Meeting report

Viral Vaccine meeting held in Barcelona, October 25–28, 2003

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The Viral Vaccine meeting was convened by the Macrae Group (New York, NY, USA) in Barcelona (Spain) from the 25th to the 28th of October 2003. It provided a platform for discussion related to vaccinology, including basic virology and epidemiology, immunology and management strategies with particular attention to licensing and economic issues.

The meeting brought together leading experts from all over the world, working on the development and use of vaccines against the most important virus infections of humans and animals. The meeting was chaired by Albert Osterhaus (Erasmus MC, Rotterdam, Netherlands) and Stanley Plotkin (University of Pennsylvania, Philadelphia, USA) and the program was established under the auspices of a scientific advisory committee consisting of Thomas Braciale (University of Virginia, Charlottesville, USA), James Crowe (Vanderbilt University School of Medicine, Nashville, USA), James Leduc (Centers for Disease Control and Prevention (CDC), Atlanta, USA), Brian Mahy (CDC, Atlanta, USA), José Melero (Instituto de Salud Carlos III, Madrid, Spain), Peter Openshaw (Imperial College School of Medicine, London, UK), Ray Spier (University of Surrey, Guilford, UK), and Hans Wigzell (Karolinska Institute, Stockholm, Sweden).

Educational grants were provided by Aventis Pasteur, Baxter Vaccines AG, GlaxoSmithKline, Intervet, Med-Immune Inc., Solvay Pharmaceuticals B.V. and Wyeth Vaccines.

The meeting was opened by an introductory keynote lecture given by Daniel Tarantola (World Health Organization (WHO), Geneva, Switzerland), who highlighted the impact of infectious diseases on the health of children especially in developing countries and the need to combat them in the most cost-effective way by the use of currently available and newly developed vaccines. The meeting was divided in 10 selected sessions, each presenting state of the art knowledge and ongoing developments in key areas of virus vaccine related research.

1. Pediatric and geriatric vaccines

Ann Arvin (Stanford University, CA, USA) presented us the latest on varicella zoster vaccine development [1]. In 1995, the Food and Drug Administration (FDA) approved the use of a live-attenuated (LA) varicella zoster virus (VZV, OKA-strain) vaccine. Vaccination has now commenced in many countries. After 6 years of clinical practice it is now clear that the vaccine is 85% effective in providing protection against infection with wild-type (wt) virus, and 95% efficacious in protection against serious disease. Breakthrough infections of vaccine virus occurred in 1–2% of the vaccinees and were generally very mild.

Zoster, caused by the reactivation of VZV from latency, is a common among hematopoietic-cell transplant recipients. Vaccination with inactivated VZV vaccine was shown to reduce the risk of zoster in these individuals, which correlated with reconstitution of VZV-specific CD4 T-cell immunity [2].

Stanley Plotkin addressed the latest developments on vaccination against cytomegalovirus (CMV) [3]. Congenital infections with CMV are the most common cause of neurological disorders in infants. In the United States, CMV is transmitted in 1% of all births causing death or sequala in 20% of these cases. To prevent these deaths as well as CMV infection in transplants recipient patients researchers have started to explore the possibilities for vaccination against CMV. Currently, four different CMV candidate vaccines are tested in phase I and II trials. The LA vaccine, based on the Towne strain, induced good immunity, including neutralizing antibodies and cell-mediated immunity, which correlate with protection. However, the immune response lagged behind that of a natural infection. To increase the immunogenicity of the Towne LA vaccine, which lacks nineteen open reading frames (ORF) and lost its capacity to establish latency, some of the ORF of the more virulent Toledo strain of CMV will be introduced into the Towne vaccine strain. In addition to these two candidate LA vaccines, a sub-unit candidate vaccine comprising of the gB glycoprotein and a canarypox vector containing the pp65 and IEI exon 4 are being tested in phase I trials. The former

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induces antibodies, whereas the latter induces a strong cellular immune response.

At the other end of the age spectrum are the elderly, which generally have a reduced immune function of the innate and adaptive immune system. This so-called immunosenescence is the cause of increased morbidity and mortality in this age group. Because of the reduced immune function, vaccine efficacies are often lower in the elderly and vaccines have to be more immunogenic to obtain similar seroconversion rates. Michael Roggendorf (University of Essen, Essen, Germany) showed that the immunogenicity of hepatitis A virus (HAV) and hepatitis B virus (HBV) vaccine is lower in the elderly, as was measured by seroconversion rates [4]. Interestingly, the efficacy of the HAV and HBV vaccine differed in individuals over 60 years of age, (71 and 37.5% seroconversion, respectively), which may be dependent on the nature of the vaccine formulations. Lluis Salleras (Autonomous Government of Catalonia, Barcelona, Spain) further elaborated on vaccine efficacy and demonstrated that HAV and HBV vaccination in pre-adolescent schoolchildren was >90% effective in protection from disease [5].

2. Vaccines for respiratory viruses—part 1

Respiratory syncytial virus (RSV) is a single-stranded negative sense RNA virus belonging to the family of Paramyxoviridae, genus *Pneumovirus*. Upon infection, the virus can cause upper respiratory tract disease in all age groups, however severe complications are more often seen in the very young, elderly and bone marrow transplant patients. In the late 1960s, vaccination experiments were conducted in young children using formalin-inactivated RSV (FI-RSV). Upon infection with a wt strain of RSV, some of these children suffered from severe complications resulting in death. This phenomenon of enhanced disease in FI-RSV vaccinated children has hampered further RSV vaccine research enormously.

Understanding the mechanisms of disease enhancement is a key issue, crucial to RSV vaccine development. Peter Openshaw and his co-workers have been studying enhanced disease in mice for many years. The possible involvement of $\gamma\delta$ T cells in human bronchiolitis has recently been highlighted [6], and depletion of these 'unconventional' T cells can prevent enhanced disease in RSV-infected mice previously sensitized by scarification with vaccinia viruses encoding single RSV proteins.

Among the target population for vaccination are very young children (under 1 year of age). The presence of maternal antibodies and the immaturity of the immune system may reduce the immunogenicity of vaccines in these children. James Crowe investigated the ability of B cells from very young children to produce neutralizing antibodies, which are important for protective immunity. Following natural infections, the immunoglobulin (Ig) region of B cells producing RSV-specific antibodies was sequenced.

Gene rearrangements resulting in a complete VDJ region in the immunoglobulin occurred in the RSV-specific B cells. However, B cells from infants less than 3 months old exhibited a striking paucity of somatic mutations in VH genes, indicating that they are unable to produce high quality neutralizing antibodies.

Potential vaccine candidates for RSV include adenovirus recombinants, immune stimulating complex (ISCOM) preparations and sub-unit vaccines, as well as LA virus vaccines. LA virus vaccines are known to induce humoral and cellular immune responses. A presentation by Miranda de Graaf (Erasmus MC, Rotterdam, Netherlands) demonstrated the possibilities of reverse genetics in creating LA viruses. This work was done using human metapneumovirus (hMPV), a virus causing similar disease as RSV. Exchanging the polymerase genes between the two different lineages of hMPV attenuated the ability to induce chloramphenicol acetyl transferase (CAT) activity from a mini-genome containing a CAT reporter gene flanked by the hMPV genomic ends. Using the full-length hMPV genome instead of a reporter gene resulted in the rescue of recombinant viable viruses [7]. This system could produce attenuated viruses that may be used for vaccine purposes.

Passive transfer of neutralizing antibodies is an available treatment for RSV. Because of the relatively short half-life of these antibodies, it is essential that the administration of the antibodies coincide with the RSV epidemic season, of which the onset varies from year-to-year. The PID-ARI network (Pediatric Infectious Diseases Network on Acute Respiratory Tract Infections) currently identifies 19 different viral and bacterial pathogens, using a multiplex PCR, in children between 0 and 16 years old. Josef Weigl (Christian Albrechts University, Kiel, Germany) showed that last year the start of the RSV epidemic was 5 weeks prior to the onset of antibody administration, unnecessarily endangering these children to an infection with RSV. These data support the need to synchronize the application of preventive antibody treatment with RSV epidemics.

Finally, Rob Moormann (Animal Sciences Group of Wageningen, Lelystad, Netherlands) presented the latest on bovine RSV vaccine development. Vaccination with FI-RSV induced enhanced disease in these animals, including eosinophil influx and an IgE response [8].

3. Vaccines for respiratory viruses—part 2

A second important respiratory pathogen is influenza virus. This segmented negative sense single-stranded RNA virus has been responsible for three major pandemics in the 20th century as well as annual epidemics. In contrast to RSV, the correlates of protection are well documented and include the presence of neutralizing antibodies and probably T-cell responses. Influenza vaccine research is focusing on two areas; the development of an influenza vaccine for a pandemic situation and the development of alternative

influenza vaccines preventing disease following infection with epidemic viruses annually.

The classical influenza vaccine is a trivalent inactivated influenza vaccine and is administered each year by injection. Harry Greenberg (Stanford University School of Medicine, CA, USA) gave an overview on the development of a cold-adapted (ca) influenza vaccine (Flumist^{TM}), which is now approved by the FDA for use in individuals between 5 and 49 years of age. Ca viruses are capable of growing at low temperatures ($25\,^{\circ}$C), and are attenuated at body temperatures. The genetic basis of attenuation for influenza A virus A/Ann Arbor/6/60 has now been identified and was limited to 4 amino-acid (aa) changes on the polymerase proteins PB1 (3 aa) and PB2 (1 aa) as well as 1 aa change on the nucleoprotein (NP) [9]. Flumist^{TM} is applied intranasally, has been shown to be effective and has a low transmission rate in highly susceptible young children in a day care setting. In the future, the medical importance of a possible association between asthma and the use of Flumist^{TM} in young children needs to be investigated. Finally, additional data confirming and extending the apparent safety and efficacy of Flumist^{TM} in individuals over 50 years of age should be obtained.

The production of influenza vaccines in embryonated chicken eggs and the generation of master reassortant vaccine strains are time consuming and inflexible procedures are hampering the rapid development of pandemic influenza vaccines. The advent of Vero and Madin–Darby canine kidney (MDCK) cell lines, registered for vaccine production, will facilitate flexible vaccine production in the future by companies like Baxter Vaccines AG and Solvay Pharmaceuticals BV [10,11]. Reverse genetics will be an alternative tool for the generation of reassortant vaccine strains. John Wood (National Institute for Biological Standards and Control, London, UK) elaborated on the possibilities of these new approaches and pointed out that licensing issues should be dealt with in order to make quick licensing of candidate vaccines possible in events of a future pandemic threat [12,13].

Catherine Ammon (MPH, Geneva, Switzerland) discussed the poor public awareness of the dangers of influenza amongst Swiss elderly [14]. Although the elderly realized that they belong to the high-risk group, bad experiences, doubt about the efficacy, and side effects, had reduced the number of individuals willing to take the vaccine. To ensure effective use of influenza vaccines, it is imperative that public awareness is increased and maintained at a high level.

4. Vaccines for flaviviruses—part 1

Dengue virus is a serious health threat to the human population infecting 50–100 million individuals each year worldwide. Robert Putnak (Walter Reed Army Institute of Research, MD, USA) presented an overview of dengue virus vaccine candidates, which are currently being tested in pre-clinical and clinical trials.

One of the major issues concerning dengue virus infections is the occurrence of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) in a small percentage of infected individuals. DHF and DSS can occur after a primary infection, but occur much more frequently after secondary dengue virus infections with a serotype different from that during primary infection. It was suggested that antibody mediated enhancement and waning immunity may play a role in the pathogenesis of these complications. Early dengue virus candidate vaccines consisted of LA virus strains, developed by Dr. A. Sabin. Vaccination studies demonstrated that LA candidate vaccines partially protected against challenge with live virus, was safe, well tolerated and immunogenic.

Since the WHO resolution on dengue virus in 1995, four different candidate vaccines are currently being evaluated in pre-clinical and clinical trials, including two candidate LA virus vaccines and two recombinant candidate vaccines. The monovalent LA vaccine candidates varied in immunogenicity and the four serotypes of dengue virus interfered with each other. Multiple doses of vaccine, given >3 months apart, were required to obtain satisfying seroconversion rates. A recombinant dengue virus serotype 4 (DEN-4Δ30) developed by the National Institute of Health is also a promising candidate, inducing a 95–100% seroconversion rate in human volunteers. A recombinant yellow-fever (YF) virus containing the prM and envelop (E) protein of dengue virus (ChimeriVax^{TM}, Konstantin Pugachev (Acambis Inc., MA, USA)) proved to have potential: A single dose with all four serotypes provided 83–100% protection against challenge infection 180 days later in *Cynomolguos macaques* [15]. Also candidate sub-unit vaccines have been developed for dengue virus. Carolyn Weeks-Levy (Hawaii Biotech Inc., HI, USA) presented data on a sub-unit candidate vaccine consisting of recombinant envelope protein (E) lacking the transmembrane region. Interestingly, monkeys vaccinated with high doses of monovalent serotype 2 dengue virus (DEN-2) E protein, became viremic upon infection with DEN-2, while lower vaccine dosages did not result in viremia. Addition of non-structural (NS)-1 protein increased the cellular immune response and improved protective immunity.

Wellington Sun (Walter Reed Army Institute of Research, MD, USA) compared candidate dengue virus vaccine efficacies observed in humans and monkeys. Monkeys are permissive for infection with dengue virus, but they do not show any disease symptoms, including DHF or DSS. Upon infection these animals clear the virus and develop antibodies that are partially cross-protective. At present it is unclear whether the monkey model is valid for predicting the outcome of vaccination in humans [16]. Seroconversion rates of mono- and tetravalent candidate vaccines were generally lower in humans than in monkeys. The DEN-4Δ30 and the ChimeriVax^{TM}-D2 (A recombinant YF virus containing the prM and envelop (E) protein of dengue virus serotype 2) candidate vaccines elicited seroconversion rates in humans of >95%. The Walter Reed group has conducted a serotype 1

dengue (DEN-1) and serotype 3 dengue (DEN-3) virus challenge study of subjects vaccinated with their tetravalent LA candidate vaccine. Results indicated that the rhesus monkey viremia correlated well with results in humans. Although the human challenge model is important in validating vaccine efficacies, its role in vaccine development remains to be defined.

Regina Kofler (University of Vienna, Vienna, Austria) described the isolation of an attenuated tick-borne encephalitis virus (TBEV), with a deletion in the capsid protein. Second-site mutations have restored the capacity of this attenuated virus to replicate in cell-culture. These mutants were shown to have the potential for the development of a live flavivirus vaccine [17].

YF virus strain 17D (YF-17D) is an attenuated virus strain, which has lost the viscerotropic, neurotropic and vector competence phenotypes of wild-type YF virus and has been used as a backbone in vaccines for the induction of immunity against other flaviviruses. Therefore full understanding of the molecular determinants for attenuation and virulence is of importance. Alan Barrett (University of Texas Medical Branch, Galveston, USA) showed that none of the 20 aa substitutions in YF-17D could be linked to neuro- and hepatotropism, however the E and NS4B protein may be important in determining the virus phenotype [18,19].

5. Vaccines for flaviviruses—part 2

The second member of the Flaviviridae family with a high impact on human health is hepatitis C virus (HCV). It was originally identified in 1989 and believed to have infected over 200 million people worldwide. Approximately 15–45% of the HCV acute infections are resolved spontaneously, which appears to be associated with strong CD4+ T-helper cell and antibody responses. Currently, the main aim of vaccination is to prevent the occurrence of chronic liver disease after infection with HCV.

Since chimpanzees are the only other species than humans susceptible for infection, they are used to evaluate the efficacy of HCV candidate vaccines. Michael Houghton (Chiron Corporation, CA, USA) demonstrated that vaccination with adjuvated recombinant glycoprotein (gp) E1 and E2 and subsequent challenge with HCV RNA, significantly reduced the incidence of chronic disease in chimpanzees [20]. In the same study, sterile immunity was associated with high levels of neutralizing antibodies. These neutralizing antibodies may prevent binding of HCV particles to CD80, as was demonstrated in vitro with hyper-immune serum from infected individuals. Currently, the gpE1 and gpE2 sub-unit candidate vaccine is being tested in phase I trials.

In 1999, West Nile virus (WNV) was introduced in the USA, which has prompted an extra effort to develop a vaccine. WNV is transmitted from birds to humans by mosquitoes and causes febrile illness in approximately 20% of infected individuals. In 0.8% of the infected individuals, infection results in neurological disorders followed by death in 5–15% of these cases. Michael Lieberman (Hawaii Biotech Inc., HI, USA) showed that vaccination with truncated E protein of WNV protected golden hamsters from infection with WNV [21,22]. Further evaluation of this candidate sub-unit vaccine, the Acambis chimeric, and the Fort Dodge killed candidate vaccine will be needed to demonstrate their safety and efficacy in humans.

6. Veterinary viral vaccines

Lorne Babiuk (University of Saskatoon, Sask., Canada) introduced the field of veterinary vaccinology. Veterinary vaccines also have to be safe, efficacious and perhaps in contrast to vaccines for human use, generally have to be cheap and fit existing practices. Furthermore, veterinary vaccines can often be evaluated directly in the targeted species. In addition to conventional live and inactivated vaccines, also genetically engineered chimeric, DNA, plant-based and replication defective veterinary vaccines are available. Some of these vaccines aim at the induction of protective immunity to human pathogens in order to prevent the contamination of food and water by these agents, which also include the bacteria *Escherichia coli* (*E. coli*) strain O157, campylobacter and *Salmonella* [23].

The ALVAC vector or canarypox vector is one of the vectors used for veterinary vaccines. Among its many advantages are the inability to replicate in mammalian cells, its large coding capacity, induction of low levels of vector immunity and its ability to overcome maternal antibodies. Huw Hughes (Merial, Lyon, France) demonstrated that a registered ALVAC vector expressing the hemagglutinin (HA) of influenza virus A/Horse/Kentucky/94 or A/Horse/Newmarket/2/93, protected horses from infection. In addition, antibody titers remained high for at least 1 year following the second vaccination.

Also coronavirus vectors have been developed as was demonstrated by Luis Enjuanes (Centro Nacional de Biotecnología, CSIC, Madrid, Spain). This single-stranded positive sense RNA virus has no DNA intermediate, minimizing the risk of chromosomal integration by the vector. In addition, variation of the spike protein can control the tropism of the virus and target certain areas of the body. Finally, reinfections with coronaviruses occur frequently, suggesting that pre-existing immunity is easily overcome by the virus. The construction of a bacterial artificial chromosome containing the full-length transmissible gastroenteritis virus (TGEV) allowed genetic manipulation of the virus [24]. It was shown that an E-protein deficient TGEV virus can only replicate on packaging cell-lines expressing the E-protein [25]. Relocation of the packaging signal between the M and E genes (a likely recombination site of E-protein deficient viruses to become propagation competent) is leading to the production of highly safe coronavirus vectors [26].

7. Vectored and DNA-based viral vaccines

Certain attenuated viruses can be used as vectors for antigen delivery of foreign (viral) antigens. Attenuation may be achieved by reduced replication capacity (e.g. YF strain 17D and modified vaccinia Ankara (MVA)) or be based on species restriction (e.g. canarypox vector (ALVAC)). These vectors can induce strong humoral and cellular immune responses specific for the protein of interest. Gerd Sutter (GSF—Institute of Molecular Virology, Muenchen, Germany) elaborated on progress made with regard to the development of MVA vector technologies. MVA can be used under conditions of biosafety level 1 because of its avirulence and its deficiency to productively grow in human cells. In animal models, MVA vaccines have been found immunogenic and protective against various infectious agents including immunodeficiency viruses. Here, data from a clinical trial were presented providing evidence for safety and immunogenicity of recombinant MVA when used as therapeutic vaccine in HIV-infected individuals [27]. Finally, it was shown that modification of immune regulatory genes, such as interferon resistance genes or interleukin-inhibitor sequences conserved within the MVA genome, might provide the basis for the development of advanced second generation MVA vaccines with even higher immunogenicity [28,29].

Another viral vector is Semliki-Forest virus (SFV), which has features of both the DNA and the RNA viral vectors. For the induction of an immune response against a foreign antigen, SFV replicons producing the foreign protein can be used for vaccination. Also naked RNA or cDNA of these replicons can be used for vaccination against foreign antigens [30]. Peter Liljeström (Karolinska Institute, Stockholm, Sweden) reported that the replicase protein of SFV plays an important role in the maturation of dendritic cells (DC). It was also shown that SFV is capable of inducing cross-priming and maturation of DC as well as interferon (IFN)-α production, providing enough danger signals to induce a vigorous immune response to foreign antigens, explaining their good immunogenicity.

The potential to modulate HCV-specific immune responses using cytokines was tested by Christine Rollier (Biomedical Primate Research Center, Rijswijk, Netherlands). The addition of DNA plasmids encoding for interleukin (IL)-12 and IL-2 did not enhance the immunogenicity of a candidate DNA vaccine based on the NS3 protein of HCV in rhesus macaques.

José Melero, using state of the art technology, has identified the fusion properties and fusion sites of RSV fusion protein (F-protein) [31]. Future research will be aimed at the development of compounds or antibodies capable of binding to the fusogenic intermediate of the F-protein.

8. Adjuvants and ISCOMs

Ideal vaccines should combine low reactogenicity with high immunogenicity profiles in order to be safe and efficacious. Unfortunately both characteristics often go hand in hand resulting in immunogenic but reactogenic vaccines. Therefore researchers have tried to enhance the immunogenicity of vaccines by prime-boost regimens or by using adjuvants or alternative methods of vaccination.

Guus Rimmelzwaan (Erasmus MC, Rotterdam, Netherlands) summarized the findings of three different studies conducted in three different species using ISCOMs containing the HA and neuraminidase (NA) of various influenza viruses. Both monkeys and chickens were protected from infection with the H3N2 subtype and the highly pathogenic H5N1 subtype of influenza A virus respectively after vaccination with an ISCOM based but not after vaccination with a conventional sub-unit vaccine [32,33]. The protection correlated with vigorous antibody and T-cell responses. In humans, who have pre-existing immunity to influenza virus, the added value of the adjuvant was less pronounced. However, the kinetics of virus-specific antibody responses was accelerated in the ISCOM vaccinated individuals compared to those who received the conventional vaccine. In addition, the ISCOM vaccine induced CTL responses, whereas the classical vaccine did not.

Vaccines are often administered intramuscularly (IM), while alternative administration methods may enhance the immunogenicity of certain vaccines. Lendon Payne (Powderject Vaccines Inc., WI, USA) demonstrated that the Powderject™ can deliver DNA and proteins to the epidermis, which is known to contain a unique population of Langerhans cells. The epidermal powder immunization can also combine certain adjuvants, like QS21 or *E. coli* heat-labile enterotoxin (LT) with DNA or sub-unit vaccines. Co-administration of LT induced strong cellular immune responses in mice [34].

Jan Wilschut (University of Groningen, Groningen, Netherlands) presented data on the use of virosomes as a possible carrier of influenza viral glycoproteins. Virosomes, which are reconstituted viral envelopes without the viral genome, have been shown to induce both humoral and cellular immunity in mice [35]. In addition, the virosomes are capable of harboring aliphatic adjuvants, further enhancing their potential to induce immunity to viral glycoproteins.

Finally, Anneke Boonstra (CoVaccine BV, Lelystad, Netherlands) informed us about the latest developments in dissacharide fatty acid sulphate ester adjuvants [36]. Experiments demonstrated that one sulphate in combination with seven lauric acids (S1L7 in squalane-in-water) induced high levels of influenza virus-specific antibodies in both pigs and humans.

9. Vaccines for HIV/AIDS

Gary Nabel (National Institute of Health, Bethesda, USA), presented recent data on human immunodeficiency virus (HIV) candidate vaccine testing. In monkeys, inclusion of the envelop gene in the candidate vaccine (plasmid

DNA—recombinant adenovirus prime-boost regiment) enhanced humoral and cellular immune responses which correlated with immune protection [37]. Using high doses of plasmid DNA (4–8 milligram), HIV-specific T-cell responses were induced in a high percentage of individuals. One of the mysteries of HIV is the transmission of CCR5-tropic viruses, and not CXCR4-tropic viruses, upon infection. New data demonstrated that the CCR5-tropic viruses can infect immature DC, whereas CXCR4-tropic viruses cannot. These immature DC can, upon maturation, mediate viral transfer to CD4+ T cells. Since this whole process is done within cells, it is highly resistant to neutralizing antibody activity. This mechanism may contribute to preferential transmission of CCR5-tropic viruses and may have implications for future vaccine design.

DNA-prime adenovirus vector boost vaccine regiments currently seem the best method to induce high CD8+ T-cell responses. Pre-existing antibodies to adenovirus however interfere with the efficacy of the vaccine. Jaap Goudsmit (Crucell NV, Leiden, Netherlands) has identified alternative adenovirus serotypes with low seroprevalence in the human population. Adenovirus serotype 35 (Ad35) was shown to have low global seroprevalence and low infection rates in both HIV positive and negative individuals, which makes Ad35 virus an attractive viral vector [38].

10. Rotavirus vaccines

Duncan Steele (WHO, Geneva, Switzerland) offered us the latest on rotavirus vaccine developments [39,40]. Rotavirus is an important pathogen of young children claiming half a million deaths each year, in particular in developing countries. Rotavirus is a complex triple layered virus with two neutralizing antigens, which are involved in the immune response of the host. Vaccine strategies differ in the need for the development of homo- and heterotypic immunity to these specific antigens. Currently, two LA rotavirus vaccines are licensed. Rotashield® (Wyeth Vaccines), a tetravalent rhesus rotavirus vaccine, demonstrated 70% protection against all rotavirus diarrhea and more than 90% against severe disease. However 25% of the vaccinees developed fever upon vaccination and there was an elevated risk of developing intussusception in 1/10,000 vaccinees. For this reason Rotashield® as a vaccine has been withdrawn. The second LA rotavirus vaccine is manufactured in China, although it efficacy in truly naïve children remains to be demonstrated.

Two candidate rotavirus vaccines are in late stages of development, Rotateq® (Merck, Research) and Rotarix® (GSK Biologicals). The efficacy of both candidate vaccines is similar in early clinical trials (approximately 70% effective in preventing rotavirus diarrhea) to that of Rotashield®. The future will reveal which of the two candidate vaccines, i.e. a two dose (Rotarix®) or the three dose oral vaccination (Rotateq®) will provide better heterologous and long-term protection. Prior to clinical use of rotavirus candidate vaccines, potential interference with live oral poliovirus vaccination and the safety, immunogenicity and efficacy in HIV-infected children should be investigated.

11. Vaccines for emerging diseases and bioterrorism

The threats of emerging viral infections and terrorist attacks with biological weapons have triggered a major effort finding cures or vaccines against these agents. James Leduc pointed out some of the issues involved in candidate vaccines for emerging and rare diseases. The difficulty with these rare viral diseases is the inability to demonstrate efficacy in humans. Should FDA approval therefore be based on data obtained in 20–50 monkeys, whose predictive value regarding efficacy and safety remain controversial. Other issues involved are; fast tracking of these vaccines by the regulatory authorities, covering international property rights, liability issues, and different viewpoints of government and industry, including willingness and justifiability to develop certain vaccines.

Gary Nabel presented data on Ebola virus vaccine development. The glycoprotein (GP) of Ebola virus causes cell rounding and detachment of human endothelial and epithelial cells. These effects require the presence of the mucin-like domain of GP and correspond with the down-regulation of Vα-integrin on the cell surface. The GP is an important vaccine candidate, however passive transfer of antibodies in both humans and monkeys did not confer protection from infection. Vaccination experiments are therefore aiming at the induction of both humoral and cellular immune responses to Ebola virus. Surprisingly, monkeys vaccinated once with an adenoviral vector encoding for the GP and NP of Ebola virus, were protected from a high dose challenge [41].

In 2001, the US government had decided that every citizen should be able to receive a smallpox vaccination. The original Dryvax® vaccine consisted of heterogeneous virion subpopulations and may have been contaminated with ruminant viruses. Richard Weltzin (Acambis Inc., MA, USA) presented data on the plaque purification of the original virus stock, yielding the ACAM2000 isolate, which proved to be immunogenic without neurovirulence in mice and monkeys [42]. Currently, this virus is being tested in phase I and II trials.

Smallpox vaccination by scarification with vaccinia virus is a crude method, which is not quantitative and requires skilled personnel. It also results in an active local lesion and shedding of live virus. Other methods of vaccination may be more quantitative and do not shed live virus. Amanda Phelps (Biomedical Sciences, Porton Down, UK) showed that intramuscular vaccination of mice provided better protection at lower vaccine doses than needle-free (microject) or scarification methods.

Severe acute respiratory syndrome (SARS) is an important emerging infectious disease caused by SARS-CoV, for which currently no cure or vaccine exists. In mice it was

shown that DNA encoding the spike protein of SARS-CoV induced spike protein-specific antibodies. These antibodies and antiserum from a recovered patient were shown by Gary Nabel to inhibit gene transfer by a pseudotype virus containing the spike protein of SARS-CoV. In addition, Kirill Kalnin (Acambis Inc., MA, USA) demonstrated that hyper immune serum from SARS-CoV infected individuals reacted with the recombinant spike protein. These data suggest that the spike protein of SARS-CoV may be a good vaccine candidate.

The viral vaccine meeting ended with a series of presentations about the emergence of SARS-CoV. On February 14th, the WHO reported the first cases of SARS-CoV. Between the 21st and 26th of March, a new coronavirus, SARS-CoV, was identified by three laboratories [43,44]. Two weeks later, the entire genome of the virus was sequenced [45], closely followed by the fulfillment of Koch's postulates [46]. Other important hallmarks for SARS-CoV were the identification of the virus in several animal species including the palm civets. Albert Osterhaus showed the ability of SARS-CoV to infect macaques [46] and different carnivore species including the house-cat and the ferret [47].

At the end of the epidemic several important conclusions could be drawn. Among the risk factors for adverse outcome of the disease were age >60 years, diabetes, heart disease and other co-morbid conditions. Early during the epidemic it was noted that health-care workers were at risk for contracting disease. This may have been due to the relatively late peak in virus-titers (day 10–16 after onset of illness), subjecting these health care workers with high doses of virus. The relatively late peak in virus titers has also hampered the diagnosis of SARS-CoV. Malik Peiris (Queens Mary Hospital, Hong Kong, SAR) showed that improved RNA extraction and real-time PCR increase the percentage positive swabs considerably (63% positive on day 1 and 88% on day 2–3) [48].

To prepare for a second introduction of SARS-CoV into the human population, antiviral drugs and protective vaccines are urgently needed. Luis Enjuanes was the first to clone the full-length genome of a coronavirus (TGEV). The deletion of the E protein and relocation of the packaging signal has generated a safe and efficacious vaccine candidate. A full-length SARS-CoV genomic clone [49], may augment the generation of candidate SARS-CoV vaccines.

The meeting was ended with a presentation by Jan Hendriks (Public Health Directorate, European Commission, Luxembourg), who on behalf of the European Commission highlighted the opportunities and stumble blocks for the development of viral vaccines of major importance to public health.

12. Conclusion

The meeting not only provided an overview of the viral vaccines that are currently used in humans and animals with varying degrees of success, but also gave a state of the art view of novel developments and opportunities in this field.

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