REPORT

Keystone Symposia "Advancing vaccines in the genomics era", Rio de Janeiro, Brazil, October 31-November 4, 2013

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ABSTRACT

The Keystone Symposia "Advancing vaccines in the genomic era" was held in the Convention Center of the Windsor Hotel, Rio de Janeiro, Brazil, from October 31 to November 4. It was part of the Keystone Global Symposia series sponsored by Bill and Melinda Gates Fundation. Two-hundred and twenty delegates and more than a hundred of others participants around the world attended. The event was subdivided into 10 workshops covering 68 lectures and 120 posters on the latest results for vaccine design, development and testing, most of them with examples of the application of the Systems Biology approach with new technologies and an integral focus. The high quality of debates and researchers meetings brought about the general concerns on the main infectious diseases. They also provided an extraordinary opportunity to exchange about new ideas and to establish collaboration programs.

Keywords: vaccine, omics, immune response, systems biology, human immunodeficiency virus, Keystone symposia

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RESUMEN

Simposio Keystone "Avances en vacunas en la era de la genómica", Río de Janeiro, Brasil, del 31 de octubre al 4 de noviembre de 2013. El Simposio Keystone "Avances en vacunas en la era de la genómica" se celebró en el Centro de Convenciones del hotel Windsor, como parte de los simposios auspiciados por la Fundación Bill y Melinda Gates. A las conferencias y sesiones de carteles asistieron 220 delegados y más de un centenar de otros participantes de varios países. El evento comprendió 10 talleres, en los que se ofrecieron 68 conferencias y expusieron 120 carteles sobre los últimos resultados en el campo de investigación de las vacunas. La mayoría de ellos con ejemplos de aplicación de la biología de sistemas, de nuevas tecnologías y enfoques más integrales. La alta calidad de los debates y de los contactos entre los investigadores demostró la preocupación general sobre las principales enfermedades infecciosas. También significó una extraordinaria oportunidad para el intercambio de nuevas ideas y el establecimiento de programas de colaboración.

Palabras clave: vacuna, ómicas, respuesta inmune, biología de sistemas, virus de inmunodeficiencia humana, simposio Keystone

*I*ntroduction

The emergency of the genome sequencing projects has led to the development of the so-called Omics (e.g., genomics, proteomics and others). These technologies, in combination with the huge knowledge generated by its application and the new research tools that have arisen in the last years, led to a more integral focus in the so-called Systems Biology approach. Its application in vaccinology has been useful to get insights on the molecular networks that orchestrate immune responses, to understand the correlates of protection and to design more rational and safer vaccines and adjuvants.

For this aim, the Keystone Symposia "Advancing vaccines in the genomic era" was held in the Convention Center of the Windsor Hotel, Río de Janeiro, Brazil from October 31 to November 4. The meeting focused on how scientists apply the Systems Biology potentials in different areas of biomedical research for the development of vaccines. The main approaches discussed were technical and bioinformatics challenges associated with the study of vaccines and immunology, and also the new technologies available to enhance the understanding of the immune system in different species.

*T*he key lecture

The keynote lecture was presented by Mr. Chriss Wilson, on behalf of the Bill and Melinda Gates Foundation, which sponsored the meeting. After the welcome words, Dr. Rino Rappuoli (Novartis Vaccines and Diagnostics, Italy) imparted the key lecture "Designing vaccines for the 21st Century Society". The conference made a reminder about the principal issues of vaccines development and focused mainly in the advance that represented the Omics technologies. Reverse vaccinology applied to the *Neisseria meningitidis* serogroup B vaccine candidates' discovery was the first example on how a successful vaccine can be developed starting from genomic information. As part of system biology, structural vaccinology was also discussed. The knowledge derived from the different crystallization processes and its application to major histocompatibility complexe-Antigen (MHC-Ag) complexes, combined with the understanding on how they interact with the immune system, allowed to address significant health issues related to malaria, the human immunodeficiency virus (HIV) and tuberculosis. Optimal design of antigens, adjuvants and delivery systems were analyzed as the principal aspects of what Dr. Rino Rappuoli considered the third wave of vaccine development.

Finally, important questions regarding the orientation of vaccine research towards different populations (pregnant woman, elderly, and population segments like travellers and people suffering from chronic illnesses) were presented, as main trends for the design of novel projects.

Lectures

Plenary sessions began on Friday, November 1. Lectures were grouped in 10 sessions regarding: Genetic dissection of immunity; Immunity to infections; Genomics of T cell responses to vaccination; System biological approaches to vaccination; Immunity to vaccines; Genomics of antibody responses to vaccination; Genomic and structural approaches to antigen and adjuvant discovery, design and validation; Lessons to be learned from other fields; Immunology of vaccination in the young and elderly; and Translation of immunological concepts to the clinic. Due to the huge number of works presented we summarize the most relevant ones in our opinion.

The first session addressed the genetic host factors that could influence the immune responses against different pathogens. Dr. Thomas Hawn (University of Washington School of Medicine, USA) discussed about the limitations of the treatment against tuberculosis caused by the partial protection conferred by the Bacillus Calmette-Guerin (BCG) vaccine and the poor understanding of the mechanism of immunity to *Mycobacterium tuberculosis* (Mtb). In this regard, the study of the possible host genetic factors, probably linked to the inadequate immune response, was examined. Innate immune genes appeared to be associated to altered *in vivo* responses to BCG and its clinical efficacy. Whole blood cells of South African newborns were stimulated *ex vivo* with BCG, ten weeks after vaccination, and a defined innate immune pathway polymorphism was found to be associated to T cell cytokines responses and susceptibility to tuberculosis. This finding provided evidence on the correlation between innate and adaptive responses after *in vivo* vaccination and has important implications for novel vaccine strategies and IFN-γ based diagnosis for tuberculosis.

Meanwhile, Dr. Mary Carrington (Frederick National Lab, USA) presented results on the influence of the differential expression levels of HLA class I for HIV control. They showed that a polymorphic microRNA target site in the 3´ untranslated region (3´-UTR) of HLA-C regulates binding of miR-148a and posttranscriptional processing, which generates different expression levels of HLA-C. They determined that this expression has a direct effect in the HIV infection outcomes. Other genes like those of natural killer immunoglobulin- and leukocyte immunoglobulin-like receptors could be implicated in this process and have an impact on host defence against the virus.The concept of system vaccinology and its potential impact on the vaccine field was discussed by Dr. Bali Pulendran Emory Vaccine Center, USA in the second day. The role of innate immune system in tuning immune responses and the identification of molecular signatures induced immediately after vaccination which correlate with later development of protective responses were presented as crucial for the understanding of vaccine efficacy. The examples of the use of Systems Biology in yellow fever vaccine YF-17D and influenza vaccines were reviewed.Similarly, Dr. Alan Aderem (Seattle Biomedical Research Institute, USA) analyzed the different approaches that had been used until now in vaccines discovery and how Systems Biology provide new tools to address the complexity of the immune system and for a more rational vaccine design. Taken together, both conferences showed the potential of this recent development to speed up not only the vaccine candidate discovery, but also the streamline of vaccines' research, manufacturing and clinical trials.

Taking advantage of new mass cytometry methodology, Dr. Mark M. Davis and colleagues (Stanford University, USA) analyzed the response to influenza vaccination in humans. They developed a method to screen blood samples for over 100 peptide-MHC tetrameters at once using combinatorial labelling of cells with many more colors than in fluorescence based analysis. In this case, they used a panel of 34 flu-peptide-class II MHC tetrameters together with CD25+ phenotypic and functional markers and find good correlation between the flu epitope specific T CD4+ response and both antibody titers and CD8+ T cell responses.

Two studies exemplified the use of the high-content genome analysis in vaccine research, presented by Dr. Danilo Casimiro (Merck Research Laboratories, USA). In one study, RNA expression profile analysis in response to marketed vaccines revealed a gene module associated with adverse effects as documented in published results. The second identified host biomarkers for hypo-responsiveness to vaccination in elderly, in a cohort of 140 65-years-old adults with several vaccines for both recall (tetanus-diphtheria booster, hepatitis A virus) and *de novo* responses (hepatitis B surface antigen; HBsAg).

Dr. Scott D. Boyd (Stanford University, USA) versed on throughput DNA sequencing of antibody gene rearrangement after an influenza vaccination. His team characterized B cell clonal expansions with that approach, combined with both single cell cloning and the expression of antibodies specific to vaccine antigens, to provide new insights on the B cell lineages responding to vaccination. Strikingly, although vaccines showed particular sequence family convergent antibody gene rearrangements, different at DNA level, they are highly similar at protein level and correlated with seroconversion. So, consistent molecular features in response to vaccination could be defined regardless of the individual gene repertoire differences found.

Dr. Antonio Lanzavecchia (Institute of Research in Biomedicine, Switzerland) delivered a lecture on the human memory B and plasma cells. His team was able to isolate unusually potent neutralizing antibodies against the human cytomegalovirus from infected donors. Their respective viral ligands were then identified and used to design an experimental vaccine. This technique was also applied to identify antibodies against pan-influenza A that neutralized conserved regions of both RSV and metapneumovirus.

Additionally, Dr. Hana Golding (Center for Biologics Evaluation and Research, USA) used whole-genome-fragment phage display libraries and surface

plasmon resonance technologies to understand humoral responses against influenza vaccines. It was found that MF59 and AS03 adjuvants induce epitope spreading from hemagglutinin HA2 to HA1 and to neuroaminidase, compared to non- or aluminium-adjuvanted vaccines. Broadening cross-clade neutralization correlated with 2-3 fold increases in antibodies avidity to properly folded HA1.

Regarding structural vaccinology, Dr. Peter D. Kwong (Vaccine Research Center, USA) presented studies that determined the structure of human neutralizing antibodies and their epitopes. They were aimed at unravelling the way in which the humoral system recognizes the fusion glycoprotein of both HIV and respiratory syncytial viruses (RSV). The major vulnerability sites to antibodies were localized at the membrane distal-apex of the pre-fusion conformation of the glycoproteins in both cases. In RSV, they were able to stabilize these conformations and use it to immunize mice and non-human primates. Protective antibody titers, many times the protective threshold, were obtained. Although the same approach had been carried out in HIV, the research step is still in progress. A similar strategy was used by Dr. Ian A. Wilson (La Jolla, California, USA) for both HIV and influenza virus. For this last, his team determined co-crystal structures of highly neutralizing antibodies and HA and identified conserved regions in the fusion domain and in the receptor binding site of the antigen. Novel epitopes for the HIV envelope (Env) protein, many of which involved glycans, remain to be identified for neutralizing antibodies against HIV-1. This glycan-dependent neutralizing antibodies are able to penetrate the glycan barrier and bind to complex regions of gp120. This information could be used to design structure-based vaccines against each infection.

The concept of reverse vaccinology was addressed by Dr. Rhea N. Coler (Infectious Disease Research Institute, USA) for tuberculosis antigen discovery. A panel of approximately 100 genes of Mtb, including those related to its growth in macrophages, up or down-regulation under hypoxic conditions, secretion, membrane association o members of the PE/PPE or Esx families, were studied. The corresponding recombinant proteins were evaluated for IFN-γ recall responses in healthy subjects previously exposed to Mtb, to identify dominant T cell antigens in humans. Antigens conferring protection in mice against Mtb were selected and expressed as fusion molecules. One of these proteins, ID93, was shown to confer both prophylactic and therapeutic protection against tuberculosis in mice, guinea pigs and non-human primate models. The remarkable topic of immunology of vaccination in young and the elderly was also discussed in several conferences. Dr. Jorg J. Goronzy (Stanford University and the Veterans Affairs Palo Alto Health Care System, USA) presented a work in which, by using a bioinformatic tool, his team found that the repertoire diversity of T cells is very robust regardless the age-related thymic involution. This contrast with the previous and well accepted idea that this last factor led to a contraction in the repertoire could severely limit the ability to recognize antigens, which only can be overcome by thymic rejuvenation. Other negative signals that affect immunological functions of T cells had also been described and become important pharmacological targets to restore T cells defects in elderly. For example, a negative feedback loop that curtails the nuclear activity of ERK and JNK kinases could be counterbalanced by silencing the controller DUSP4 expression in elderly CD4+ T cells, restoring their helper activity for B cells differentiation and antibody production.

In an interesting talk, Dr. Bonnie B. Blomberg (University of Miami Miller School of Medicine, USA) addressed the issue of biomarkers of vaccine efficacy in elderly. She showed that aged B cells secrete increased levels of TNF-α and this correlates with their decreased function. This rise in the proinflammatory status (increased levels of TNF-α, IL-6, C-reactive protein, and CMV replication) could directly impact in B cell function, thus impairing the capacity of individuals to respond to vaccination. Differences like those in immune responses in elderly could be assessed by an *in vitro* system proposed by Dr. Ofer Levy (Boston Children's Hospital and Harvard Medical School, USA). A novel human microphysiologic assay system was developed, using primary newborn, infant and adult leukocytes cultured in autologous plasma containing soluble factors that vary with age and exert deep effects on the magnitude and nature of immune responses.

Taken together all the results presented led to a better and hopeful understanding of immune responses mechanisms triggered by vaccination, which was further complemented by the intense discussion generated during poster sessions.

Poster sessions

The posters sessions were divided in three discussion days due to the high number of works presented. The immunogenicity test of another vaccine candidate against HIV was presented by Dr. Gina L. Thede (Akshaya Inc., Canada) and colleagues. They used the Chemigen platform to express six engineered HIV antigens: Gag, Env, Tat, Rev, Vpr, and Vpu, as well as an N-terminal gp64 signal sequence and 6×His tag, and a C-terminal murine Fc fragment. Preliminary results demonstrated that the vaccine binds to immature dendritic cells (DCs) and induces CD4+ and CD8+ T cell activation and proliferation. In addition, stimulation with vaccine-loaded DCs was found to promote the increased production of IFN-γ and TNF-α from both CD4+ and CD8+ T cells. Furthermore, IgM was detected in cell culture medium when B cells were stimulated with antigen-loaded DCs, indicating that B cells were able to differentiate when exposed to the vaccine. Taken together, these results suggested that this novel DC receptor-targeted HIV vaccine is able to elicit humoral and cellular immune responses and, therefore, shows potential for development as a prophylactic/therapeutic vaccine against HIV infections.

Also, Dr. Arno C. Andeweg (Vrije Universiteit Brussel, Belgium) presented a work related to HIV vaccination, in this case using autologous DCs expressing the HIV-1 Tat, Rev and Nev proteins in seropositive patients, followed by antiretroviral therapy (ART) interruption. The study of blood transcriptome and proteome timelines revealed that genes involved

in HIV replication, and also in innate and adaptive immune pathway activation, were up-regulated. Immunotherapy could be related to a change in cellular defence and immune responses, while ART interruption after vaccination resulted in the up regulation of genes specific for antiviral and stress responses.

The use of Omics technologies to discover vaccine candidates against different pathogens was also a common topic in the works presented. Dr. Juan C. Alvarez (Universidad de Antioquia, Colombia) used proteomics to identify antigen candidates in order to design a molecularly defined vaccine against *Leishmania panamensis*. In this work, the use of CpG motifs as adjuvant showed advantage over other Toll-like receptor ligands and drove the immune response towards a Th1 pattern, with the induction of IFN-γ and IgG2a titers. The study of humoral immunoproteome by proteomics techniques revealed five hypothetical proteins, two heat shock proteins, an autoantigen-like protein, four proteins oxidative stress-related proteins and 12 proteins with other fuctions, all of them possibly involved in the protective humoral response.

An interesting work was presented by Dr. Jordana G. A. Coelho-dos-Reis (Rockefeller University, USA). They used an adeno-associated virus (AAV)-based gene delivery system to achieve reconstitution of human myeloid DCs in NOD/SCID/IL-2r gamma null mice (NSG). Before the infection with the AAV vectors that encoded HLA-A2 (AAV-A2) and human cytokines, the researcher team performed a human hematopoietic stem cell transfusion. The result was that the infected mice displayed a superior reconstitution of human mononuclear cells than the respective transgenic mice or NSG mice infected with AAV-A2 vectors alone. This demonstrated that the delivery system was an efficient method to transfer human genes to a mice model.

An important topic was the use of adjuvants and new formulations. Dr. Elizabeth De Gaspari (Adolfo Lutz Institute, Brazil) presented the use of bilayer fragments of the cationic lipid dioctadecylmethylammonium bromide (DDA-BF) as adjuvant for a *N. meningitidis* serogroup B outer membrane vesicle (OMV) vaccine. Delayed-type hypersensitivity responses and IgG titers could be achieved by a single dose of OMV/DDA-BF while two doses of the vaccine adjuvanted in Alum were required for the same result with a mixed Th2/Th1 pattern in both cases. Meanwhile, Dr. Franco Piazza (Infectious Disease Research Institute, USA) selected a glucopyranosyl lipid adjuvant o/w emulsion combined with another o/w emulsion (GLA-SE) as a novel adjuvant for ID93 Mtb vaccine. In a phase I clinical assay in healthy subjects, three doses of the vaccine induced no serious adverse effects, being reported headache, fatigue and myalgia as the most common adverse reactions. Adequate CD4+ T cell responses also supported the decision to begin a second trial in South Africa. A third adjuvant (rASP-1), in this case a secreted protein derived from *Onchocerca volvulus*, was used by Dr. Sara Lustigman (Lindsley F. Kimball Research Institute, USA) for the administration of a trivalent-inactivated flu vaccine. The explored formulation resulted in improved flu-specific antibody responses, but also conferred an increased protection in a mouse model. Moreover, crossreactivity increased against other flu virus strains by using that method, requiring a lower antigen dose.

In this session, a work from our group was presented. It consisted in a successful generation of a Th1-type response in Balb/c mice, in spite of an ongoing-Th2 response, after immunization with the TERAVAC-HIV vaccine candidate. This is a vaccine formulation comprising an HIV-1-derived multiantigenic polypeptide named CR3, carrying HIV-1 Th and cytotoxic T cell epitopes, formulated together with HBsAg and the hepatitis B core antigen (HBcAg). Mice primarily immunized with placebo, the mixture of the HBsAg and HBcAg, HIV-1 lysates or TERAVAC-HIV were further boosted five times with HBsAg + HBcAg or TERAVAC-HIV either by intranasal or simultaneously by intranasal and subcutaneous routes. Primary Th2 responses recognizing the CR3 protein were evidenced in mice immunized with viral lysates or THERAVAC-HIV, with significant IL-4 and IL-10 secretion and negligible levels of IFN-γ and IgG2a antibodies. Significantly, TERAVAC-HIV booster immunization of the same animal groups subverted the Th2 profile and developed an equivalent Th1 profile of high levels of IFN-γ secretion and IgG2a antibody production in serum. These results suggested that the equivalent strategy could be applied for the potential therapeutic restoration of the Th1-type HIV-specific cellular response in seropositive patients.

Concluding remarks

As shown in the lectures and poster sessions of this conference, vaccination is one of the most successful methods employed by mankind in his fight against illness. However, up to our current knowledge, new approaches will be required to prevent HIV,tuberculosis, malaria and other emergent and re-emergent diseases. Well-established together with wider approaches in immunology and technology, need to be integrated for a systemic view aiding for a more rational vaccine design.

Although substantial funds are required to develop and use most of such technologies, collaboration projects between different research institutions, information management and databases access through free digital resources and the effort of national and public sectors, could provide joint solutions for a successful outcome. This Keystone symposia conference focused on a number of the most important issues in vaccine research, helping to identify opportunities to productively integrate immunology and vaccinology. The development of novel concepts is required to speed up vaccine discovery, development and fulfill regulatory approval demands for entering clinical trials. This would be achieved in the near future with the proper collaboration on information, bioengineering and biomaterials science scientific communities.

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