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ABSTRACT BOOK

Edited by
Anna Maria Marella and Giuseppina Mandarino

Laboratory of Bacteriology and Medical Mycology

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This second international Conference on Vaccines is devoted to report on research on anti-infectious vaccines and vaccinations. It focuses upon novel strategies in the search of vaccine candidates, in particular through post-genomics and proteomics, adjuvants and vaccination modalities. Special emphasis is on the impact exerted by the recent exciting biotechnological achievements on the generation of novel vaccine components, addressing emergent and re-emergent infections, as well as on improved approaches and implementation of vaccination policy. The Conference wishes to provide some answers to the complex, hot questions arising from the progress in the different areas impacting on vaccines and vaccinations in the next future, mostly on the context of public health actions.

Key words: Vaccines, Vaccination, Infections, Biotechnology

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Questa seconda Conferenza sui Vaccini è dedicata ad illustrare i recenti progressi nel campo dei vaccini e delle vaccinazioni antinfettive. Sono messe a fuoco le nuove strategie per la ricerca di candidati vaccinali, adiuvanti e modalità di vaccinazione. Enfasi speciale viene posta sull'impatto esercitato dalle più recenti acquisizioni biotecnologiche, soprattutto in termini di ricerca post-genomica e proteomica, sulla generazione di nuovi vaccini contro le infezioni emergenti e riemergenti come pure sui miglioramenti e sulle nuove applicazioni di politica vaccinale. La Conferenza vuole fornire alcune risposte alle più complesse problematiche derivanti dai progressi nelle diverse aree che condizionano lo sviluppo dei vaccini, soprattutto quelle rivolte ad orientare le necessarie azioni di sanità pubblica.

Key words: Vaccini, Vaccinazioni, Infezioni, Biotecnologie

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Opening lecture

COMBINATION VACCINES: CHALLENGES AND REWARDS

Kathryn Edwards
Vanderbilt University, Nashville (TN), USA

The continuing increase in the number of effective vaccines suitable for use in infancy and early childhood has posed substantial economic and logistical difficulties. Providing these vaccines as separate injections not only is expensive but also requires multiple needle sticks, distressing parents, providers, and patients alike. Scheduling additional vaccination visits to reduce the number of injections per visit increases costs, burdens staff, and jeopardizes the entire immunization program by increasing the likelihood of missed vaccinations. The shipping, handling, and storage of a plethora of vaccines are burdensome and expensive and increase the possibility of error. These problems have stimulated continuing efforts to develop new combination vaccines. However, the development and evaluation of combination vaccines can pose complex issues.

The combining of multiple related or unrelated antigens into a single vaccine is not a new concept; combination vaccines have long been a bedrock of our pediatric and adult immunization programs. Those combination vaccines in common use include diphtheria and tetanus toxoids, available alone (DT or Td) or with whole-cell (DTwP) or acellular (DTaP) pertussis vaccine; inactivated (IPV) or live oral (OPV) trivalent polio vaccine; and measles and rubella vaccine, available alone (MR) or with mumps vaccine (MMR).

The first combination vaccine licensed in the United States was trivalent influenza vaccine, approved in November 1945, and the second was a hexavalent pneumococcal vaccine, licensed in 1947. DTwP, although developed in 1943, was not licensed until March 1948. IPV was licensed in 1955, and the individual OPV serotypes were licensed from 1961 to 1962. Efforts to overcome the interference seen with simultaneous administration of three live vaccines delayed the licensure of trivalent OPV until June 1963. MMR and MR were licensed in April 1971, and quadrivalent meningococcal vaccine in 1978.

As the number of safe and effective pediatric vaccines has grown, efforts have intensified to develop increasingly complex combination vaccines. Most such pediatric combination vaccines begin with a DTwP or DTaP vaccine and add such antigens as IPV, conjugate *Haemophilus influenzae* type b (Hib), and hepatitis B (HB). As development efforts for the DT(a)P-based combinations have matured, some manufacturers have turned their efforts toward developing so-called second-shot combinations that incorporate conjugate pneumococcal (PnC) and conjugate meningococcal (MnC) antigens. A third developmental stream has been directed toward combination vaccines targeted principally at travelers, typically based on HB or hepatitis A (HA) components.

The primary focus of this presentation will be on the newer combination vaccines that merge products such as HB or Hib vaccine with each other or with one or more of the traditional combination vaccines. Issues that complicate the development, evaluation, and licensure of combination vaccines will be discussed.

Special lecture

GENOMICS AND REVERSE VACCINOLOGY

Rino Rappuoli
Chiron SpA, Siena, Italy

Since the discovery that microbes cause infectious diseases and that vaccination can be used to prevent them, the first task of every scientist working in vaccine development has been to grow the infectious agent. The *in vitro* grown microorganisms then could be used as starting material for the identification of protective antigens or could be manipulated, usually by multiple *in vitro* passages, in order to obtain a live-attenuated microorganism.

The DNA sequence of the whole genomes of microorganisms allowed for the first time to tackle vaccine development starting from the computer prediction of protective antigens and to obtain vaccine candidates without the need of growing microorganisms. In order to underline the different path to vaccine discovery that has been made possible by the genomic sequencing, this new method has been named “reverse vaccinology”.

Reverse vaccinology is not just a different method to practice vaccine development, but it is a powerful tool to tackle those vaccines that have been difficult or impossible so far. The first example of a genome-based vaccine development has been serogroup B meningococcus. In this case, forty years of vaccine research using the conventional approaches had discovered 15-20 potential antigens, which unfortunately had been ineffective in providing a vaccine that could universally protect against the disease. The availability of the genomic sequence made available at once all potential antigens encoded by the bacterium. 600 novel potential antigens were identified by computer prediction, expressed as recombinant proteins in *Escherichia coli* and tested as vaccines within 18 months. 29 novel antigens were found to be good vaccine candidates. The best among the novel antigens have been used in subsequent studies to design a universal vaccine to be tested in clinical trials. The same approach used for meningococcus B is now being applied to several microorganisms and is likely to lead to the development of many novel vaccines.

In conclusion, the present is one of the best moments in history for vaccine development. The information provided by the genomic era has made all possible antigens of nearly all pathogenic microorganisms available in databases. The progress in immunology and vaccine delivery is making possible to target all arms of the immune system. GAVI (Global Alliance for Vaccines and Immunization) and the vaccine fund are making available an unprecedented amount of money.

Nevertheless, vaccines are not a priority for industry and this may jeopardize the future of vaccines unless properly addressed on a global basis.

Session I
Improving immune responses to vaccination

Chairpersons
Maria Teresa De Magistris, Sergio Romagnani

BACTERIAL CPG SIGNALS VIA TOLL-LIKE RECEPTOR 9 (TLR9)

Hermann Wagner
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Bacterial DNA and certain Oligonucleotides (ODN) containing unmethylated CpG dinucleotides stimulate innate immune cells, whereas eukaryotic DNA and methylated oligonucleotides can not. CpG-DNA directly stimulate B cells, macrophages and Dendritic Cells (DCs) to secrete cytokines, specially Th1 like cytokines such as IL-12 and IL-18, TNF-α and IL-1β. The cells express co-stimulatory molecules and show increased antigen presentation. *In vivo* CpG-DNA induces strong Th1 responses to soluble proteins and protective Th1 responses in Th2 driven infectious diseases. To understand the molecular mechanism by which CpG-DNA exerts its biological effects we analysed together with Akira's group (Osaka/Japan) the signalling pathways involved. CpG-DNA activates the stress kinase JNK1/2, p38 and ERK, via MyD88 and TRAF-6. While TLR2 and TLR4 deficient mice respond normally to CpG-DNA, TLR9 deficient mice are completely defective in their response to CpG-DNA. Genetic complementation of human non-responder cells with either human (h) or murine (m) TLR9 conferred responsiveness to CpG-DNA in a CD14 and MD2 independent manner, yet required species specific CpG-DNA motifs for initiation of the Toll/IL-1R signal pathway via MyD88. Greenfluorescence protein (GFP) tagged MyD88 became recruited to endosome like structures in response to CpG-DNA, colocalising with CpG-DNA and TLR9. In contrast, LPS recruited GFP-tagged MyD88 to the cell membrane. Human CD123+ pDC2 express TLR9, respond to CpG-DNA but not to LPS and produce large amounts of type 1 Interferon. In contrast, human CD11c positive pDC-1 respond to LPS (and express TLR4) but not to CpG-DNA (and don't express TLR9). Thus human DCs segregate in CpG-DNA and LPS responsive subpopulations, the former producing type 1 Interferon and the latter Interleukin 12.

TOWARDS THE RATIONAL DESIGN OF TH1 ADJUVANTS

Philippe Moingeon, Jean Haensler, Alf Lindberg
Department of Research and Development, Aventis Pasteur, Marcy l'Etoile, France

Finding adjuvants in order to enhance immune responses against target immunogens has been a major and recurrent issue for the vaccine industry. Yet, it is to be solved, most particularly in the context of a growing interest in designing new types of vaccines capable of eliciting Th1 immune responses. A review of synthetic adjuvants which have been (or are being) tested in clinical studies will be presented. Importantly, recent advances in our understanding of the physiology of immune responses offer new avenues to design and test candidate adjuvants, based on either synthetic or natural molecules, with the aim to mimic and recapitulate pro-inflammatory signals initiating both innate and adaptative immune effector mechanisms. Thus, adjuvants of the future might be a mixture of molecules selected singularly for a capacity to either attract, target or activate professional antigen presenting cells. Used as a combination, such molecules should facilitate antigen presentation by professional APCs and lead to a potent induction of T cell-mediated effector and immune memory mechanisms.

HOW IS MEMORY MAINTAINED IN THE IMMUNE SYSTEM?

Antonio Lanzavecchia
Institute for Research in Biomedicine, Bellinzona, Switzerland

Immunological memory confers to primed individuals a certain level of immediate protection as well as the capacity to mount secondary responses. These aspects have a distinct cellular basis. Protective memory is mediated in peripheral tissues by "effector memory" T cells and by antibodies, while reactive memory is mediated by "central memory" T cells and memory B cells that mount recall responses in secondary lymphoid organs. I will propose that in the absence of antigen central memory T and B cells function as "stem cells" continuously generating low levels of effector T cells and plasma cells in response to homeostatic cytokines and other polyclonal stimuli. The "stem cell model" of immunological memory has implications for vaccination, immune reconstitution and autoimmunity.

IMPROVING IMMUNE RESPONSES TO NEONATAL VACCINATION: WHICH CHALLENGES?

Claire-Anne Siegrist
World Health Organization Collaborating Centre for Neonatal Vaccinology, University of Geneva, Switzerland

Limitations of neonatal and early life immune defence mechanisms increase the risk of certain infections and may limit vaccine responses. Immunological challenges include induction of generally weaker primary Ab responses to both polysaccharide and protein antigens and of limited clearance capacity of intracellular pathogens, possibly related to weaker IFN-γ and cytotoxic T cell responses than later in life, in addition to the potentially inhibitory influence of maternal antibodies. The relative role of the maturation of Antigen-Presenting-Cells, B and T cells in these limitations is progressively being deciphered, both in human and animal models. It has already become clear that, upon appropriate immunological stimulation, strong Th1/CTL responses could be elicited in the neonatal period, and that even high titers of maternal antibodies do not prevent induction of $CD4^+/CD8^+$ responses. Induction of memory may also be achieved early after neonatal immunization, even in absence of detectable antigen-specific effectors, which could possibly allow earlier disease control. In contrast, limitations in the induction of primary Ab responses persist despite the use of strong adjuvants/efficient delivery systems, prompting for a better understanding of limiting factors. Our recent work on the influence of the postnatal development of the spleen/lymph node micro-architecture in the shaping of primary Ab responses suggests that unresponsiveness to lymphoid-mediated signals at the neonatal follicular-dendritic-cell precursor level delays germinal center induction and Ab responses to T-dependent antigens.

NERVE GROWTH FACTOR AND IMMUNODEFICIENCY IN THE ELDERLY

Federico Cozzolino
University of Rome "Tor Vergata" and INeMM, National Research Council, Rome, Italy

The autocrine production of Nerve Growth Factor (NGF) by memory B cells is a critical mechanism that preserves their long-term survival, by maintaining the structural and functional integrity of the Bcl-2 protein, the most important anti-apoptotic factor in memory B cells. Neutralization of this autocrine circuit by anti-NGF antibodies leads to apoptotic death of memory B cells *in vitro* and to a decrease of specific IgG responses against immunizing antigens in a mouse model, while the primary IgM response is not affected. The same pattern of IgM *vs* IgG humoral response is typical of senescent individuals. We therefore considered the possibility that in aged animals a reduced availability of NGF occurs for the maturing memory B cell population after the somatic hypermutation process within the germinal center. In particular, we investigated whether substantial abnormalities in the generation of memory B cells could be detected in ageing mice and, if so, whether the autocrine NGF circuit was involved in the metabolic derangements. We also designed experiments to check whether the induction of endogenous NGF production might attenuate or correct the humoral response abnormalities of aged mice. Here, we describe that ageing is associated with a sharp decline in the number of cells bearing the phenotype of memory B cells. A reduced production/availability of NGF is at the basis of such condition, as memory B cells, isolated from spleens of elderly animals, produce minimal amounts of NGF, if any, and die faster than their counterpart in young animals. The humoral immune response is severely compromised in populations of aged mice and treatment of old animals with inducers of endogenous NGF gene expression – like recombinant NGF itself – is sufficient to restore the quantity and even the quality of the humoral response against simple or complex immunizing antigens, at levels comparable to those of young populations. Conversely, the humoral response of young immunocompetent mice can be made "old-senescent", in terms of quantity and affinity of serum specific IgG, by treatment with neutralizing anti-NGF antibodies, that induce apoptosis of differentiating memory B cells. These findings indicate that induction of NGF expression in the maturing population of memory B cells may represent a novel strategy for treatment of humoral immunodeficiency of ageing.

Session II

The impact of recently marketed vaccines and present status of vaccines under development

Chairpersons
Vincenzo Barnaba, Paola Verani

PERTUSSIS VACCINES: LESSONS, QUESTIONS AND PERSPECTIVES

Nicole Guiso
Unité des Bordetella, Institut Pasteur, Paris, France

Whooping cough is a very recent disease, described for the first time in 1578. Its agent is a Gram negative bacterium, *Bordetella pertussis*, discovered by Bordet and Gengou in 1906. The first vaccines, Pertussis whole-cell vaccines (Pwv) were composed of bacterial suspensions inactivated by heat. Generalized vaccination was introduced in many developed countries in the 50's. Pertussis vaccination of infants has dramatically reduced disease, complications and deaths in infancy and early childhood. However, the efficacy of Pwv was variable, they were not well tolerated and vaccination was stopped in some countries. Furthermore, forty years after the introduction of vaccination in countries with high coverage and efficacious Pwv, a resurgence of the disease is now observed with a shift in the age distribution of pertussis cases, adolescents and adults being identified as a major source of infection of non-vaccinated infants. In these countries, the observed disease resurgence seems to be attributable to a reduction of natural boosters following contact with wild-type *Bordetella pertussis*, and the lack of vaccinal boosters for adolescents and adults. This hypothesis is supported by different studies suggesting that immunity following pertussis infection or Pwv vaccination is not life-long, most of the hospitalised infants are contaminated by adults, and prevalence of pertussis infection in the adult population is high. For all these reasons better tolerated vaccines, but as efficacious as Pwv, were necessary. Vaccines composed of purified inactivated bacterial proteins, Pertussis acellular vaccines (Pav), were developed after the characterization of *Bordetella pertussis* virulence factors. Pav composed of one to five components were found efficacious and better tolerated than Pwv in infants. These vaccines are now used for primo-vaccination in different countries but also for adolescents and Health Care Workers boosters already in a few countries. Furthermore, it was recently shown that *Bordetella pertussis* isolates circulating within European populations are different from those strains that form the basis of either Pwv or Pav. Is the emergence of these variants due to vaccine pressure? Will Pav protect against all variants? There is no conclusive evidence of a causal relationship between variations in circulating isolates, vaccination practices or vaccine types. However, evolution of *Bordetella pertussis* population has to be monitored carefully and the surveillance of the disease must continue.

PERSPECTIVES IN THE DEVELOPMENT OF A VACCINE AGAINST *HELICOBACTER PYLORI*

Giuseppe Del Giudice
Chiron SpA, Siena, Italy

Helicobacter pylori causes one of the most widespread infections worldwide: it affects more than 50% of the human population, and is responsible for serious gastric pathologies such as chronic gastritis, peptic ulcer, atrophic gastritis and, in some individuals, gastric cancer. Current treatments with antibiotics are efficacious, but encounters several drawbacks at the level of compliance, side effects, antibiotic resistance, etc. The availability of vaccines could contribute in reducing the burden of *H. pylori* associated diseases. Several bacterial antigens have been identified as virulence factors and proposed as potential vaccine candidates. Some of these antigens have been tested in experimental animal models of challenge with *H. pylori*. The experiments in animals have shown that prophylactic and therapeutic vaccination against *H. pylori* is indeed feasible. Several open questions still remain concerning the understanding of the host-microbe relationship and the quality of the immune response which should be induced in order to confer protective immunity in humans. The answers to these questions will be crucial in helping the preparation of appropriate vaccine formulations able to efficaciously protect humans both prophylactically and therapeutically. Some clinical trials have been carried out so far with limited results using orally delivered vaccines. Trials are in progress in humans using antigen combinations delivered parenterally. The hope is that these new vaccines will show the expected efficacy against *H. pylori* and will permit the elimination of this pathogen which has cohabited with humans for more than 100,000 years.

TACKLING HCV INFECTION

Sergio Abrignani
Chiron SpA, Siena, Italy

Abstract not received

GLYCOCONJUGATE VACCINES FOR *STREPTOCOCCUS PNEUMONIAE*: PRESENT AND FUTURE

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The development of glycoconjugate vaccines for encapsulated bacterial pathogens has resulted in the control of invasive diseases caused by *Haemophilus influenzae* type b and *Neisseria meningitidis* group C. The recent introduction of a highly efficacious, 7-valent vaccine (Prevenar®) against the major serotypes of *Streptococcus pneumoniae* (pneumococcus) is having a similar impact on disease in the US where vaccine use in children is universal. It appears from early post-introduction studies that the impact of this vaccine may be even greater than anticipated due to a herd effect on unvaccinated populations of children and adults. The success of Prevenar has lead to the development of future generations of this product that will have expanded serotype and serogroup coverage and therefore greater utility in many regions around the world. Recent data from South Africa have demonstrated the efficacy of a 9-valent formulation (adding serotypes 1 and 5) in young children in Soweto. An assessment of the global epidemiology of pneumococcal disease suggests that a vaccine with 11-13 conjugates will achieve over 70% disease coverage in most regions of the world. The next step beyond expanding the serotype coverage for pneumococcus is the evaluation of combinations of this vaccine with other glycoconjugates. The most important in this area is to combine the pneumococcal conjugates with conjugates for the meningococci. The recent success of meningococcal group C conjugate vaccines in Europe has lead us to develop a combination vaccine that contains 9 pneumococcal serotypes along with our meningococcal group C conjugate (Meningitec®). Data from this combination demonstrate that these vaccines can be delivered safely and without interference. In the future, it will be important to include meningococcal serogroups A, Y and W-135 to an expanded pneumococcal conjugate vaccine formulation. It is our hope that this can be accomplished in a single formulation. And lastly, in the field of pneumococcal disease control, the potential of glycoconjugate vaccines to improve on the efficacy of polysaccharide vaccines in the elderly will be an important area of future research.

NEW INSIGHTS INTO THE MECHANISM(S) AND FUNCTION OF ADAPTIVE HUMORAL IMMUNITY

Arturo Casadevall
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The mechanism of antibody-mediated protection has been studied for over 100 years and there has been a general consensus in the field of immunology that it is well understood. Classically, specific antibody is believed to protect against pathogenic microbes by opsonization, complement activation, virus and toxin neutralization, and antibody-dependent cellular cytotoxicity. However, antibody-mediated protection against the facultative intracellular pathogen *Cryptococcus neoformans* cannot be explained by any of these mechanisms. Studies of the immune response to *C. neoformans* infection in mice in the presence and absence of specific antibody reveal changes in cytokine expression associated with antibody-mediated protection. Antibody-mediated protection was associated with elevations in Th2 cytokines that appear to result in a more effective cell-mediated response, possibly by reducing host damage resulting from the vigorous Th1-polarized response to *C. neoformans* infection. For *C. neoformans* a new mechanism of antibody-mediated opsonization was discovered whereby antibody binding to the polysaccharide capsule mediated changes that allowed phagocytosis through CR3 (CD18/CD11b) in the absence of complement. Other studies revealed a critical relationship between the amount of antibody present and the outcome of infection with the paradoxical result of 'prozone-like' effects at high concentrations of antibody whereby a protective antibody lost protective efficacy or became disease enhancing. The mechanism of action of 'prozone-like' effects may involve interference with fungal killing and/or the immune response elicited though both specific and non-specific effects of high antibody doses. Another unexpected finding is that for certain antibodies the constant region can alter fine specificity thus challenging the long-standing dogma that antibody specificity is solely the purview of antibody-antigen interactions mediated by the variable region. From these observations a new synthesis of antibody-function will be proposed that includes a major role in modulating the intensity of cellular immune response.

Session III
Modern approaches and technologies for vaccine design

Chairpersons
Carlo Pini, Gianni Pozzi

DENDRITIC CELLS AND PATHOGENS

Paola Ricciardi-Castagnoli, Francesca Granucci
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Global gene expression analysis has found large application in the field of immunology, as it has clearly emerged that the study of a single immunological parameter at a time it is not sufficient to reach a general vision of how the immune system faces a particular pathogen, maintains self tolerance or remembers past infections. An interesting feature of Dendritic Cells (DC) is that they respond to perturbations (invading pathogens) without destroying self-tissues. To exert this function DC use at least three different strategies. They respond, in a few hours, to infectious agents (innate immunity) by recognizing molecular patterns typical of microorganisms and absent in self-tissues; they mount a late response that discriminates among different microbes giving rise to memory (adaptive immunity), and, finally, they maintain tolerance against self-proteins. The global gene expression analysis of DC in response to various pathogens will be discussed and a predictable DC signature will be presented.

DNA VACCINE

Karin Moelling, Jan Schultz, Jochen Heinrich, Jovan Pavlovic
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We and others have previously reported on profound anti-tumor and long-lasting anti-metastatic efficiency elicited by interleukin 12 (IL-12)-encoding plasmid DNA, which acts by the induction of a positive cytokine feedback loop. In contrast to protein therapy IL-12-encoding DNA was non-toxic in small and large animal models. In an ongoing compassionate trial a patient with abdominal tumor showed some reduction of tumor size after local treatment with no toxicity. A clinical trial with 9 patients is ongoing. A follow-up of an HIV-DNA vaccine for 3 years (4 patients) shows no anti-DNA antibodies. We investigated a number of cytokine-encoding DNAs. In combination with IL-12- and interferon-induced protein 10 (IP-10)-encoding DNA the anti-tumor and anti-metastatic efficiencies were enhanced. Furthermore, we designed a new DNA construct by using a prokaryotic protelomerase, a cleaving-joining activity, which forms linear prophage DNA with closed ends in lysogenic bacteria. Such doggy-bone DNA, a linear covalently closed dumbbell-shaped DNA, coding for a cytokine has been analyzed in a tumor model in mice and showed some efficiency.

DNA-MICROARRAY TECHNOLOGY: BENEFITS AND PROBLEMS

Jörg D. Hoheisel
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The Division of Functional Genome Analysis at the DKFZ is involved in the development of technologies for the analysis of large genomic areas to entire genomes with respect to the encoded functions and their regulation. Based on technical advances, various functional aspects are being analysed. One emphasis is work on DNA-, protein- and peptide-microarrays. Apart from addressing chemical and biophysical issues, the resulting methods are immediately put to the test in relevant, biologically driven projects on various organisms. Beside other applications, analyses are performed on the detection and use of disease-relevant polymorphisms and epigenetic variations in the area of molecular epidemiology as well as comparative studies on transcript levels and the actual protein expression by means of complex DNA- and antibody microarrays. Also, systems are being developed toward early diagnosis, prognosis and evaluation of the success of disease treatment.

Session IV
Vaccines of poverty

Chairpersons
Fernando Aiuti, Giancarlo Majori

VACCINATION STRATEGIES AGAINST TUBERCULOSIS

Stefan H.E. Kaufmann
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Tuberculosis remains on the top list of microbial killers. Its threat is further worsened by the increasing incidences of multi-drug-resistant strains and the dangerous liaison with HIV. General agreement exists that tuberculosis can only be conquered successfully by novel vaccination strategies in adjunct to improved chemotherapy. Currently, a vaccine against tuberculosis exists named bacille-Calmette-Guérin (BCG). Although this vaccine prevents miliary tuberculosis in newborns satisfactorily, it is inefficient in protecting against adult pulmonary tuberculosis. Novel vaccination strategies either focus on subunit vaccines including naked DNA-vaccines or viable attenuated vaccines. Subunit vaccination strategies are based on the assumption that one or few antigens suffice for an efficient immune response. Hence, the identification of protective antigens represents an essential prerequisite for the success of this type of vaccines. Identification of protective antigens is best performed on the global level using transcriptome and proteome approaches. Subunit vaccines come in two forms: protein/adjuvant formulations or naked DNA constructs. Viable attenuated vaccines are based on the assumption that multiple antigens are required for efficacious protection. Two major strategies are being pursued: knockout mutants of *Mycobacterium tuberculosis* and improved recombinant (r)-BCG vaccines. Crippled versions of *M. tuberculosis* should not only be deleted of genes involved in virulence/persistence, but also in genes that manipulate the protective immune response. Improved r-BCG should first be endowed with a higher immunogenicity and second may need a broader antigenic repertoire. The rational design of novel vaccine candidates will be described and the pros and cons of the different vaccine types discussed.

DEVELOPMENT OF HIV/AIDS VACCINE BASED ON TAT: FROM BASIC SCIENCE TO HUMAN TRIALS

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Tat, the transactivator of HIV-1, is produced very early after infection, is key to the virus life cycle and to AIDS pathogenesis, is immunogenic and well conserved among HIV subtypes. A Tat-specific immune response in humans or monkeys correlates with nonprogression to AIDS. In addition, our recent studies indicate that active Tat activates DC (Dendritic Cells) function and drives Th-1 type immune responses that are key to virus control. We have recently shown that vaccination of cynomolgus monkeys with biologically active HIV-1 Tat protein or DNA is safe, immunogenic and contains primary infection with the SHIV89.6P. No signs of virus replication, nor CD4 decline or disease onset was found in 9 of 11 vaccinated monks in 2 years of follow-up. Protection correlated with the presence of CD8+ CTLs and CD8-mediated antiviral activity. No residual virus hidden in resting cells was detected by QC-RT-PCR in any of the protected monkey either in the plasma or in lymph nodes, upon two boosts with tetanus toxoid, a stimulus known to activate virus replication. In addition, upon challenge vaccinated animals had a boost of anti-Tat responses and developed anti-Gag immune response. Safety and immunogenicity data have been confirmed by two new protocols and Tat (protein or DNA) was safe also in monkeys with AIDS. Based on these data, preventive and therapeutic phase I clinical trials are being started in Italy. In addition, immunological, virological and feasibility studies for phase III trials are being conducted in South Africa and Uganda. The results indicate that there is a high sequence homology and an extensive immune cross-recognition between Tat B clade, that is used for phase I studies in humans, and Tat from clades A, C and D that are found in Uganda and South Africa, strongly supporting the concept that a Tat-based vaccine can be universally applied.

MALARIA: FROM BASIC RESEARCH TO VACCINE

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Malaria caused by the parasite *Plasmodium falciparum* contributes with 200 million cases and 1,5 million deaths per year mainly amongst children under the age of 5 years. During the last years, the emergence of drug resistant parasites together with poverty and the lack of adequate logistics have caused an increase in the number of malaria case in Africa and Asia. Experiments carried out in the laboratory as well as observations on people living in malaria endemic countries indicate that immunity against the parasite can protect from subsequent infections. These observations have provided the rationale for a series of attempts aimed at developing a vaccine against malaria. This has proven to be a very complex and difficult task. After the development and assessment of about 40 different experimental vaccines there is not yet an effective malaria vaccine. Progress in understanding how the immune response ultimately controls the infectivity and the growth of the malaria parasite has been slow due to the lack of suitable animal models and *in vitro* assays for measuring level of protective immunity *in vivo*. None of the immune responses identified in humans against *P. falciparum* antigens correlates unequivocally with either protection from infection or decreased morbidity. This lack of basic knowledge has hampered the development of an effective vaccine. Parasite components have been evaluated for vaccine development with a priority that was dictated by the order in which they have been discovered rather than for their role as targets of protective immunity. The sequencing of the *P. falciparum* genome has revealed that the parasite might produce about 5,000 different proteins and contain multiple variants of many of the corresponding genes. New experimental approaches must be sought to identify amongst this challenging number of microbial molecules, those that are responsible for inducing the natural acquired immunity observed in the field. Genomic information, transcription profile and proteomic analysis will be extremely helpful in predicting protein function and the likely sub-cellular localisation as well as to identify genes that contribute to virulence and pathogenesis. However, all these data combined together will provide little information about the ability of microbial molecules to elicit a protective immune response. High throughput assays for measuring the cell mediated and humoral immune responses against a vast repertoire of microbial proteins in large groups of individuals would be needed to fill the gap between genomic data and vaccine development.

DEVELOPMENT AND FIELD EFFICACY OF AS02A ADJUVANT: MALARIA VACCINE AS AN EXAMPLE

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During the past decade, various means of vaccination have emerged, including DNA vaccination, live vectors and adjuvanted proteins. All have been shown efficacious in inducing one or both arms of the immune response in animal models, but did not so far demonstrate the same efficacy in humans. Indeed, DNA vaccination requires high amount of material to induce CTL responses and provides only weak antibody responses, live vectors have been shown to be efficacious but are impaired by safety concerns, and adjuvanted proteins have not been shown so far to induce consistent CTL in humans. Over the past 6 years, we have established the capacity of an adjuvanted protein vaccine against malaria (RTS,s and AS02A), to protect adult volunteers against experimental as well as field challenge. The immune response induced with this vaccine was shown to be both humoral and cell mediated. This vaccine has been further tested in the field. Data on the development of the formulation, the immune response induced and its efficacy in the field challenges will be presented.

DIARRHOIC DISEASES

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Abstract not received

Concluding remarks

PUBLIC HEALTH PERSPECTIVES OF ANTI-INFECTIOUS VACCINATION

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As stated in the 2002 edition of the Jordan Report (NIAID, NIH), the last 20 years have witnessed an explosive upsurge of interest and progress in the area of vaccines and vaccination against infectious (and non-infectious) diseases. Yet we continue to be incapable of controlling such devastating diseases such as malaria, tuberculosis, AIDS and others worldwide. Will the fruits of post-genomic research be harvested during the next ten years or ancient and will modern plagues of humankind continue their dreadful course? Will the threat of bioterrorism persuade government authorities, public health stakeholders and granting agencies to commit themselves to the off-market vaccine generation such as those against anthrax or tularemia? Will the anti-vaccine movements find more voice among politicians and pseudoscientists to rise the challenges posed by safety problems to an unbearable level? And will the scientists proficiently use all the biotechnological tools and resources that the last decade has generated for improvements in all areas of vaccines and vaccination?

At the end of the First Vaccine Conference held in this Institute in 1998, there was a clear recognition that the "easy" vaccines had been already developed, and that the next ten years would have seen great challenges but also significant advances in the area of "difficult" vaccines. Since then, some progress has been made in the generation and licensing of new vaccines (for example, the pneumococcal conjugates), in the area of live attenuated vectors, genetic engineering, gene immunization and delivery systems, nanotechnologies and adjuvant immunology. Great enthusiasm is also derived from basic immunological and microbiological research disclosing vaccine-exploitable features of host-parasite relationship as well as from the rather recent and exciting discoveries of the very close links between innate (adjuvants) and adaptive (antigens) immunity.

In substance, much remains to be accomplished and the "difficult" vaccines are not yet available. Vaccinologists, however, should be always aware of two memorable and unsurpassed scientific and ethical issues resting on their work and commitment: 1) the science of vaccines sums up the best of all other sciences and progress in its absolute interdisciplinarity; 2) only vaccines can eradicate diseases. No other discipline can offer to their devotees and public health such a stimulating scientific reward coupled with such an immense human benefit.

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