were ideal to promote informal discussions on free time and animated the debates at poster sessions.

*T*he key lecture

The venue at Ottawa Convention Center on December 13th was preceded by the key lecture of Dr. Bruce Beutler (University of Texas Southwestern Medical Center, USA) versed on Genetics and Innate Immunity. Dr. Beutler showed the application of forward genetics to disclose proteins essential for immune function, and suggested targets for pharmacotherapy. His group produced and screened mice with germline point mutations. This methodology allows the identification of hundreds of phenotypes after a given stimulus. Then, the chromosome is mapped and the gene-associated causative mutation could be identified. This is a practical way to attribute new functions to a given protein linked to stimuli-associated immune pathways.

*T*he conference

Oral presentations were grouped in seven sessions according to a common topic.

Innate sensing of pathogens and vaccines

The session took place on Friday, December 14th with Dr. Stephanie C Eisenbarth (Yale University, USA),

✍ Corresponding author

who centered her talk on NOD-like receptors (NLRs) and their role in dendritic cell (DC) activation after vaccination. In addition to the known properties of NLRp3 inflammasome in the adjuvant function of aluminum hydroxide and in the novel vaccine delivery system of nanoparticles, another NLR (NLRp10) has a critical role in the DC migration to lymph nodes after stimulation with different innate activators. She defined this molecule as a gatekeeper for DCs. In NLRp10 knockout mice, CD11b+ DCs displayed impaired migration to the draining lymph node, positioning NLRp10 as a fundamental molecule in the steps required for adaptive immune response following vaccination. Going deeper into aluminum hydroxide adjuvant properties, Dr. Yan Shi (University of Calgary, Canada) used atomic force microscopy and single cell force spectroscopy to study ligand-receptor interactions. Using a synthetic platform, they found that alum binds DC plasma membrane lipids, facilitating non-phagocytic antigen uptake. Then, activated DC, without further association with alum can stably bind to CD4+T cells inducing its activation. Another interesting work on DC receptors and T cell stimulation was presented by Dr. William R Heath (University of Melbourne, Australia). He identified DEC-205 as a surface receptor on splenic CD8+ DCs, involved in the recognition of CpG oligonucleotides. It is required for optimal B cell maturation, IL-6 secretion from B cells and for priming cytotoxic T lymphocyte (CTL) responses to CpG. Targeting of another CD8+ DC receptor, Clec9A, leads to the induction of a potent antibody response without adjuvants and facilitates T follicular helper (TFH) generation. All these experiments emphasize the value in studying DC surface molecules for vaccine development and basic biology. Finally, Dr. Taryn L Osmond (Victoria University of Wellington, New Zealand) confirmed by flow cytometry that splenic CD8α+, langerin+ DC is the main subset important for CTL cell cross-priming. The ability of these DCs to produce high IL-12p40 and CD40 co-stimulation and antigen presentation provides and enhances immune environment for optimal CD8+ T cell stimulation.

Augmenting immune response to vaccines

This session highlighted the role of different approaches for potentiating the immune response against viral infections. Dr. Robert A Seder (NIAID, USA) proposed the use of adeno and poxviral vectors as vaccine candidates for optimizing CD8+ T cell response against the human immunodeficiency virus (HIV) and malaria infections, specifically using heterologous prime-boost schedules. His group made a filogenetic comparison between chimpanzee (chAd) and human (hAd) adenovirus looking for low seroprevalence and efficient induction of CD8+ T cell response. Immunization of non-human primates with recombinant chAd3 and hAd5 achieved 100 % survival after virus challenge. In addition, flow cytometry analysis demonstrated the recruitment of similar proportion of DC subsets that differentially primed CD8+ or CD4+ T cell response.

Dr. Tanya Watts (University of Toronto, Canada) proposed the use of 4-1BBL (CD137L), a member of the tumor necrosis factor (TNF) family, as an immune stimulant in acute and chronic viral infections. She concluded that the use of this molecule in a boost phase of a prime-boost strategy increases the size of functional CD8 memory pool to influenza virus. In contrast to its effects in acute infection, during chronic viral infection with Lymphocytic Choriomeningitis Virus (LCMV) in mice or HIV in humans, the 4-1BB signaling pathway become desensitized due to loss of a key signaling adaptor TRAF1. This event could be reverted with the combination of IL-7 therapy in order to restore TRAF1, followed by a 4-1BBL containing vaccine. This approach represents a promising avenue for immune therapy of chronic viral infections.

The final talk, by Dr. Daniel D Pinschewer (University of Geneva, Switzerland), reviewed the immunobiology of wild-type LCMV and explained the advantages of the replication defective recombinant vectors (rLCMV) as a platform for human vaccines. He stated that rL CMV induce antibodies and long-lasting CD8+ T cell responses. Besides, they are three times more immunogenic and efficient than Ad5 and its seroprevalence in humans is globally below 5 %. Also, rLCMV can efficiently be re-administered in homologous prime-boost vaccinations. All these together strengthen the use of rLCMV as a useful approach to generate vaccine candidates against global threats.

T and B cell memory to vaccines sessions

These two sessions took place on Saturday, December 15th, focused on. The former began with Dr. Ton N Schumacher (Cancer Institute, The Netherlands) who developed an MHC-based technology to measure T cell reactivity against hundreds of potential T cell epitopes. A personalized immunomonitoring and reproducible T cell responses at the single cell level demonstrate that the fate of individual naïve T cell is extremely discordant. From these experiments they conclude that the reproducibility of T cell responses that we see at the cell population level is due to the average of highly divergent cellular behaviors. Similar results were presented by Dr. Marc K Jenkins (University of Minnesota Medical School, USA), demonstrating that the origins of CD4+ memory T cells are largely driven by TCR-peptide interactions and the signaling pathways they triggers on naïve T cells. Using single cell methods, he proved that naïve T cells are intrinsically predisposed to produce one type of effector cells. These results explain in part why some people manage infections better than others due to the difference on their TCR repertoire which determines the type of effector cells that it will produce. Dr. John T Harty (University of Iowa, USA) and Dr. David Masopust (University of Minnesota, USA) talked about effective strategies to induce memory $CD8^+$ T cells by prime-boost immunizations. Dr. John T Harty, using the model of influenza virus infection, showed that it is possible to generate protective numbers of memory CD8+ T cells shorting the time between inflammatory priming and booster immunizations. Although, he assayed homologous and heterologous schedules, the last one showed evidences of the highest response. Particularly, he identified that CXCR3+ memory CD8+ T have superior protective capacity against influenza because of their

ability to populate the respiratory tract. This CXCR3 expression is affected by the presence of IL-12 during priming, a fact to considerer for the selection of adjuvants for future influenza virus vaccines. In this sense, the group of Dr. David Masopust, working with models of infection in mice, has exploited heterologous prime boost vaccination. They demonstrated that anamnestic memory CD8+ T cell differentiation is flexible, and an abundant quantity can be achieved while maximizing protective efficacy and preserving proliferative potential. Moreover, they described an additional function for non lymphoid memory CD8+ T cells, as local sensors of previously encountered antigens that precipitate innate-like alarm signals and draw circulating memory CD8+ T cells into the tissue. Anamnestic responses into the tissue are thus robust because of the collaboration between nonlymphoid and circulating populations of memory CD8+ T cells. Vaccines must thus establish memory CD8 T+ cells in both compartments to maximized responses at common portals of pathogen exposure. In this regard, they are currently studying the number and polyfunctionality of SIVgag specific memory CD8+ T cells in rhesus macaques and their protective efficacy against high dose intravaginal challenges with SIVmac251.

Finally, Dr. Ross M Kedl (University of Colorado Health Sciences Center, USA) proposed the use of an innate receptor agonist (i.e. polyIC, Pam3cys) for the administration of subunit vaccines. This strategy favors antigen persistence on lymphatic endothelial cells, a cell type not previously known for its capacity to capture and hold antigen for extended periods. This persistence correlates directly to the induction of protective cellular immunity after vaccination. The data shown have significant implications on antigen dosing for clinical vaccine formulations.

Two remarkable works started the *B cell Memory to Vaccines*, which were delivered by Dr. Shane Crotty (La Jolla Institute for Allergy and Immunology, USA) and Dr. Peter Sage (Harvard Medical School, USA). They studied the antibody response to vaccines in terms of CD4+ T cell interactions with B cell populations. It is known that TFH cells are potentially useful biomarkers in human vaccine clinical trials as predictors of long term humoral immunity and antibody quality. In this sense, Dr. Shane Croty emphasized the role of TFH cells as a limiting factor for the development of germinal centers, affinity matured antibodies and memory B cells and plasma cells. On the other side, a tight regulation of this TFH-mediated humoral immunity is attributed to another population: the T follicular regulatory (TFR) cells. Dr. Peter Sage confirmed that programmed cell death 1 (PD-1) expression inhibits lymph node TFR cells but not TFH cells. Experiments using PD-1 deficient mice and adoptive transfer experiments suggest that manipulating blood TFR and TFH subsets is an efficient strategy for the selective and effective control of antibody response to vaccines.

Understanding signatures of vaccine protective efficacy

The session began with the talk of Dr. Bali Pulendran (Emory University, USA). He have recently used a system biology approach to identify early gene signatures that correlate with, and predict the later immune response in humans vaccinated with the live attenuated yellow fever vaccine YV-17D, or with the influenza vaccines. He explained and discussed the role of system biology in the prediction of vaccine immunogenicity, particularly amongst high risk populations such as infants or the elderly. Then, Dr. Ennio de Gregorio (Novartis Vaccines and Diagnostics, Italy) talked about the immune profiling of vaccine adjuvants, basically the oil-in-water emulsion MF59 and the novel TLR-dependent agonists Small Molecule Immune Potentiators (SMIPs). MF59 injection promotes the local expression of chemokine genes leading to the recruitment of innate immune cells and the resulting antigen uptake by DCs and their migration to the proximal lymph nodes. This process is independent of NLRp3 inflammasome and type I interferons but depends on MyD88. The goal now is to identify the relative contribution of different components of MF59 to its adjuvanticity. In addition, Dr. Ennio de Gregorio presented data of optimized formulations of SMIPs as promising adjuvants for safe and efficacious vaccines.

In a Short Oral Presentation (SOP), Dr. Magali Matsumiya (Jenner Institute, UK), looked for associations between early changes in gene expression and long term immunogenicity in BCG-vaccinated volunteers immunized with Modified Vaccinia virus Ankara expressing antigen 85A (MVA85A) using the DNA microarray technology. The results showed that Toll-Like Receptor 1 (TLR-1) levels on the day of vaccination correlates with IFN-γ ELISPOT to mycobacterial antigen 85A over 24 weeks. Along with two other genes, CD14 and TICAM2, TLR-1 mRNA levels can predict high and low ELISPOT responders with 80 % accuracy. In addition, when stimulating PBMC *in vitro* with MVA85A, production of CXCL2 is significantly lower in cells also exposed to neutralizing antibodies to TLRs 1, 2 or 6. Together, this data suggest the TLR-1, 2 and 6 axis is particularly important in the initial response to vaccination with MVA85A and affects the strength of the subsequent adaptive response.

Translating immunity to vaccines

The session started in the afternoon on Sunday, December 16th, with the lecture by Dr. Dan H Barouch (Harvard Medical School, USA) on novel HIV vaccine strategies. Despite the failure of Ad5 vector-based HIV vaccine in the STEP study, his group has been focused in the development of Ad vectors derived from alternative serotypes, such as Ad26, Ad35 and Ad48. Vaccines using the above mentioned vectors conferred protection against intra-rectal SIV challenge in rhesus monkeys. In comparison with Ad5 immunizations, memory T cells elicited by Ad26, Ad35 and Ad48 are low in magnitude but exhibited prolonged functionality and increased recall capacity following antigen re-exposure. Additionally, the cytokine profile elicited after vaccination differs among Ad vectors and may provide the basis for their varied protective efficacy. In humans, Ad26 vector expressing HIV-1 Env protein has proven to be save and immunogenic. The next step Dr. Dan H Barouch proposed is to ad-

vance into clinical trials with Ad26/MVA expressing bioinformatically-optimized mosaic HIV-1 antigens and administer it in prime-boost regimes.

The next talk was delivered by Dr. Helen McShane (University of Oxford, UK) about the clinical development of tuberculosis (TB) vaccines. Her group works with MVA85A TB vaccine designed to boost the effects of BCG, but the proof-of-concept efficacy trial to demonstrate this is currently ongoing. Besides, they are now conducting a clinical trial to evaluate the safety and immunogenicity of MVA85A in spray as a promising delivery route for new TB vaccines. This strategy guarantees that immunogen was delivered directly to the respiratory mucosa.

Two SOP on Dengue and HIV closed the morning session. The first one, conducted by Dr. Daniela Weiskopf (La Jolla Institute of Allergy and Immunology, USA), showed the analysis of dengue virus-specific response after primary infection. Their data of *ex vivo* IFN-γ responses from the Sri Lankan hyperendemic area suggest that multi-functional CD8+ T cells are associated with protection from dengue virus disease instead of a pathogenic role linked to original antigenic sin. This theory stated that skewing of T cell responses induced by primary infection with a given serotype causes less effective response upon secondary infection with a different serotype, predisposing to severe disease. Skewing of responses toward secondary infecting viruses was detected but not associated with impaired responses either qualitatively or quantitatively. Furthermore, they demonstrated higher magnitude and more polyfunctional response for Human Leukocyte Antigen (HLA) alleles associated with decreased susceptibility to severe disease, suggesting that multi-functional CD8+ T cells are associated with protection from dengue virus disease.

Then, Dr. Ma Luo presented a novel anti-HIV vaccination strategy based on overlapping peptides targeting the twelve protease cleavage sites on HIV-1. They conducted a pilot study in cynomolgus macaques using a prime-boost approach with Vesicular stomatitis virus-peptide/nanopackaged peptides.

Immunity and vaccines against global threats

This session took place on monday, December 17th, and started with the talk by Dr. Willen A Hanekon (University of Cape Town, South Africa) who studied 6363 local adolescents analyzing whole blood gene expression every six months. During two years of follow up, 35 adolescents developed TB disease (case) and 65 remained healthy (controls). His team identified a differential expression of multiple genes that allow prospective discrimination between case and controls 18 months prior to TB diagnosis, when all adolescents were completely healthy. Up-regulation of inflammatory and myeloid cell gene expression was prominent in adolescents at risk of TB long before disease manifest. These findings allow targeted intervention in the future and will guide studies of vaccination-induced correlates of protection against TB disease. Another work on TB vaccine was presented by Dr. David M Lewinsohn (Portland VA Medical Center, USA) who shared the lessons from a human phase I trial using BCG prime-Ad boost (AERAS 402 vaccine) in healthy adults' volunteers. They concluded that the measurement of vaccine induced CD8+ T cells using peptide pools does not give an accurate reflection of the ability of the vaccine to recognize the infected target.

In the field of influenza vaccines Dr. Rafi Ahmed (Emory Vaccine Center USA) demonstrated that it is possible to achieve heterosubtypic protection using a vaccine designed to elicit broadly cross-reactive antibodies. His group analyzed B cell response in 24 healthy volunteers immunized with the monovalent subunit pandemic H1N1 2009 vaccine. A rapid IgG-producing plasmablast response was observed in 100 % of cases. Over half of single cell PCR-generated monoclonal antibodies from isolated plasmablasts were virus specific and most of them neutralized more than one influenza strain. One antibody was found to recognize not only H1 and H5 but also H3 influenza viruses suggesting that cross-reacting response might be induced by vaccination.

Then, Dr. Mark Y Sangster (University of Rochester, USA) and Dr. David Furman (Stanford University, USA) demonstrate how age and immune status predisposed the influenza-specific immune response following vaccination. Dr. David Furman identified 15 parameters that predict the antibody response to the influenza vaccine with approximately 80 % accuracy, providing new insights into what variables may be most important for immune health.

An update in the Sanofi Pasteur's tetravalent dengue vaccine was explained by Dr. Dany De Grave (R&D Sanofi Pasteur, USA). He showed the most recent vaccine data, including results of the first proof of concept efficacy trial in 4000 Thai vaccines between four and eleven years old.

Finally, Dr. Louis J Picker (Oregon Health and science University, USA) talked about the vaccination efficacy of the SIV protein-expressing CMV vectors (Rh CMV/SIV). He highlighted the unique characteristics to this approach related to their proved efficacy in experimental models. He achieved a 50 % protection of animals after intrarectal or intravaginal challenge with highly pathogenic SIVmac239. Besides, Rh CMV/SIV vector elicited high frequency SIV-specific memory T cells in lymphoid and extra lymphoid tissues. In comparison with the current best alternative approaches, this vector develops CD8+ T cell responses with 3-fold breath, regardless pre-existing CMV immunity. This approach represents a promising strategy in the field of HIV vaccines.

Workshops and poster session

The poster session included more than 240 posters. Some of them illustrated the main results of vaccine clinical trials and other ones showed preclinical results about immunogenicity of new vaccine candidates against diverse infectious agents.

In the afternoon of Sunday, December 16, the authors of the present report discuss a work entitled *Novel adjuvant formulations for a quimeric protein of HIV-1*. We presented the results of the preclinical evaluation in mice of TERAVAC-HIV. This formulation developed by the Center for Genetic Engineer and Biotechnology (CIGB, Havana, Cuba) includes three antigens: the recombinant HIV-quimeric protein CR3 comprising Th and CTL epitope-rich regions from dif-

ferent HIV proteins and the hepatitis B virus (HBV) nucleocapsid and surface antigens virus-like particles (VLPs). The adjuvant effect of HBV VLPs on the induction of a Th1 HIV-specific cellular response was compared with another formulation based on CR3 adjuvated with the IMS 4112 nanoparticle (Seppic, France) after intranasal, subcutaneous or intranasal/subcutaneous co-administration. Our results in mice suggested that HBV VLPs are better adjuvants than the Seppic nanoparticle IMS 4112 to generate an anti-CR3 Th1 cellular response, which was characterized by CR3-specific IFN-γ-secreting cells and the proliferation of CR3-specific CD4+ and CD8+ T lymphocytes.

Considering that the majority of the posters were focused on novel adjuvants and how to improve vaccine delivery, two workshops were scheduled in view of the aforementioned topics and some posters were selected for SOP. In the following are summarized the most remarkable works.

Novel adjuvants workshop

The workshop began with the presentation by Dr. Frank Wegmann (University of Oxford, UK), on the use of polyethyleneimine (PEI) as a mucosal adjuvant in conjunction with viral subunits glycoproteins. Using HIV-1 Env gp120 as a model protein, he demonstrated antigen uptake by DCs and endothelial cells after intranasal (i.n.) immunizations in mice and rabbits. This stimulation leads to the induction of a Th2-biased cytokine production. Moreover, PEI association protects the antigen from proteolotic cleavage and induces anti-gp120 IgG response in sera. After a single i.n. administration of antigen-PEI immunogen, animals were protected from lethal intravaginal challenge. These results merit further investigation in order to use PEI as a mucosal adjuvant for humans in the near future. A new alternative of mucosal adjuvant was presented by Dr. Karen Smith (Statens Serum Institut, Denmark) who used immunostimulating glycolipid, monomycolyl glycerol (MMG) in cationic liposomal vaccine adjuvant. The immune response induced after immunization was characterized by Th1/Th17 cells and high levels of antibodies. They also carried out stability studies using these liposomes concluding that those containing 18-31 mole % MMG displayed the highest immunoestimulatory capacity and stability. Next speech corresponded to Dr. Sang Mu Shim (Korea Research Institute of Bioscience and Biotechnology, South Korea) who presented PC nanogel as an efficient adjuvant for H1N1 influenza vaccine. Compared with alum, the PC nanogel-adjuvanted vaccine showed a dramatically-enhanced protective efficacy, exhibiting increased pandemic HA-specific antibody production, higher neutralization activity, and earlier virus clearance after the pandemic influenza virus challenge. Both, *in vitro* and *in vivo* experiments demonstrated that the efficacy of PC nanogel is TLR-4 dependent. Another TLR-mediated adjuvant activity was achieved using alphavirus replicons encoding the TLR-5 ligand flagellin. This work, presented by Dr. Maria L Knudsen (Karolinska Institutet, Sweden), showed a strong IgG response characterized by a broad isotype profile and immune activation of several innate intracellular pathways. Dr. Silvia Vendetti (Instituto Superiore di Sanità, Italy) demonstrated in mice, the properties of retinoic acid (RA) to improve mucosal vaccination. Mice were pretreated with RA, primed i.n. with tetanus toxoid (TT) and systemically boosted with TT plus alum. This strategy resulted in a higher titer of both systemic TT-specific IgG and mucosal IgA, representing an advantageous approach to induce mucosal immunity in the absence of mucosal adjuvants. Additionally, given the crucial role of RA in imprinting a mucosal homing capacity on T and B cells, the use of this compound could improve the effectiveness of mucosal delivered vaccines.

Vaccine delivery workshop

Although nanotechnology and chemical synthesis are useful tools currently exploited in the development/delivery of new vaccine candidates, the use of viral vectors constitute an efficient way for the induction of potent transgene-specific immune response. In that way, this workshop began with the talk of Dr. Ana Carolina reis Albuquerque Cajaraville (Fiocruz, Brazil), about the characterization of a malaria vaccine candidate based in the yellow fever 17D viral vector. This strategy combines in one immunogen two diseases affecting the major endemic areas in the Americas and Africa. Two recombinant viruses were generated, expressing the heterologous merozoite surface protein 1 (MSP1) of *Plasmodium falciparum* and *P. vivax* respectively. After immunization of mice and non-human primates with the viral vectors, neutralizing antibodies against yellow fever and anti-MSP1 antibodies were induced. Finally, they concluded that the *Saimiri sciureus* monkeys are a good animal model to evaluate malaria vaccine candidates based on the yellow fever platform. Another interesting works, using Ad5 as vector, were presented by Dr. Matthew DJ Dicks (University of Oxford, UK) and Dr. Allan R Thomsen (University of Copenhagen, Denmark). Dr. Matthew DJ Dicks explained how using 'recombineering' (recombination mediated genetic engineering) they generated and modified new Ad vectors to enable a reliable comparative assessment of immunogenicity. Their approach used bacterial artificial chromosomes in vector construction facilitating the exchange of virus-associated ARN and fiber sequence between serotypes. This methodology allows investigating which elements of the viral genome influenced vector immunogenicity, a key element to improve future Ad vectors for clinical use. Experimental data presented by Dr. Allan R Thomsen using Ad vectors, demonstrated the increase of transgene-specific CD8+ T cells by co-expression of transgene and IL-2 in the same vector. Additionally, the presence of IL-2 increased the survival of tumor bearing mice after immunization. All this together strengthens the application of Ad vectors as tools for antigen delivery during infectious or malignant diseases. Dr. Even Fossum (Institute of Immunology, Norway) designed the so-called Vaccibodies by fusing the Xcl1 chemokine to a given antigen. After immunization, the Vaccibody specifically targeted cross-presenting DCs, also known as resident CD8+ DC expressing the Xcr chemokine receptor that binds Xcl1. The effectiveness of this approach was evaluated at the influenza infection model in mice. Animals developed hemagglutinin (HA)-specific IFN-γ secreting cells detected

by ELISPOT and a highly selective IgG2a antibody response. Additionally, CD8+ T cells generated after vaccination protected mice against a lethal dose of influenza A virus, as depletion of this population rendered the mice susceptible to infection. Using influenza as a target for vaccination, Dr. Masaru Kanekiyo (NIAID, USA) designed a self-assembling nanoparticle fusing viral HA to ferritin, a protein that naturally forms nanoparticles composed of 24 identical polypeptides. Thereby, the resulting nanoparticles were able to induce 10-fold higher anti-HA titers and neutralizing antibodies against two highly conserved vulnerable HA structures after immunization. Theses spontaneously-assembled nanoparticles improve the potency and breadth of influenza virus immunity and served as a platform for new vaccines against emerging influenza viruses. Finally, Dr. Torben Knuschke (Institute for Medical Microbiology, Germany) discussed their results using biodegradable calcium phosphate nanoparticles (CaPNPs) functionalized with CpG and encapsulating virus derived peptides. Functionalized CaPNPs were efficiently taken up by DCs *in vivo* and elicited a potent T cell-mediated immune response in immunized mice with high number of antiviral IFN-γ-producing CD4+ and CD8+ effector T cells. In addition, these molecules successfully prime cellular immunity for prophylactic and therapeutic immunization.

Concluding remarks

Vaccines are remarkably successful in reducing morbidity and mortality from infectious diseases in both developed and developing countries. Globally, mortality from infectious diseases still exceeds ten million deaths annually, reflecting a need for new, more effective and more deployable vaccination approaches. As many of the relatively easy vaccines have already been made, vaccinology is now tackling more difficult diseases, often caused by complex and genetically variable pathogens. A better understanding of the immunological mechanisms underpinning existing and novel vaccines is therefore required.

The advances in immunology, genomics and systems biology are beginning to offer a deeper understanding of the molecular and cellular mechanisms of host immunity, and the pivotal role played by the innate immune system in shaping the adaptive immune response. This is providing new opportunities to identify new signatures of vaccine immunogenicity and protective efficacy, which should facilitate a vaccine development and guide improved vaccine design.

Scientists are confident on those new strategies for the development of novel vaccine candidates will result in obtaining effective vaccines that can be used in humans. The quality of the papers presented at this conference Immunological Mechanisms of Vaccination shows that there is a global concern in eradicating chronic and re-emerging infectious diseases. Currently, a special attention is focused to the search for new and potent adjuvants and delivery systems that allows the generation of the immune response at the systemic and mucosal compartments.