Report on the International Symposium on Vaccinology, November 18-20, Paris, France, organized by the Académie des Sciences and the Marcel Mérieux Foundation.

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Meeting report

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Sometimes, numbers say more than long sentences. Even if the lives of 3 million children are saved each year by the World Health Organization expanded programme of immunization (EPI), 17 million people died from infectious diseases in 1997. One can draw two conclusions from these numbers: one is that vaccination is an effective tool for disease prevention and control. The other one is grimmer: vaccination does not reach the populations that most need it.

Although vaccination led to the control of major diseases during the past 200 years, new (HIV, hepatitis C) and old (tuberculosis, malaria, acute respiratory infections, etc.) diseases alike are still killing millions of people. Vaccines, from the medical and economic point of view, are among the most cost-effective tools available to prevent and control diseases. The development of new vaccines, as well as new vaccine strategies, was one of the issues discussed at the International Symposium on Vaccinology.

Vaccines were developed originally on an empirical basis, using mostly attenuated or inactivated pathogens. With the considerable progress made in immunology, genetics, and genomics, and the emergence of new technologies, new strategies, such as DNA vaccines, peptide analogues, viral and bacterial vectors, etc. are now designed to produce vaccines eliciting specific immune responses. These new approaches also offer exciting perspectives to prevent noninfectious pathologies such as autoimmune and chronic diseases as well as cancer.

However, as vaccines are becoming more effective, they are also widening the gap between the 'haves' and 'have-nots'. As Dr Charles Mérieux and Prof. Gros emphasized in their opening remarks, developing countries should be helped to become scientifically less dependent on the Northern hemisphere. Such a shift to self-reliance of the third world could allow a better distribution of the vaccines where they are needed.

It is clear that immense socioeconomic discrepancies between countries exist in terms of public health. World cooperation is necessary to overcome these discrepancies in order to give more people access to vaccination. Vaccination needs public education, and mothers in this regard are a very potent target.

Vaccination also calls for acceptance of some risk. With the decrease of diseases comes the decrease of disease perception, followed by a rise in the concern about vaccines and vaccine safety.

Finally vaccination is no longer a field restricted to science and medicine. Economic, social, political, and cultural aspects of vaccination must be studied extensively in order to assess and maximize its impact on human welfare.

1. T cells as key targets for new vaccines

T cells are quite important in the immune response: these cells regulate antibody production, they give rise to cytotxic T cells (CTLs), and they are essential for immune memory. Ph. Kourilsky showed that with as many as a million different T cells with different T-cell receptors, a naive mouse has a very diverse T-cell repertoire, and the repertoires of two different animals share some, though rather few, overlaps. In the response to a single antigenic epitope, the number of precursors is small (less than 50). After immunization with the Cw3 peptide, the few specific CD8 T clones which respond all grow at the same speed, which may reflect the stochastic mode in which specific T cells encounter the antigen. If this appears to be limiting, it would raise the question of vaccination at multiple sites.

Localization of the antigen is a key factor for induction of the T- and B-cell response. The responses can range from ignorance, when the antigen is confined outside secondary lymphoid organs, to exhaustion or tolerance when the antigen has massively invaded an organism (R. Zinkernagel).

Cross-talk between innate (NK cells, dendritic cells, macrophages) and adaptive (T and B cells) responses through the production of various cytokines takes place during an immune response. Modulation of the response towards cell-mediated or humoral immunity is achieved

by changing the balance between cytokines produced by the different cells involved in the immune response. Antigen presented in the presence of interleukin (IL)-12 (an essentially innate immune system cytokine) induces the generation of CD4 T helper cells involved in cell-mediated immunity (Th-1) and cytotoxic CD8 T cells, while in the absence of IL-12, a Th-2 response is obtained. The orientation of the T-cell response towards a Th-1 or a Th-2 phenotype by infection also depends on the genetic background. The treatment with IL-12 of a BALB/c mouse infected with *Leishmania* will allow the animal to survive the infection by switching the Th-2 response towards a Th-1 response. Thus, the modulation of the balance between a Th-1 and a Th-2 T-cell response might be a key factor in helping an organism to fight a specific disease (G. Trinchieri).

Vaccines so far have not been very efficient against chronic diseases or persistent infections. Pathogens with latent life cycles can hide in cells for long periods of time, and although they might raise a large CTL response at the beginning of the infection, this response decreases over time to reach a small percentage of remaining specific cells. Detection and sorting of very low numbers of CTLs in Epstein-Barr persistent viral infection have been achieved by using tetramers of major histocompatibility complex/specific peptide. Interestingly, all the CTLs detected by the specific tetramers are functionnal. The presence of remaining functional CTLs in chronic infections such as HIV is overcome by the ability of the virus to mutate its genome (A. McMichael).

2. Mucosal immune response

J. Mestecky defined the mucosal surface as a battlefield of 400 m^2, with the mucosal immune system overstimulated every day by multiple pathogens. Mucosal colonization by pathogens results in strong immune responses taking place in the exposed and adjacent regions, and a weak response in remote mucosal tissues. This route of immunization can induce local antibody production, local cell-mediated immunity, tolerance, and most importantly, generalized secreted antibody responses. Oronasal, rectal, and vaginal epithelia contain antigen-presenting cells which maintain a collaboration with lymphoid cells. In turn, those cells produce immunoglobulin A (IgA) and immune factors like cytokines, and present receptors to pathogens and cytokines. Mucosal vaccine applications range from controlling allergies to viral, bacterial, parasitic, and even autoimmune diseases. Delivery systems such as naked DNA, liposomes, and live vectors must be specifically designed to avoid acidic degradation and proteolysis and to allow interaction with macrophages (e.g., vaccines against cholera) (J. Mestecky). Mucosal immunity is best stimulated by topical immunization, and oral vaccines would have many advantages: they are easier to produce, easier to deliver, and safer (J. Holmgren).

Vaccines against mucosal infections such as influenza often prevent the infection process more than the disease itself. Because the influenza surface glycoproteins (haemagglutinin and neuraminidase) are very prone to mutations, periodic drifts occur for each influenza virus strain, interspersed by yearly shift of subtypes. Therefore, the design of attenuated vaccines against the influenza virus must be carefully evaluated by surveillance through a map of mutations in the gene encoding the viral haemagglutinin in different parts of the world (J. Skehel).

3. Immune response to specific antigens

While protein-based vaccines are T-cell dependent, can be boosted, and give a good response in children under two years old, polysaccharide-based vaccines are T-cell independent, raising mainly an IgM response, cannot be boosted, and give a poor response in children less than 2 years old. Vaccines containing a mix of polysaccharides conjugated to a carrier and protein might provide a wider immune response even in infants. Hib polysaccharides in the form of conjugates are efficient type-specific vaccines. However, polysaccharides from other pathogens differ in their immunization capabilities. Multivalent vaccines (such as a 7- to 11-valent vaccine against the most pathogenic serotypes of *Streptococcus pneumoniae*) could enable a wide protective response to infants and young children (A. Lindberg).

Other pathogenic antigens of nonpeptidic origin can also raise immune responses: mycobacterial antigens include esters (which include 2-methylbutylaldehyde) called phosphoantigens. They induce proliferation and cytolytic function of the γ9 δ2 T cells in humans, as well as inducing a Th-1-type cytokine secretion. These properties could confer a potential protective role on this particular T-cell population. The chemical properties and the relatively small size of the phosphoantigens comparatively to the T-cell receptor suggest that they may covalently bind to the γ9 δ2 T-cell receptor and/or that the latter could act as catalytic antibodies (J.-J. Fournié).

4. The need for new vaccines

At the millennium's end, smallpox virus has been eradicated, and poliomyelitis is on the path to eradication. Vaccination has used all the arms of the immune sytem to induce protection, but the principal means of success has been serum antibodies. In the future, we must learn how to better induce secretory antibodies and cellular immunity of various types. Emergence of new agents, resistance to antibotics, and age-barrier immunity lead to a need for new vaccines (S. Plotkin).

About three million people die from tuberculosis every year. BCG vaccination against adult tuberculosis is rather inefficient, probably because of a failure to activate the right T cells for optimal immunization. The immune response appears to be very complex, with CD4 T cells and γ-interferon playing a central role in protection against the disease, and CD8, γδ, and NK T cells as well as tumour necrosis factor and IL-6 also being involved. In a comparative study of the genomes of tuberculosis and BCG, more

than 16 genes, specific to tuberculosis, have been isolated. Naked DNA, recombinant *Mycobacterium tuberculosis* antigens, mutant *M. tuberculosis* strains, and various other strategies should be evaluated for inducing protection in adults (S. Kaufmann).

The design of a malaria vaccine has to conform to two different goals: keeping travellers free from the disease, and preventing severe disease in children and pregnant women in endemic regions. Understanding the life cycle of *Plasmodium falciparum* is fundamental to the development of an efficient vaccine. A vaccine involving a CD8 T-cell response can be provided at the liver stage of malaria, because infected hepatocytes elicit CTL responses. At the blood stage of the disease, erythrocytes are infected and generate an antibody response. To reach the first target group (noninfected travellers) $CD8^+$ T-cell responses and γ-interferon secretion should be effective at the liver stage. Immunization with irradiated sporozoïtes protects about 100% of this group for at least nine months. Nevertheless, this requires exposure to high quantities of irradiated infected mosquitoes. On the other hand, inducing antibody responses in individuals to their blood-stage infection may lead to the control of parasitaemia and prevent the transmission of the disease. The efficacy of future malaria vaccines depends on the identification of antigen targets and the design of a delivery system that induces the correct immune response at each stage of the disease (S. Hoffman).

With the increase of antibiotic use throughout the world to treat bacterial infections responsible for diarrhoeal diseases, there is an increase in antibiotic resistance. Vaccination might help, if not to treat various serious bacterial diseases, at least to minimize their severity. However, the design of a single vaccination approach is often hampered by the diversity of aetiological agents, serotypes, immune mechanisms of protection, and the populations of vaccinees. Live attenuated vaccines delivered orally against rotaviruses, vibrio cholera, shigella, enterotoxigeneic *Escherichia coli* and *Salmonella typhimurium* seem, in the light of the experimental results, the most promising option (Ph. Sansonnetti).

The protective role of IgE antibodies in schistosomiasis infection has been demonstrated in mice and rats. The rat is a good model for humans. Severe forms of infection correlate with the lack of expression of the receptor to IgE on the surface of macrophages and eosinophiles. Neutralizing antibodies against the glutathione S transferase (GST) enzyme can control the disease progression by reducing the production of eggs by females, their hatching capacity, and their viability. Future vaccines to control schistosomiasis should induce the production of IgE antibodies against the GST enzyme. A clinical trial using GST enzyme as the antigen will soon be launched (A. Capron).

The number of deaths (more than two million in 1997) due to AIDS makes clear the urgent need for an effective HIV vaccine. Because HIV changes its use of receptor during the course of infection, and glycosylation limits accessibility to immunogenic epitopes, an HIV vaccine based only on neutralizing antibodies is not a good option. Vaccination of nonhuman primates with simian immune deficiency virus (SIV), while not clearing the infection, induces a CTL activity that correlates with the containment of viral infection due to a virulent SIV challenge. Thus, it seems that controlling the replication of the virus before the infection has settled is the main objective in designing a vaccine (W. Paul). CTLs also play an important role in response to HIV-1 infection, with cross-clade reactivity. A new vaccination approach should include a priming vaccine to induce CTL response, followed by a boosting to induce neutralizing anti-gp120 (M. Girard).

Infants suffer more severe, more prolonged, and more recurrent diseases than adults because in early life innate and specific immunities are weaker than those of adults. In developed countries, vaccination against measles is performed at 12 to 18 months so that the vaccine is not neutralized by maternal antibodies. However, in developing countries and especially in endemic areas, many children would die if the vaccine was not given earlier. Conventional vaccines induce in infants less γ-interferon and CTL response than in adults and are inefficient in the presence of maternal antibodies – the antigen delivery system and adjuvants having no influence on this inhibition. However, conventional vaccines do prime in the presence of antibodies, since a second dose of vaccine very efficiently boosts primary response. On the other hand, DNA vaccines induce similar CTL responses for both adults and infants. An adjuvant like CpG oligonucleotides can also have an influence on vaccine efficacy in the infant by restoring adult-like cytokine production and by activating neonatal antigen-presenting cells to adult-level response. Vaccines for infants should be designed to induce a better Th-1 response in order to optimize their efficacy (A.-C. Sigriest).

Several chronic diseases have been found to have a microbial origin (e.g., ulcers, heart diseases, cervical cancer...). As traditional vaccines are inefficient against chronic diseases, new vaccine design must be thought of. DNA vaccination carries no risk of infection due to the vaccine, and it induces humoral and cellular immunue responses. DNA immunization with complex mixtures of genes can be followed by challenge with the pathogen as an approach to identifying protective antigens without any a priori assumption. Genes can be efficiently delivered to the dendritic cells through the use of a 'gene gun'. Furthermore, inducible vaccines could be designed where a primary vaccination would be followed by a boost with drugs that induce gene transcription (S. Johnston).

Vaccination against respiratory syncytial virus (RSV) would save about seven million lives a year. Many difficulties in obtaining classical vaccines led to the exploration of DNA vaccination. Studies of the viral genome by recombinant approaches have many advantages (such as the identification of viral mutations and the introduction of new specific mutations). This approach is being used to develop a live attenuated anti-RSV (P.L. Collins).

Human vaccination is insufficient to control and eradicate infectious diseases if it is not paired with animal vaccination. Development of new veterinary vaccines is also needed to reach this goal (P. Pastoret).

5. Vaccines against chronic diseases

Acute respiratory tract infections generated by Gram⁻ bacterium *Chlamydia pneumoniae* can lead to atherosclerosis. CD8 T cells and γ-interferon are important in acquired protection against chlamydia. Vaccine design should use stimulation of CD8 cells as a target. DNA vaccination with omp2 gene is currently being tested (Mäkelä and colleagues).

The multiplication of *Helicobacter pylori* in the gastric mucosa of humans can cause gastritis, peptic ulcer, and eventually gastric carcinoma. Current therapies have variable efficacies and face growing antibiotic resistance. The infection raises a high humoral and mucosal response, but the pathogen is generally not cleared. A protective antigen has been identified (the metalloenzyme urease). It is common to all *Helicobacter* strains, highly conserved among species, and essential for the survival of the bacteria. Therapeutic immunization with urease and a mucosal adjuvant is promising, although improvements in the efficacy of the vaccine have to be made. On the other hand, a dozen candidate antigens selected by a genomic strategy will be evaluated for inclusion in a multivalent vaccine (A. Labigne).

Active immunotherapy against cancer is promising. Specific CTLs are found in almost all tumour-infiltrating lymphocytes, from which autologous tumour-specific CTL clones can be derived in vitro. A number of antigenic peptides specific for tumours have already been identified. However, tumour cells are able to escape immune recognition by various mechanisms (downregulation of class I molecules, release of inhibitory cytokines, expression of Fas antigen, etc.). New approaches involving appropriate responses to specific antigens through injections of peptide alone or of peptide pulsed on dendritic cells should be devised. Increasing the immunogenicity of peptides by linking them to lipids or by substituting important amino acids could improve vaccine-based therapy of cancer (J.C. Cerrottini). Immunological memory is a key to preventing metastasis (Ph. Kourilsky).

Experimental allergic encephalomyelitis, the mouse model for human multiple sclerosis, can be suppressed by vaccination with copolymer-1 (a mixture of poly GLAT amino acids). In humans, once the disease is established, copolymer-1 dramatically reduces the attacks. Copolymer-1 shares a cross-reactivity with myelin basic protein peptides, and it can displace the latter's binding on antigen-presenting cells. Its mechanisms of action might involve antigen spreading and bystander suppression. A good response has been observed in the treatment of another autoimmune disease, myasthenia gravis, with a vaccine based on peptide analogues linked together (M. Sela).

6. Science, vaccines, and society

In many different cultures, vaccination was soon associated with prophylactic rituals. Later, the myth of vaccination persisted, and was further enhanced by Pasteur's treatment of J. Meister. When vaccination was accompanied by colonial power, it was sometimes perceived as another burden, and was rejected in order to manifest resistance to local foreign authority. Governments later started taking an interest in vaccination to promote welfare of their people, but it also became clear that vaccination failures or accidents may occur, which resulted in rejection and fear of the vaccinal process (A.-M. Moulin).

Vaccines were designed to curb infectious diseases by lowering the number of infected persons. Vaccination also offers protection not only to vaccinees, but to nonvaccinees in the vicinity of the former because of lowered exposure. Protection can also come from passive vaccination by contact of nonvaccinees with vaccinees excreting live, attenuated organisms (S. Plotkin). Like economic or financial programmes, the design of vaccination programmes can be translated into mathematical equations including different parameters: age, rate of infection, probability of dying... According to this model, in order to block an infection, 90% of the population should be vaccinated, while eradication requires that number to be above 97% (R. Anderson).

The inclusion of vaccination programmes as part of public health calls for public education. People need to understand why vaccination is necessary and to accept it. Vaccination implies the notion of taking a risk in a healthy state, and people rightfully need to be informed of possible adverse effects. However, causal relationships between vaccines and diseases attributed to vaccination must be carefully assessed (N. Halsey).

Importantly, the impact of vaccine on the population of a developed society (like France) is the reflection of the acceptability of this vaccine by physician practitioners. This fact emphasizes the necessity of implicating doctors in vaccination programmes much more deeply and of improving their public health background (J. Drucker). The acceptance of a vaccine also takes place through the medium of popular newspapers, radio, and television, and vaccination programmes should target specific populations that can have influence on future generations, for instance, mothers (D. Salisbury). It is also of importance to determine whether a vaccine is needed, as well as why, and where. Today, cost-benefit, cost-effectiveness, and cost-utility analyses are integrated into the design of vaccines. Often, time, availability of affordable product and adequate financial returns can be achieved simultaneously (A.R. Hinman).

Disease has become a surrogate for alien invasion. Vaccination is a unique interface between science and society. In order to have been able to vaccinate 121 million children in India against polio in a couple of days, world cooperation was needed. To obtain a continuous impact of vaccination also requires improvement in social cooperation. Vaccines, as the 'tugboat' of other public health programs, should ideally bring more social justice (W. Foege). However, the discrepancies between developed and developing countries are getting bigger: a child from a developing country is forty times more likely to die from an infectious disease than a child from a developed country. On the other hand, the diversity in economies, in epidemiological vectors, and in climates, varied sanitation levels, and even the occurrence of conflicts have to be

taken into account when vaccination programmes are to be introduced into developing countries. We need then to answer a rather cynical question: 'Where are the vaccines needed?' (R. Widdus). The expanded programme of vaccination has been set up in developing countries 'at risk', bringing along with it infrastuctures including medical assistance and health education. How not to lose the benefit of these campaigns is another question that requires an urgent answer, along with how to devise a follow-up programme aimed at providing boosters after primary vaccination (A. Da Silva).

In fact, it is clear that social, political, and cultural aspects have to be taken into account before, during, and after vaccination campaigns. Nonscientific issues specific to developing countries – such as rapid growth, development, and urbanization, which lead to overcrowding and poor sanitation, along with political instability and financial crises, which might destroy primary care systems – must be considered, together with scientific issues such as excessive use of antibiotics, emerging pathogens, and disappointing results in some vaccine trials. Solutions including affordability and access to vaccination, as well as acceptance and awareness by promoting education, call for worldwide research cooperation at the level of epidemic surveillance, basic medical sciences, vaccine delivery, and improved vaccine development (T. Pang).

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