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Policy paper

INNAMORA, a European Workshop focussed on the mechanisms of innate immunity in pathogen–host interaction and their exploitation in novel mucosal immunisation strategies

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1. The Euroconference/Workshop on "Novel Strategies of Mucosal Immunisation through Exploitation of Mechanisms of Innate Immunity in Pathogen–Host Interaction"

Aims of the conference

The Euroconference/Workshop "Novel Strategies of Mucosal Immunisation through Exploitation of Mechanisms of Innate Immunity in Pathogen–Host Interaction" (acronym INNAMORA) was held in Siena, 6–9 November 2002, at the Research Centre of Chiron Vaccines. The aim of the meeting was the implementation of European efforts towards novel vaccination strategies within a global vision, with many objectives:

1. To gather the co-ordinators of the most relevant EU-funded projects on vaccine strategies, mucosal immunisation, and innate immunity, in order to establish the state of the art of the European research in this field.
2. To be updated on the newest trends and directions of research on the mechanisms of innate immunity and their exploitation in the design of new vaccination strategies, with a series of high-level overviews and lectures from top scientists and opinion leaders.
3. To encourage involvement of younger investigators in the field of research and development of vaccines, through data presentation, discussion with the world leaders in the field, and direct contact with an industrial research environment.
4. To confront and compare international policies in the vaccine field, with presentations and discussion among representatives of NIAID (the US National Institute of Allergy and Infectious Diseases), EU Commission, and international organisations (WHO, IVI), to dissect the future trends of mucosal immunisation strategies within the global health policies.

Programme of the conference

OPENING SESSION
Funding Strategies for Vaccine Research

Aldo Tagliabue (IVI, Seoul, Korea): Introduction
Donata Medaglini (EU Commission, Bruxelles, Belgium): European Funding through the sixth FP
Katherine Taylor (NIAID, Bethesda, MD, USA): NIAID Biodefence Research Agenda

KEYNOTE ADDRESS
Thomas Lehner (London, UK): Innate and Adaptive Immunity against HIV Infection

EUROCONFERENCE
Novel Strategies of Mucosal Immunisation through Exploitation of Mechanisms of Innate Immunity in Pathogen–Host Interaction

Session 1: *INNATE IMMUNITY AND PATHOGEN–HOST INTERACTION*
Overview: John Holton; Chair: Ivan Roitt

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Projects:

- ADRI—Novel inhibitors of adhesin/receptor interactions involved in microbial infection at mucosal surfaces. QLK2-2001-01216. Ivan Roitt.
- POLYCARB—Treatment and prevention of anti-bacterial infections by anti-adhesion compounds. QLK2-2001-01852. Roland Pieters.
- INVADERS—Innate immunity and vaccine development: role of soluble mediators. QLK2-2001-02103. Sandra Gessani.
- IFN-ALPHA and HBV VA—Evaluation of the adjuvant activity of IFN-α in vaccination strategies against HBV. QLK2-2001-02114 (Demo). Filippo Belardelli.
- HOSPATH—Towards control of septic shock induced by gram-positive bacteria: host pathogen interactions. QLK2-2000-00336. Terje Espevik.
- INNATE DEFENCE—Experimental and clinical dissection of innate immunity against intracellular pathogens. QLK2-2002-00846. Martín Rottenberg.

Session 2: *VACCINATION STRATEGIES*

Overview and Chair: Giuseppe Del Giudice

Projects:

- DC STRATEGIES—Immunological mechanisms of T cell activation by dendritic cells: a novel strategy for immune intervention. QLK2-2000-00470. Paola Castagnoli.
- PROTARVAC—Development of prophylactic and therapeutic vaccines optimised for cellular processing and presentation to T lymphocytes and targeted to professional antigen presenting cells. QLK2-2001-01167. Peter van Endert.
- CTLATVAX—Priming protective MHC class I-restricted T cell immunity by novel vaccine delivery strategies that target alternative epitope repertoires. QLK2-2002-00700. Jörg Reimann.
- MEMOVAX—Immunological memory and vaccination. QLK2-2001-01205. Federica Sallusto.
- NEOVAC-EC—Improving vaccination in early life. QLK2-1999-00429. Michel Goldman.

Session 3: *MUCOSAL IMMUNISATION*

Overview and Chair: Jan Holmgren

Projects:

- MUCIMM—Mucosal immunisation—cluster project. QLK2-1999-00228. Aldo Tagliabue.
- MUCIMM—First project: MUCIMM/DEL. Living and non-living delivery systems for immunisation. Aldo Tagliabue.
- MUCIMM—Second project: MUCIMM/EXP. Understanding immunomodulatory pathways at mucosal surfaces to improve vaccine adjuvant effects. Nils Lycke.
- MUCIMM—Third project: MUCIMM/CLIN. Mucosal immunisation and vaccine development in humans. Jan Holmgren.
- MUCADJ—Prove the mucosal adjuvanticity of LT mutants with influenza antigens for intranasal immunisation. QLK2-1999-00070 (Demo). Audino Podda.
- MUCOSAL TB VACCINE—Role of mucosal immunity for protection against tuberculosis. QLK2-1999-00367. Juraj Ivanyi.
- ISCOTB—Novel approaches to induce mucosal immunity against TB using the combined adjuvant strategy of CTA1-DD and ISCOMS. QLK2-2001-01702. Nils Lycke.
- DETEC—SIV/HIV VACCINES: detecting efficacy and explaining inefficacy. QLK2-1999-01215. Paul Racz.
- MUVADEN—Mucosal vaccines against Human and Simian Immunodeficiency Viruses based on dendritic cells. QLK2-2002-00882. Paul Racz.

Session 4: *VACCINE DELIVERY*

Overview and Chair: Gianni Pozzi

Projects:

- VACACT—Detoxified adenylate cyclase toxin: a major improvement for the development of safe, efficient, and multipurpose vaccines. QLK2-1999-00556. Claude Leclerc.
- CTL DELIVERY—Non-replication particles as immunogen carrier. QLK2-1999-00318. Polly Roy.
- Lipopeptide—Defined lipopeptide vaccines. QLK2-1999-00772. Kristian Dalsgaard.
- PEPSAC-MIMIC—Recombinant polypeptides for antigenic mimicry of bacterial surface polysaccharides. QLK2-1999-00854. Marco R. Oggioni.
- YERSINIA CARRIER VACCINES—Development of novel live carrier vaccines by targeted attenuation of *Yersinia*: genetic engineering and immunological evaluation. QLK2-1999-00780. Jürgen Heesemann.
- BIOSAFE-VACCINE-VECT—Biosafe coronavirus vaccine vector for the prevention of human infections of the enteric and respiratory tract. QLK2-2001-00874. Luis Enjuanes.
- LABDEL—Oral delivery of vaccine and therapeutic products using non-pathogenic lactic acid bacteria. QLK3-2000-00340. Jerry Wells.
- TCS-TARGETS—Bacterial two-component systems as targets for the development of novel anti-bacterials and anti-infectives. QLK2-2000-00543. Jerry Wells.
- EUROAMP—European Network for development of novel safe vaccines based on new generation amplicons and other defective HSV-1 derived vectors as foreign antigen delivery systems. QLK2-1999-00055. Alberto Epstein and Thomas Brocker.

Session 5: *VACCINES FOR POVERTY-RELATED DISEASES*

Overview and Chair: Brigitte Gicquel

Projects on AIDS:

- ENVEP—European network for vaccine evaluation in primates: combined vector immunisation for AIDS vaccine development. QLK2-1999-00871. Gerhard Hunsmann.

- EUROVAC—European vaccine effort against HIV/AIDS. I. QLK2-1999-01321. Giuseppe Pantaleo.
- EUROVAC-II—European vaccine effort against HIV/AIDS. II. QLK2-2001-01316. Giuseppe Pantaleo.
- EUROVAC-III—European vaccine effort against HIV/AIDS. III. QLRT-2001-01431. Giuseppe Pantaleo.

Projects on MALARIA:

- EUROMALVAC-1—A European malaria vaccine development consortium. QLK2-1999-01293. David Arnot.
- EUROMALVAC-2—The second phase of a European malaria vaccine development consortium. QLK2-2002-01197. David Arnot.
- PAMVAC—The development of a vaccine against pregnancy-associated malaria. QLK2-2001-01302. Christoph Lindenthal.

Projects on TUBERCULOSIS:

- TB VACCINE CLUSTER—A cluster for tuberculosis vaccine development. QLK2-1999-01093. Brigitte Gicquel.
- TB VACCINE CLUSTER—Project 1. Optimisation and pre-clinical evaluation of TB VACCINE. Mark Doherty.
- TB VACCINE CLUSTER—Project 2. Development of new live attenuate vaccines against tuberculosis. Brigitte Gicquel.
- TB VACCINE CLUSTER—Project 3. Non-protein antigens. Fabrizio Poccia.
- TB VACCINE CLUSTER—Project 4. Identification of mechanisms and correlates of protective immunity to *M. tuberculosis*. Tom H. M. Ottenhoff.
- AFTBVAC—Development of a tuberculosis vaccine in Africa. QLK2-2002-01613. Kris Huygen.

EUROPEAN WORKSHOP

Innate Immunity and Pathogen–Host Interaction

Session 1: *Innate Immunity and Interface with Adaptive Immunity*

Chair: Angela Santoni and Alberto Mantovani

Lorenzo Moretta (Genova, Italy): NK cells: their receptors and their interactions with DC

Charles A. Dinarello (Denver, USA): Cytokines in innate immune responses: benefits vs. run-away diseases

Session 2: *Dynamic Interaction between Pathogens and Host Cells*

Chair: Michael U. Martin and Antonio Lanzavecchia

Antonio Lanzavecchia (Bellinzona, CH): Innate immunity: from DC activation to T and B cell memory

Manfred P. Dierich (Innsbruck, Austria): Role of complement in the control of HIV dynamics and pathogenesis

Alberto Mantovani (Milan, Italy): PTX3, a new non-redundant pattern recognition receptor

Session 2: *Dynamic Interaction between Pathogens and Host Cells* (Part 2)

Chair: Diana Boraschi and Terje Espevik

Sergio Abrignani (Siena, Italy): Innate immune responses and liver infections

Douglas T. Golenbock (Boston, USA): Recognition of bacterial and viral pathogens by TLRs: mechanism of TLR activation

Hermann Wagner (München, Germany): Bacterial CpG-DNA/TRL9 interactions bridge innate and adaptive immune responses

Session 3: *Novel Vaccination Strategies*

Chair: Werner Falk and Aldo Tagliabue

Trinad Chakraborty (Giessen, Germany): Pathogen–host interaction as system to deliver antigens and vaccines at the mucosal level

Rino Rappuoli (Siena, Italy): Reverse vaccinology

John D. Clemens (Seoul, South Korea): Research challenges for introducing new vaccines to impoverished populations

Workshop A: *Innate Immunity and Pathogen–Host Interaction*

Chair: Diana Boraschi and Michael U. Martin

Lee M. Wetzler (Boston, USA): Immune stimulation by Neisserial porins is mediated by TLR2

Seung Hyun Han (Birmingham, USA): LTA from *S. penumoniae* R36A stimulates TLR2 through its lipid moieties

Huamei Fu (Göteborg, Sweden): OmpA-deficient *Escherichia coli* activate neutrophils to produce superoxide and show increased susceptibility to anti-bacterial peptides

Marcello Chieppa (Milan, Italy): Appropriate triggering of the mannose receptor elicits maturation of monocyte-derived dendritic cells in an anti-inflammatory, tolerogenic mode

Maria Rescigno (Milan, Italy): Dendritic cells and epithelial cells cross-talk during host–pathogen interaction

Alison Kerr (Glasgow, Scotland): Natural killer cell activity can be detrimental during pneumococcal pneumonia

Workshop B: *Vaccination Strategies*

Chair: Werner Falk and Aldo Tagliabue

Klaus Heeg (Marburg, Germany): Poly-guanosine motifs confer enhanced uptake and immunostimulative properties to phosphodiester CpG oligonucleotides

Catherine Rush (Glasgow, Scotland): Dissecting the mechanisms underlying DNA vaccination

Detlef Neumann (Hannover, Germany): Amelioration of the autoimmune pathology of MRL *lpr*/*lpr* mice by intramuscular delivery of plasmids encoding IL-12 and IL-18

Jeffrey Ulmer (Emeryville, USA): Delivery systems for DNA vaccines and adjuvants

Annalisa Ciabattini (Siena, Italy): Oral priming of mice using recombinant spores of *B. subtilis*

Myriam Francotte (Rixensart, Belgium): Intranasal administration of enterotoxins induces reactogenicity in the brain of different mouse strains

Poster Session A: *Innate Immunity and Pathogen–Host Interaction*

Lucia Conti (Rome, Italy): Soluble factor(s) released by HIV-infected T cells render immature DCs permissive to T-tropic HIV-1 infection

Andrea Doni (Milan, Italy): Production of pentraxin 3 (PTX3) by dendritic cells

Alison Kerr (Glasgow, Scotland): A role for complement in pulmonary defence during pneumococcal pneumonia

Caroline Lassnig (Wien, Austria): Human CD13-transgenic mice—an in vivo infection model for Coronaviruses

Karel Otero-Gutiérrez (Milan, Italy): Rapid and transient induction of the orphan chemokine receptor HCR and its putative murine ortholog L-CCR in maturing dendritic cells

Patrick Perrier (Milan, Italy): Gene expression analysis of human monocyte-derived dendritic cells treated concomitantly with a pro- and an anti-inflammatory stimulus

Poster Session B: *Vaccination Strategies*

Uday Kishore (Oxford, UK): Therapeutic effects of natural and recombinant forms of human surfactant proteins, SP-A and SP-D, in lung infection and allergy

Maurilio Sampaolesi (Milan, Italy): Application of stem cells in a model of muscular dystrophy induces expression of sarcoglycan-specific antibodies but no immune reaction

Karen Smith (Glasgow, Scotland): ICOS-B7RP1 interactions are important for the clonal expansion and B cell helper function responses of naïve, Th1, and Th2 cells

Sandra Prior (Potters Bar, UK): Adenylate cyclase toxin as a multipurpose vaccine: a study on the interactions with host cells

Enrico Proietti (Rome, Italy): Type I IFN as a mucosal adjuvant in an influenza vaccine model

Yufei Wang (London, UK): Stimulation of maturation of DC and adjuvant function by the peptide-binding fragment of HSP70

2. Global need for vaccines

The use of vaccines has been generally introduced at the beginning of last century and has greatly contributed to abating the incidence of a series of infectious disease that had previously caused great part of the mortality of the human population [1]. Vaccines are among the most effective types of medical intervention [2] and it is generally agreed that their use caused and will cause the disappearance from the globe of many of the great killers of the past, such as smallpox and plague.

At the present time, the rapid progress in research and a continuously increasing public awareness of the social value of vaccination are setting the bases for an unprecedented favourable situation for implementing vaccination strategies world-wide. In this context, the concept set forth in 1983 by the World Bank that absence of health is the main obstacle to economic development in poor countries has placed vaccination as the first action for the economic improvement of less-developed countries [3]. Likewise, the World Health Organisation (WHO) together with UNICEF (United Nations Children's Fund) launched in 1981 a global campaign of immunisation (Expanded Programme of Immunisation, EPI) to vaccinate 80% of children world-wide with five basic vaccines (against polio, diphtheria, measles, pertussis, tetanus). The Global Alliance for Vaccines and Immunisation (GAVI) was founded in January 2000, to unify public and private efforts towards enlarging vaccine availability particularly in less-developed countries. The financial body of GAVI, the Vaccine Fund, has received excellent support through private donations (up to US$ 1.1 billion), including a contribution of US$ 750 million from the Bill and Melinda Gates Foundation. Furthermore, during the World Economic Forum held in Davos in January 2003, the Bill and Melinda Gates Foundation has announced a further contribution of US$ 200 million to establish the "Grand Challenges in Global Health" initiative, to support and accelerate research and vaccine development for AIDS, malaria, diarrhoeal diseases and other infections affecting the most impoverished countries.

The main problem faced in the practical implementation of all these enthusiastic public and private initiatives is the progressive disinterest of vaccine industries, with the consequent risk of inadequate vaccine supplies. In fact, the economic value of vaccines is negligible from the industrial viewpoint, where its potential business amounts to only about 2% of the global pharmaceutical market [4]. Other problems that make the vaccine business unattractive for companies include the high risk of liability actions, the pressing requests from humanitarian organisations for decreasing vaccine prices, the recalcitrant attitude of some public health administrations to meet the vaccine costs to the full coverage of the population. As a consequence, the number of industries producing and manufacturing vaccines has dramatically dropped in recent years. In the USA, vaccine companies have decreased from 37 in 1967 to 10, bringing about a significant shortage in vaccine supply that would expose the population to the risks of uncontrolled outbreaks of vaccine-preventable diseases [2,5,6]. Even more dramatic would be the situation in less-developed countries, where vaccine coverage is most needed.

A comprehensive organisation of the global efforts towards vaccine implementation is clearly needed at multiple levels. The programmes of GAVI and other similar programmes need to be paralleled by a clear policy towards encouraging industrial involvement in vaccine research, development and manufacturing. Incentives for industrial research and investments in the vaccine field should be

devised, such as de-taxation policies, insurances against liability risks, more attractive patent coverage, waiving the pressure of public-sector agencies (the principal buyers of vaccines) for lowering the vaccine price. Without genuine involvement of vaccine companies, no social or humanitarian effort towards vaccination in less-developed countries could be hoped to be successful.

3. Vaccines for developing countries

Generally, the need of vaccines in developing countries involves vaccines already available and vaccines still to be developed, which are directed to diseases that do not affect developed countries and are not of interest to industry.

3.1. Old vaccines

In the case of vaccines that are already available, the recent effort for global vaccination coverage (e.g. EPI, GAVI) is facing an increasing shortage in vaccine supply caused by lack of industrial interest. If the problem is true for developed countries, it is greatly exacerbated in the poorest countries that do not have the financial capacity of meeting the cost of vaccines. However, decreasing vaccine price for these countries does not offer a reasonable solution, as price could always be too high for them, and companies are increasingly discouraged in maintaining the vaccine business. Moreover, vaccines developed and tested in developed countries might need to be adapted to the widely different geographical conditions and infrastructure present in other countries. To make some simple examples, vaccines that need to be conserved at $+4\,^{\circ}C$ may pose insurmountable difficulties of distribution in sub-equatorial countries, vaccines that need multiple administrations will face problems of compliance, injectable vaccines will pose the problem of supply of sterile syringes and or their safe disposal to avoid spreading of infections, oral or nasal vaccines will face problems of proper absorption in populations stricken by diarrhoeal and lung diseases with generalised mucosal inflammation and abundant mucus secretion.

3.2. New vaccines

Development of new vaccines is progressing slowly. From the point of view of more basic research, the enthusiastic availability of funding from charities and private organisations makes possible to invest also in areas considered of low priority in developed countries. This is the case of the International Vaccine Institute (IVI), an international organisation located in South Korea, that relies upon a substantial funding received from GAVI for running top-level basic research and epidemiological studies on vaccines for diarrhoeal diseases (e.g. *Shigella*, rotaviruses), and for running a large training programme for Asian countries such as Bangladesh, China, India, Pakistan, Philippines, Thailand, Vietnam [7]. Again, the main problem is not that of devising an effective new vaccine, but that of adequate vaccine manufacturing and of proper vaccine distribution and administration. From this point of view, there is a tendency by international organisations of supporting the independent development of vaccine production and distribution in each country. This is an issue that further bothers vaccine companies, as they fear unforeseen competition risks with little possibility of enforcing their patent rights without raising strong public reaction and risking a generalised damage to their business. However, this does not appear as a consistent or imminent risk for companies. Indeed, implementation of independent vaccine production in less-developed countries will need a more basic and long-term strategy in supporting the social and economic development of the country.

3.3. Need for higher education, training and technology transfer

Social and economic development is necessary before industrial initiatives such as independent vaccine manufacturing could be safely established. Organisations such as the Human Frontier Science Programme Organisation (HFSPO), the Wellcome Trust, the Third-World Academy of Sciences (TWAS), and European Molecular Biology Organisation (EMBO) are actively tackling the issue of higher education and basic research in life sciences in less-developed countries. Although apparently of little immediate practical outcome, the establishment of higher education programmes in these countries and the formation of a class of young independent scientists is the basis for any future social and economic development. Specific training and technology transfer will be needed in order to implement independent vaccine manufacturing and distribution. In this sense, the involvement of vaccine companies, on the basis of international cooperation and licensing agreements, would be of great importance. Other initiatives, as that of IVI, are moving in this direction by establishing facilities for small-scale vaccine production devoted to training of third-world personnel and technology transfer.

3.4. International policies

Co-ordination of international policies and forging of new treaties for implementing development in poor countries is at the basis of the successful outcome of global vaccination and health improvement.

USA policies on vaccination have undergone major change in direction after 11 September, due to the threat of bioterrorism. Large financial resources have been contracted to vaccine companies for resuming/increasing the production of old vaccines (e.g. anthrax and smallpox) in spite of their inadequate safety profile. In addition, large financial support for research is being made available to scientists

world-wide. Three areas of biodefence vaccine research will be tackled: innate and adaptive immunity, human immunology, unusually susceptible civilian populations (children, elderly, immunosuppressed people).

The EU Commission, on the other hand, has focussed efforts in the vaccine field of the new 6th Framework Programme almost exclusively on dealing with three major poverty-related diseases: a viral one (HIV/AIDS), a bacterial one (tuberculosis, TB), and a protozoan one (malaria). The programme promotes international collaboration with direct involvement of third-world scientists, a major involvement of vaccine companies in the R&D activities, and strong training programmes for young investigators of developing countries.

A wealth of other initiatives are taken by individual countries, e.g. countries of northern Europe such as Sweden and Denmark. Furthermore, international organisations have vaccine programmes in poverty-stricken countries (WHO, UNICEF, IVI).

Large and small initiatives world-wide are flourishing, given the excellent social perception of vaccines as tools for improving human health and advancing social and economic development. As a consequence, private foundations and charities are sustaining many of these initiatives, as is the case of the Vaccine Fund of GAVI. However, the progress in basic research and in vaccination strategies needs to be pursued in parallel by a concomitant progress in the industrial development and manufacturing activities. No practical progress can take place if the current shortage in vaccine supplies due to the lack of industrial interest is not overcome and if industrial investment into new vaccine development is not implemented (Table 1).

4. EU efforts for new vaccine development

Within the new 6th Framework Programme (2002–2006), the EU Commission specifically aims at poverty-related diseases, and this follows the concept that improving health is the way of fighting poverty [8].

A specific line of research will be funded, entitled "Confronting the major communicable diseases linked to poverty". The strategic objective for this action is to develop new vaccines, drugs and other innovative interventions to fight the three major infectious diseases linked to poverty: HIV/AIDS, malaria and tuberculosis. These three diseases are both the cause and consequence of poverty in many developing countries, in particular in Sub-Saharan Africa. In its programme for action "Accelerated action on HIV/AIDS, malaria and tuberculosis in the context of poverty reduction"

Table 1
Old vaccines in jeopardy, new vaccines in need

Disease	Cases in 2001[a]	Reduction (%)[b]	Problems
Old vaccines			
Smallpox	0	100.00	Inadequate supply
Diptheria	2	99.99	Supply emergency due to discontinuation of production by some manufacturers
Pertussis	4788	98.20	Supply emergency due to discontinuation of production by some manufacturers
Tetanus	26	98.34	Supply emergency due to discontinuation of production by some manufacturers
Poliomyelitis	0	100.00	Eradication based on the oral vaccine, supplies of injectable vaccine insufficient to complete it
Measles	96	99.99	Shortage of the trivalent MMR vaccine in the USA
Rubella	19	99.97	Shortage of the trivalent MMR vaccine in the USA
Mumps	216	99.86	Shortage of the trivalent MMR vaccine in the USA
H. influenzae	51	99.75	Insufficient capacity for global implementation
New vaccines			
Cholera	120000 deaths		Current vaccine poorly effective
Thypoid fever	12–17 million infected; 600000 deaths		Available vaccines poorly effective
Shigellosis	1100000 deaths		No vaccines available
Rotavirus	800000 deaths		A vaccine withdrawn because of intussusception, others in trial
Dengue fever	50 million new infections		First generation vaccine currently in phase II trials
Schistosomiasis	200 million infected		No strategies of control available
Leishmaniasis	12 million infected		Vaccine under development, preventive measures need implementation
Tuberculosis	8.7 million new infections; 2 million deaths		No effective vaccine available, drug treatment expensive, frequent induction of resistance
HIV/AIDS	40 million infected; 3 million deaths		Vaccines under development
Malaria	273 million infected; 3 million deaths		No vaccine available

[a] Cases in USA only.
[b] Reduction calculated vs. the highest incidence registered in the 20th century.

[9], the EU Commission provided a broad policy framework for a comprehensive global and multidisciplinary approach against the three poverty-linked diseases.

Concerning research, the overall strategy is to develop new effective interventions against these three diseases. In order to realise this goal, the action is organised in two major components: (i) developing new promising candidates up to the pre-clinical and early human testing (phase I clinical trials), and (ii) establishing a clinical trial programme to support phases II and III clinical trials [10].

4.1. Developing new promising candidate vaccines, drugs and microbicides

It is planned to develop new effective interventions harnessing the full spectrum of basic molecular research through to pre-clinical and proof-of-principle testing (early human testing, safety trials) using the integration of different disciplines and approaches, while pursuing rational and systematic concepts and comparative evaluation procedures. The emphasis will be in translating new knowledge effectively into the development of promising new candidate vaccines, drugs or microbicides. The involvement of relevant research groups from developing countries is highly encouraged.

4.2. Establishing clinical trial programmes

The second component of this action is the implementation of the European and Developing Countries Clinical Trials Partnership (EDCTP) [10]. One of the main goals of the EDCTP is to support phases II and III clinical trials of promising candidates in developing countries. Within EU policies, the EDCTP is part of the overall strategy of the FP6 but is an independent legal and financial entity, specifically dedicated to supporting clinical trials in developing countries.

Thus, the most relevant effort of the EU Commission not only puts the emphasis on basic research, but it strongly encourages transfer of scientific results into practice, which needs an important industrial involvement. Advanced training activities are emphasised as an essential component of these projects and should be specifically directed at the professional development of third-world fellows for implementing their formation as researchers in basic science and clinical research, as research managers and as industrial executives or users of the knowledge produced within the project.

5. Strategies for new vaccines: dissecting the mechanisms of pathogen–host interaction

The first approach in the strategies for designing innovative vaccines is that of understanding the paths of interaction of the pathogen with host cells. This interaction is dynamic, in that both the pathogen and the host cell upon encounter change their physiological features in the attempt to survive. Escape mechanisms of pathogen and the host's mechanisms of defence have evolved concomitantly in an astonishingly dynamic relationship of co-evolution and reciprocal adaptation. Knowing the mechanisms of escape built by the pathogen, and the mechanisms of defence used by the host is the basis for designing effective and better targeted interventions of protection.

The innate mechanisms used by host cells to fight and neutralise the pathogen invasion, and the mechanisms used by micro-organisms to escape host surveillance, are comprehensively described in the excellent review of Basset et al. [11].

5.1. Host interaction with pathogen: defence mechanisms

The first interaction between a pathogen and the host usually takes place at the mucosal level, since mucosal linings represent the mechanical barrier between external environment and internal space. Thus, the first defensive activities take place at the mucosal surfaces, to avoid adhesion of micro-organisms to the cell surface (cilial movement, mucus secretion). The epithelial cells also express defence receptors (e.g. the Toll-like receptors, TLR) that recognise and bind specific pathogen associated molecular patterns (PAMP) and initiate defensive cell activation. Epithelial cells can synthesise and secrete a wide array of anti-microbial peptides (including defensins, cathelicidins and histatins) that contribute to the host defence. Cell activation leads to the production of chemokines, cytokines and other agents signalling cell injury [12]. Chemokines have the role of attracting immune cells (neutrophils, macrophages, NK cells and lymphocytes) to the infection site. There, phagocytic cells (neutrophils, macrophages) can ingest and kill the micro-organisms into phagolysosomes. Other cytokines produced at the infection site, together with TLR triggering by microbial or endogenous agents, can activate immune cells to better effect their inflammatory defence action and to initiate the triggering of specific acquired immunity [13–15]. Factors with a potent anti-microbial role produced during the inflammatory reaction include cytokines and chemokines, acute phase proteins and long pentraxins, complement components [16,17]. Dendritic cells and NK cells are also involved in pathogen recognition, antigen uptake and presentation, and cytocidal activities, contributing both to the innate defence response and to the initiation of the subsequent acquired type of specific immunity and establishment of immunological memory [18–20].

5.2. Pathogen interaction with host: escape mechanisms

Micro-organisms can attach to the epithelial cells through specialised surface structures (adhesins) or through other

receptors. To avoid recognition by TLR and anti-microbial peptide action, several pathogens have evolved modifications in their surface components, and have devised inhibitory molecules against defensins. To pass the epithelial barrier, micro-organisms can either damage (induction of cell necrosis or apoptosis or degradation of the extracellular matrix) or invade the mucosal cells. After entering the cell, micro-organisms can survive in membrane-linked vacuoles and spread to the underlying and surrounding tissue. To survive the innate immunity surveillance, some micro-organisms have devised systems to inhibit phagocyte-mediated killing through different mechanisms of avoidance, e.g. by interfering with uptake or by inhibition of phagolysosome formation. Other infective agents, e.g. some viruses, are able to produce anti-cytokine proteins (such as mimics of soluble cytokine receptors) that can capture and neutralise cytokines, thus avoiding activation of the immune response.

6. Strategies for new vaccines: mucosal immunisation

In the last two decades, it has been thought that vaccines administered via oral, nasal, vaginal and rectal routes (i.e. mucosal vaccines) could solve many of the problems related to parenteral vaccination. The interest in developing mucosal vaccines is based on a series of considerations [21]. In the first place, establishment of protective immunity at the mucosal sites would greatly increase vaccine effectiveness, in light of the fact that the vast majority of infections occur or begin at the mucosal surface. Moreover, mucosal immunisation could overcome the problems of vaccine efficacy in immunosuppressed people (e.g. HIV-infected), in people previously vaccinated with parenteral vaccines and in young children with circulating maternal antibodies [22]. Other more practical reasons are the easy administration, and the highly reduced risk of transmitting infections (as with syringes in the case of injectable vaccines).

Since in the experimental models mucosal vaccines showed promising results it was expected that within a shorttime mucosal vaccines could be developed for human use. However, this was not the case. In the last few years, the most important oral vaccine, the attenuated anti-polio vaccine developed by Sabin in the 1950s, has been progressively abandoned in developed countries to avoid the few cases of disease caused by the vaccine. Furthermore, two recently developed mucosal vaccines for human use, against rotavirus diarrhoea and influenza, were withdrawn after a short period in the market because of adverse reactions among the vaccinees. However, the first evidence that a nasal vaccine against flu with LTK63 mucosal adjuvant could be safe and effective in humans was presented at this meeting [23].

It is extremely important to further develop mucosal vaccination, as it would be of paramount importance in eradicating diseases in developing countries. At first, the paradigm of immunity versus tolerance (particularly important at the gastro-intestinal level) must be solved. Gastro-intestinal tolerance allows us to co-exist with our normal flora and to ingest large amounts of foreign food proteins without inducing harmful systemic immune responses. If we want to immunise at the mucosal level we have to invent new delivery systems and effective mucosal adjuvants.

6.1. The mucosal immune system: tolerance versus immunity

The characteristics of the mucosal immune system are different from those of the systemic immune response, and can be exploited to design effective mucosal vaccines [24].

The mucosal immune system (mucosal associated lymphoid system (MALT)) has been well defined in terms of inductive and effector sites in the gastro-intestinal tract (GALT), and in the nasal tract (NALT). Less known is the corresponding genito-rectal associated lymphoid system (GERALT). Inductive sites are the regional lymph nodes and aggregates of lymphoid tissue (e.g. Peyer's patches in the gut) where immunisation occurs and from which lymphocytes migrate to the effector sites (e.g. mucosal *lamina propria*). MALT is a common, communicating system as, for instance, genital mucosal immunity can be induced by nasal or oral immunisation [25].

Defence at the mucosal level includes both mechanical and innate mechanisms effected by epithelial cells, and innate and adaptive immunity involving leukocytes, dendritic cells, and lymphocytes. Secretory IgA, produced at the mucosal surface, participate both in killing invading micro-organisms (by opsonisation and neutrophil activation, and by arming intestinal lymphocytes in an antibody-dependent cellular cytotoxicity mechanism) [26], and in inhibiting microbial adhesion.

The route and features of antigen interaction with immune cells at the mucosal surface can determine the type of immune response elicited. Whereas mucosal delivery of microbial antigens (e.g. cholera) can elicit significant response also in immunosuppressed patients, a particular characteristic of the mucosal system is the induction of tolerance (especially to T-dependent antigens able to elicit delayed type hypersensitivity reactions, DTH). The phenomenon of oral tolerance (or ignorance) is a very important physiological mechanism to avoid development of DTH and other allergic reactions to ingested food proteins and other antigens. Oral tolerance and induction of mucosal immunity usually involve different types of antigens, oral tolerance being difficult or impossible to induce with strong immunogens, while the reverse is true for the mucosal immune response. However, the two phenomena are not mutually exclusive and sometimes the same mucosal immunisation procedure may concomitantly give rise both to a local significant IgA antibody formation and to tolerance or suppressed peripheral immune response.

Therefore, mucosal immunisation and induction of oral tolerance may represent promising approaches to induce protection against mucosal infectious agents and against systemic inflammatory autoimmune disorders, respectively.

However, in practice it has been very difficult to elicit strong protective immune response and IgA production by oral administration of soluble antigens. The successful oral vaccines for human use are therefore very few: the Sabin polio vaccine, two cholera vaccines (one killed, one attenuated), an adenovirus vaccine, and the Ty21a thypoid vaccine.

6.2. Mucosal antigen vectors

To overcome the difficulties in inducing effective and protective immunity by mucosal administration of antigen, different vectors are being studied for appropriate delivery at the mucosal sites [21].

Several live bacterial vectors have been developed to target and deliver relevant antigens at the mucosal sites. These are either based on attenuated or mutated pathogens (e.g. *Salmonella*, *Shigella*, BCG, *Bordetella*) or on commensal bacteria (lactobacilli, certain streptococci and staphylococci). The tropism of these bacteria for the human gut or respiratory mucosa is essential for adequate delivery to the mucosal sites. The system using *Bacillus* spores expressing recombinant foreign antigens is proving very effective and safe in inducing mucosal immunity and protection in experimental models [27]. The use of attenuated bacterial vectors is being tested in severe infections, such as that of the intracellular pathogen *Listeria monocytogenes*, exploiting the same pathways of infection followed by the virulent bacterium [28].

Among viral vectors, the early vaccinia vector is being replaced by other poxviruses (e.g. canary poxvirus) and by adenoviruses. Psudoviruses appear rather promising, as do virus-like particles (VLP), recombinant self-assembling non-replicating viral core structures from non-enveloped viruses. Their high immunogenicity, together with the possibility of expressing recombinant antigens on their surface, make VLP excellent candidates for mucosal vaccine delivery. In addition, VLP prepared from pathogenic viruses can be used safely when administered by the natural route of infection to elicit mucosal immunity and IgA antibodies.

These vectors, besides actual antigen delivery, serve also the function of adjuvants, as they usually elicit strong activation of innate response mechanisms and of immunostimulatory cytokine production.

6.3. Mucosal adjuvants

In experimental systems, the most effective and well studied mucosal adjuvants are cholera toxin (CT) and the closely related *E. coli* heat labile enterotoxin (LT). Both toxins are pentamers of cell-binding B subunits, associated with a single toxic A subunit. CT and LT can potently enhance immunogenicity at the mucosal level, by increasing antigen uptake and presentation, and inducing B cell maturation and immunostimulatory cytokine production [21]. Moreover, CT and LT fail to induce tolerance and can even abrogate tolerance induction. However, their toxicity has prevented the possibility of human use [29]. Development of non-toxic CT and LT derivatives was at first based on isolated B subunits (CTB and LTB), which however worked only when physically coupled to the antigen and delivered intranasally. Non-toxic recombinant mutated forms of CT and LT retain partial adjuvant capacity as compared to the native toxins. Mutants and modifications of the A subunit of CT (CTA) are also being tested with some promising results, although in general adjuvanticity decreases with toxicity [30].

Agents stimulating innate immunity and triggering Toll-like receptors are excellent adjuvants, in that they induce production of cytokines and co-stimulatory molecules, contributing not only to the non-specific amplification of the immune response but also to the induction of the specific adaptive immunity [31]. These agents are essentially microbial products able to induce strong inflammation (e.g. bacterial LPS). However, of particular interest as potential adjuvants are synthetic oligodeoxynucleotides containing non-methylated cytosine-phosphate-guanosine (CpG) motifs, which are particularly abundant in bacterial DNA. CpG motifs bind to TLR9 and trigger strong, predominantly Th1 immune response and cytokine production [32]. CpG oligodeoxynucleotides, besides good systemic adjuvants, are quite effective also as mucosal adjuvants in several experimental systems. Intranasal, oral, or vaginal administration of CpG oligodeoxynucleotides together with the antigen can induce mucosal IgA production, systemic Th1 response, cytokine production, cytotoxic T lymphocyte (CTL) induction, and effective protection from subsequent infectious challenge [21].

Since a significant part of the effect of many adjuvants is due to induction of inflammatory cytokines, combinations of cytokines have been tested to obtain an adjuvant effect in the absence of toxicity. IL-1 is well known for its potent adjuvant activity and, in combination with Th1-stimulating cytokines (in particular IL-18), it can elicit a mucosal immune response (CTL, IFN-γ, IgA) against the antigen as potent as that obtained with CT [33]. Use of chemokines has also proven useful in stimulating effective mucosal immunity after mucosal administration with antigens in experimental models, although a thorough study on the efficacy of chemokines as mucosal adjuvants is not available.

6.4. Routes of immunisation versus routes of infection

One of the great advantages of mucosal vaccination against pathogens is the exploitation of the same routes used by pathogens for entering and invading the body. Mucosal vaccines therefore can take advantage of the mucosal route for eliciting the same type of defensive reactions that are mounted against the pathogen (both innate local reactions

and subsequent specific local and systemic immunity and generation of memory), i.e. the optimal response against the pathogen. This, together with the peculiar features of communication in the mucosal associated lymphoid system, makes mucosal vaccination the choice vaccination for all infections occurring or beginning at mucosal surfaces, which account for the majority of infectious diseases.

7. Conclusions

The notion that health is necessary for the economic and social progress of a nation has given rise to numerous private and public initiatives world-wide to improve health conditions in developing countries. Vaccination is the first objective to be tackled and it needs a renewed effort to develop novel vaccines and to make old vaccines available. To this end, a global strategy of support of the vaccine business should be designed, in order to attract the interest of industries. This would not only ensure the adequate supply of vaccines (both for developed and developing countries) but it would also encourage new investments and research in the vaccine field [34].

Special emphasis should also be devoted to supporting higher education and training, and research in basic science in developing countries. This will pose the basis for future independent development, thus allowing implementation of technology transfer and independent vaccine production, manufacturing and distribution.

The need for vaccines in developed countries should be implemented through several parallel strategies: (1) re-establish the production of old vaccines and implement national policies of vaccination; (2) support research and development of new vaccines (e.g. AIDS); (3) optimise old vaccines (to increase their efficacy and safety, e.g. new routes of administration).

The need for vaccines in developing countries has to be dealt with by different objectives: (1) immediate use of old vaccines, even if their efficacy is not complete, to reduce disease burden; (2) research to develop “tailored” vaccines adapted to the different conditions of the population, such as health state (considering ongoing infections and/or mucosal inflammation, e.g. diarrhoea or respiratory infections), immunodepression (in conditions of starvation and disease), age (newborn, young children, elderly people), geographical considerations (climate, genetic factors).

Mucosal vaccines are one of the most promising avenues for future vaccination. Their advantages include high efficacy due to exploitation of the same routes/mechanisms of immune activation as infective micro-organisms, effectiveness also in immunosuppressed people and in the presence of interfering immunity, easy administration, low cross-infection risk. Despite the limited success obtained so far in developing mucosal vaccines for human use, the ongoing and future research effort in this direction are bound to yield important results in the fight against disease and poverty.

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