Challenges for Vaccine Development: Medical Needs and Social Implications

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CHALLENGES FOR VACCINE DEVELOPMENT: MEDICAL NEEDS AND SOCIAL IMPLICATIONS

3rd Semmering Conference: April 12 – 15, 07; Baden

Vienna Vaccines is an independent non-profit organization devoted to building worldwide Vaccine Networks. Its goals are to support the cooperation between academia, governmental/non-governmental organizations, vaccine industry and financial institutions in the vaccine arena and to present Austria as a country with a high potential in terms of innovation and research. Vienna Vaccines wants to explain and illustrate the relevance of biotech in terms of healthcare and emphasize the significance of Vaccines. Vienna Vaccines is entirely funded by sponsors and by the participation fees for the conference.

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Challenges for Vaccine Development: Medical Needs and Social Implications

Alexander von Gabain

Vienna Vaccines is delighted to welcome all participants to the third Semmering Vaccine Symposium held in the outskirts of Vienna during the spring season of the last four years, this time in the wonderful health spa resort of the City of Baden.

The two previous symposia were dealing with the themes: “The Future of Vaccines– Cancer Meets Infectious Diseases” (2003) and “Novel Vaccines against Infectious Diseases – Developed Countries meet Developing Countries” (2005). In the tradition of the of the two previous events which were extremely well accepted by the participants and the public opinion, we hope that we will be able to create again an atmosphere stimulating constructive discussions between academia, vaccine industries, financial institutions, public and private organizations engaged in the vaccine arena, specialized and general journalists and interested laymen.

Vaccines and vaccination strategies have a documented come back and growth in the global attempt to improve worldwide health care for the benefit of all humans. There are many forces at work forming the vaccine field that comprises, to name a few, outstanding academic research, biotech and pharma industries, institutional investors, NGOs, public organization and media dealing with fears and non-acceptance of a medical intervention that normally is applied when the customer feels well. Most conferences in the field are either too large to get opinion leaders and experts with different background into constructive discussions. Other vaccines conferences and workshops are too specialized and often cope only with one disease target or a too narrow selection of experts needed for the launch of novel vaccines. Our symposia are trying to fill this gap and to include as many as possible expertise opinions into the program, but still to keep a size that allows during and between the official program to exchange views and to build networks that - we hope - will last.

Vienna Vaccines is an independent non-profit organization devoted to building global vaccine networks. The goal of Vienna Vaccines is to initiate and to support contacts and cooperation between all kind of key players and parties engaged in the vaccine arena, but also to position Austria as a country with a high potential in terms of innovation and biomedical research. Vienna Vaccines wants to spread knowledge, to illustrate the relevance of biotech for healthcare and to emphasize the significance of vaccines. The organization is entirely funded by sponsors and by the fees of the conference participants. At this place I like to thank all sponsors, the SAB members, other not mentioned supporters and above all the engaged members of the organization team without their total devotion the symposium could not take place: Johannes Fuchs, Kerstin von Gabain, Eva Grasböck, Mike Hanny, Astrid Meinl, Barbara Strutz-Grell and Martina Thyringer.

CONFERENCE PROGRAM

Thursday, 12 April, 2007

12.00 – 14.00 Check In

14.00 – 14.20 Opening Statement by Alexander von Gabain

SESSION I: New Vaccines in the Light of Novel and Old Targets

Chair: Franz-Xaver Heinz

14.20 – 14.50 Allergy Vaccines. Rudolf Valenta (Medical University Vienna)

14.50 – 15.20 Therapeutic Vaccination Against Chronic Disease: Clinical Experience. Martin Bachmann (Cytos Biotechnology)

15.20 – 15.40 Break

15.40 – 16.10 Vaccines Against *Staphylococcus aureus*. John Shiver (Merck & Co)

16.10 – 16.40 Therapeutic Vaccine Against HPV. Cornelis Melief (Leiden University Medical Center)

16.40 – 17.00 Results from a First Clinical Safety and Immunogenicity Phase I Trial With a New Subunit Vaccine Against Tuberculosis. Tom Ottenhoff (Leiden University Medical Center)

KEY NOTE LECTURE

Chair: Beatrix Grubeck-Loebenstein

17.00 – 17.30 Human Monoclonal Antibodies and Analytic Vaccinology. Antonio Lanzavecchia (Institute for Research in Biomedicine)

18.30 – 19.00 Opening of Poster Session

19.00 Get Together

Friday, 13 April, 2007

07.00 – 09.00 Breakfast

SESSION IIA: Vaccines in the Light of Microbial Exposure, Life Stage and Immune Status

Chair: Hubert Blum

09.00 – 09.30 The PD-1:PD-1 Ligand Pathway. Gordon J. Freeman (Harvard Medical School)

09.30 – 10.00 Role of the Cell-Mediated Immune Response in HCV Pathogenesis. Carlo Ferrari (Azienda Ospedaliero Universitaria di Parma)

10.00 – 10.30 "Immunity and Aging". Beatrix Grubeck-Loebenstein (Inst. f. Biomedical Aging Research of the Austrian Academy of Sciences, Innsbruck)

10.30 – 11.00 Break

11.00 – 11.30 The Challenge of Early Life Vaccination. Paul-Henri Lambert (University of Geneva)

11.30 – 12.00 Dilemma of the Oral Administration. A Case Study of a Shigella Vaccine. Philippe Sansonetti (Institut Pasteur)

12.00 – 13.30 Lunch Break

SESSION IIB: Vaccines in the Light of Microbial Exposure, Life Stage and Immune Status

Chair: Philippe Sansonetti

13.30 – 14.00 Bacterial Sneak Attack: Subversion of the Host Cell Cycle. Eric Oswald (INRA, Toulouse)

14.00 – 14.30 Dynamic Immune Responses in Tuberculosis. JoAnne L. Flynn (University of Pittsburgh)

14.30 – 15.00 The Population-Level Impact of Routine Infant Immunization with Pneumococcal Conjugate Vaccine in the U.S. Arthur Reingold (Berkeley School of Public Health)

19.30 Austrian Evening

Saturday, 14 April 2007

07.00 – 08.30 Breakfast

SESSION III: Novel Vaccines in the Light of Markets and Social Implications

Chair: Regina Rabinovich

08.30 – 09.00 Primary Prevention of Cervical Cancer with GARDSIL® (Quadrivalent Human Papillomavirus [HPV] Vaccine). Mark Feinberg (Merck & Co)

09.00 – 09.30 Case Study: Rotarix™, a Human Attenuated Oral Rotavirus Vaccine for Global Use. Beatrice De Vos (GSK)

09.30 – 10.00 Break

10.00 – 10.30 Development of a Cell-Culture Derived H5N1 Pandemic Influenza Vaccine. Noel Barrett (Baxter)

10.30 – 11.00 Japanese Encephalitis: A Global Vaccine Approach for a Global Immunization Need. Erich Tauber (Intercell)

11.00 – 12.30 Lunch Break

SESSION IV: Novel Vaccine Strategies in the Light of Advancing Technologies

Chair: Thomas Decker

12.30 – 13.00 The Induction of Immunity or Tolerance – A challenge for Mucosal Vaccine Development. Jan Holmgren (Göteborg University)

13.00 – 13.30 Toll-Like Receptor (TLR) Agonists and ZOT-Derived Peptides as Vaccine Adjuvants. Sefik S. Alkan (3M and ALBA Therapeutics)

13.30 – 14.00 Monoclonal Antibodies: A New Paradigm in Infectious Diseases. Eszter Nagy (Intercell)

14.00 – 14.30 Break

14.30 – 15.00 IL-15 as a Vaccine Adjuvant to Induce Long-Lived, High Avidity Memory $CD8^+$ T Cells, Even in CD4-Deficient Hosts. Jay A. Berzofsky (NCI, Bethesda)

15.00 – 15.30 M2e-Based Universal Influenza A Vaccine. Walter Fiers (University of Ghent)

15.30 – 16.00 It's Prime Time for Clinical Trials of Plasmid DNA Vaccine. Ron B. Moss (Vical)

16.00 – 16.30 Break

PANEL DISCUSSION: The Future of Vaccines in the Light of Commercial, Social and Disease Challenges

Moderator: Gerd Zettlmeissl

16.30 – 18.30 Jeffrey Almond (Sanofi Pasteur)

Alexander von Gabain (Intercell)

Michael Greco (Parteurop Développement)

John Hodgson (Nature Biotechnology)

Jacques-François Martin (Parteurop Développement)

Regina Rabinovich (Gates Foundation)

Ray Spier (Vaccines)

Mike Ward (BioCentury)

19.00 Gala Dinner

ALLERGY VACCINES

Rudolf Valenta

Christian Doppler Laboratory for Allergy Research, Div. of Immunopathology,
Dept. of Pathophysiology, Center for Physiology and Pathophysiology,
Medical University of Vienna, Austria.

Immunoglobulin E (IgE)-mediated allergy affects more than 25% of the population in industrialized countries. During the last years the cDNAs coding for most of the relevant disease-eliciting allergens have been isolated and expressed as recombinant allergens. Based on recombinant allergens it has become possible to reconstruct the epitope complexity of the most common allergen sources and novel diagnostic tests have been developed which allow the dissection of patients reactivity profiles down to the single molecules. Furthermore it has become possible to develop by recombinant DNA technology and peptide chemistry new types of allergy vaccines with reduced allergenic activity. The engineering and characterization of vaccines for the most common allergen sources will be discussed. Results from vaccination studies, in particular with hypoallergenic recombinant derivatives of the major birch pollen allergen, Bet v 1, will be reported. Active treatment with the derivatives induced protective IgG antibodies which inhibited allergen-induced release of inflammatory mediators. Furthermore a reduction of skin and nasal sensitivity as well as an improvement of symptoms in actively treated patients was observed. Most important, rises of allergen-specific IgE induced by seasonal birch pollen exposure were significantly reduced in vaccinated patients. The new allergy vaccine based on genetically engineered allergen derivatives not only ameliorate allergic reactions, but also reduce the IgE production underlying the disease. According to the data it can be envisioned, that it will be possible to develop therapeutic and prophylactic vaccines based on recombinant DNA technology and synthetic peptide chemistry against the most common forms of IgE-mediated allergies.

Review articles:

1. Valenta R, Kraft D. From allergen structure to new forms of allergen-specific immunotherapy. Curr. Opin. Immunol. 2002, 14: 718-727.
2. Valenta R. The future of antigen-specific immunotherapy of allergy. Nat. Rev. Immunol. 2002, 2: 446-453.
3. Westritschnig K, Valenta R. Can we genetically engineer safer and more effective immunotherapy reagents? Curr. Opin. Allergy Clin. Immunol. 2003, 3: 495-500.
4. Recombinant allergens: from production and characterization to diagnosis, treatment, and prevention of allergy. Methods, Volume 32 edited by: Valenta R. Kraft D.

5. Valenta R, Ball T, Focke M, Linhart B, Mothes N, Niederberger V, Spitzauer S, Swoboda I, Vrtala S, Westritschnig K, Kraft D. Immunotherapy of allergic disease. Adv. Immunol. 2004, 82: 105-153.
6. Linhart B, Valenta R. Molecular design of allergy vaccines. Curr. Opin. Immunol. 2005, 17: 646-655.
7. Larche M, Akdis CA, Valenta R. Immunological mechanisms of allergen-specific immunotherapy. Nat. Rev. Immunol. 2006, 6:761-771.

Notes

THERAPEUTIC VACCINATION AGAINST CHRONIC DISEASE: CLINICAL EXPERIENCE

Martin Bachmann

Cytos Biotechnology AG, Zürich-Schlieren, Switzerland

Non-communicable, chronic diseases are currently the major cause of death and disability worldwide and many of these maladies have reached epidemic proportions. According to WHO these disorders, including cardiovascular and respiratory diseases, diabetes, obesity and cancer, now account for 59% of the 57 million deaths annually and almost half of the global disease burden. WHO identifies comparatively few risk factors, namely smoking, alcohol abuse, obesity, high cholesterol, and high blood pressure as the cause of many of these chronic conditions. We are developing a new class of medicine, based on vaccines approaches, to treat both risk factors and their associated chronic diseases. Two such vaccines, targeting smoking cessation and hypertension, have now clinical proof-of-concept and preclinical as well as clinical results will be presented for both vaccines. The current data indicate that therapeutic vaccination may indeed be a new modality to treat chronic diseases.

Notes

VACCINES AGAINST *STAPHYLOCOCCUS AUREUS*

John W. Shiver

Merck Research Laboratories, West Point, PA 19486

S. aureus is a leading cause of nosocomial- and community acquired- infections and has developed resistance to most currently available antibiotics. As yet no effective vaccine has been identified to protect against these infections. Factors that may contribute to this problem include immune evasion and the presence of pre-existing anti-*S. aureus* antibodies which may result from antigen decoys in humans. I*n vitro* and *in vivo* assays were used to study *S. aureus* antigens, including IsdB, to identify possible immune correlates with the aim of addressing whether vaccines that target *S. aureus* can be effective at preventing disease in humans. Vaccination with IsdB affords specific protection in *S. aureus* infection models. Passive transfer studies using anti-IsdB monoclonal antibodies point to the mechanism of protection as being antibody mediated. IsdB differs from other *S. aureus* vaccine candidates in that it is rapidly expressed *in vivo* and seems to be accessible to antibodies early during infection. Vaccination of animals with IsdB results in a rapid rise in titer to IsdB, and protection from lethal challenge is associated with higher titers of IsdB-specific antibody. How these results point to correlates for protection in humans will be discussed.

Notes

THERAPEUTIC VACCINE AGAINST HPV

CJM Melief[1]. MJP Welters[1], MJG Löwik[2], APG Vloon[1], JW Drijfhout[1], ARPM Valentijn[3], AR Wafelman[3], GJ Fleuren[4], R Offringa[1], SH van der Burg[5] and GG Kenter[2]

Depts. of [1]Immunohematology and Blood Transfusion, [2]Gynaecology, [3]Pharmacy, [4]Pathology and [5]Clinical Oncology, Leiden University Medical Center, Leiden, The Netherlands

A therapeutic vaccine was designed based on long overlapping peptides covering the complete amino acid sequence of the HPV16 E6 and E7 oncogenic proteins, thereby harboring all potential T helper and CTL epitopes. Previously, we demonstrated that HPV16 specific T-cell immunity induced by this vaccine, delivered in Montanide ISA 51 adjuvant was able to terminate persistent infections and eradicate established HPV16+ tumors inrabbits.

Currently, 11 patients with histologically proven HPV16+ vulvar intraepithelial neoplasia (VIN) grade III were vaccinated 4 times with a 3-week interval by s.c. injection of the long peptides emulsified in Montanide ISA 51. Immunological monitoring was performed at the systemic level by the analysis of blood samples, drawn before each vaccination and after the last vaccination, and at the local level by the analysis of HPV16-specific T-cells in tissue biopsies of the VIN lesion (before and after vaccination) as well as a biopsy from the last vaccination site.

In all 11 patients, already after 2 vaccinations strong and broad vaccine-induced systemic proliferative responses, accompanied with the production of IFNg and IL-5 were detected. This type of response is similar to the memory T-cell responses observed in healthy individuals with HPV16-specific immunity. Importantly, circulating HPV16 E6 and E7 specific T-cells produced IFNg upon stimulation with naturally processed and presented antigen. Notably, vaccination resulted in the induction of both CD4+ and CD8+ HPV16-specific T-cells. Multiple epitopes were recognized in each patient. Analysis of the local immune response demonstrated the presence of HPV16-specific Th1/Th2 cells infiltrating both the vaccination site and the VIN lesion after vaccination in 6 out of 9 patients analyzed.A complete clinical response was seen in 4 out of 11 patients,as determined by complete clearance of lesions by macroscopy and microscopy.In 3 of these patients HPV 16 was also cleared as determined by PCR.

In conclusion, our peptide-based vaccine elicits a strong and broad HPV16-specific T-cell response that displays the capacity to migrate into the persistently HPV16-infected lesion of patients with high grade VIN and causes complete regressions in a substantial proportion of patients.

Literature

1. Antigen presentation by an immature myeloid dendritic cell line does not cause CTL deletion in vivo, but generates CD8+ central memory-like T cells that can be rescued for full effector function. Dumortier H., van Mierlo G.J., Egan D., van Ewijk W., Toes R.E., Offringa R., Melief C.J. J Immunol 175: 855-863, 2005.
2. Established human papillomavirus type 16-expressing tumors are effectively eradicated following vaccination with long peptides. Zwaveling S., Ferreira Mota S.C., Nouta J., Johnson M., Lipford G.B., Offringa R., van der Burg S.H., Melief C.J. J Immunol 169: 350-358, 2002
3. Therapeutic vaccination with papillomavirus E6 and E7 long peptides results in the control of both established virus-induced lesions and latently infected sites in a pre-clinical cottontail rabbit papillomavirus model. Vambutas A., DeVoti J., Nouri M., Drijfhout J.W., Lipford G.B., Bonagura V.R., van der Burg S.H., Melief C.J. Vaccine 23: 5271-5280, 2005
4. Human papillomavirus type 16-positive cervical cancer is associated with impaired CD4+ T-cell immunity against early antigens E2 and E6. de Jong A., van Poelgeest M.I., van der Hulst J.M., Drijfhout J.W., Fleuren G.J., Melief C.J., Kenter G., Offringa R., van der Burg S.H. Cancer Res 64: 5449-5455, 2004
5. Distinct regulation and impact of type 1 T-cell immunity against HPV16 L1, E2 and E6 antigens during HPV16-induced cervical infection and neoplasia. van Poelgeest M.I., Nijhuis E.R., Kwappenberg K.M., Hamming I.E., Drijfhout J.W., Fleuren G.J., van der Zee A.G., Melief C.J., Kenter G.G., Nijman H.W., Offringa R., van der Burg S.H. Int J Cancer 118: 675-683, 2006
6. Detection of human papillomavirus (HPV) 16-specific CD4+ T-cell immunity in patients with persistent HPV16-induced vulvar intraepithelial neoplasia in relation to clinical impact of imiquimod treatment. van Poelgeest M.I., van Seters M., van Beurden M., Kwappenberg K.M., Heijmans-Antonissen C., Drijfhout J.W., Melief C.J., Kenter G.G., Helmerhorst T.J., Offringa R., van der Burg S.H. Clin Cancer Res 11: 5273-5280, 2005
7. Cat and mouse games. Melief C.J. Nature 437: 41-42.

Notes

RESULTS FROM A FIRST CLINICAL SAFETY AND IMMUNOGENICITY PHASE I TRIAL WITH A NEW SUBUNIT VACCINE AGAINST TUBERCULOSIS.

Tom H. M. Ottenhoff*, Jaap T. van Dissel*, Jan Nouta*, Corine Prins*, Peter Bang#, Alex von Gabain@, Ingrid Kroman# and Peter Andersen#.

*Leiden University Medical Center, The Netherlands; #Statens Serum Institute, Copenhagen, Denmark; @Intercell AG, Vienna, Austria.

Tuberculosis is an important global health problem, causing 2 million deaths and 8 million new cases each year. One third of the world's population is infected with *Mycobacterium tuberculosis* (MTB). Although the current vaccine *M.bovis* BCG protects infants against severe forms of TB, BCG fails to afford consistent and adequate protection against pulmonary TB in adults, which is the main, contagious form of the disease. The development and testing of new TB vaccines is a key priority of the global TB research agenda.

A recombinant fusion protein consisting of two dominant T cell stimulating antigens of MTB, Ag85B and ESAT6 has been shown to induce significant protection against TB in various small animal models and in non human primates. This protein has now been tested in a phase I clinical trial, admixed either with no, low dose or high dose IC31 adjuvant. Both fusion protein and adjuvant were tested for the first time in humans. Results will be presented regarding safety as the primary variable and immunogenicity as the secondary variable.

Notes

HUMAN MONOCLONAL ANTIBODIES AND ANALYTIC VACCINOLOGY

Antonio Lanzavecchia

Institute for Research in Biomedicine, Bellinzona, Switzerland

Following appropriate priming by infection or vaccination memory B cells and serum antibody levels are sustained for a lifetime conferring immediate protection upon secondary encounter with the pathogen. I will first discuss the differential requirements for activation of human naïve and memory B cells and propose a homeostatic model for the maintenance of the memory B cell pool and of serum antibody levels. I will then describe two methods that can be used to interrogate the human memory B cell repertoire. The first is based on limiting dilution analysis of polyclonally stimulated mononuclear cells. Using this method we measured the frequency and fine specificity of memory B cells in serial samples under steady state conditions and after vaccination. In particular we found that only a small fraction of virus-specific memory B cells produce neutralizing antibodies, while the majority recognizes internal or denatured antigens. The second method is based on the efficient immortalization and cloning of memory B cells. Using this method we have been able to isolate from the human memory repertoire several potent and broadly neutralizing monoclonal antibodies against viruses such as SARS, Dengue, H5N1 and HCMV. I will illustrate how such antibodies can be used not only to provide immediate protection, but also as probes for epitope discovery and vaccine design.

Notes

THE PD-1:PD-1 LIGAND PATHWAY

Gordon J. Freeman

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Harvard Medical School, Boston, MA, USA.

The PD-1:PD-1 ligand (PD-L) pathway, which is part of the B7:CD28 family, consists of the PD-1 receptor and its two ligands, PD-L1 and PD-L2. Engagement of PD-1 by its ligands inhibits T cell responses. The cytoplasmic domain of PD-1 contains two tyrosine signaling motifs, which are phosphorylated upon receptor engagement and recruit the tyrosine phosphatase SHP-2. Recruitment of these phosphatases leads to dephosphorylation of TCR proximal signaling molecules leading to attenuation of the TCR/CD28 signal. The PD-1 ligands have distinct patterns of expression. PD-L2 is inducibly expressed only on dendritic cells (DCs) and macrophages, whereas PD-L1 is broadly expressed on both professional and non-professional antigen presenting cells. Interferons a, b, and g are powerful upregulators of PD-L1 expression. During pro-inflammatory immune responses, such as infection, PD-L1 expression is intense and extensive. PD-L1 expression is also found on many solid tumors and high PD-L1 expression is associated with poor prognosis. Recent studies have shown that PD-1:PD-L interactions control the induction and maintenance of peripheral T cell tolerance, and point to a novel role for PD-L1 on non-hematopoietic cells in protecting tissues from autoimmune attack. PD-1 and its ligands have also been exploited by a variety of microorganisms to attenuate antimicrobial immunity and facilitate chronic infection. PD-1 is expressed after T cell activation but expression declines to low levels on memory T cells. In contrast, in chronic infections, PD-1 remains highly expressed on T cells and these T cells have an “exhausted” phenotype with limited capacity to proliferate, produce cytokines, or lyse target cells. Blockade of this pathway with mAbs reinvigorates the exhausted T cells, allowing them to expand and produce effector cytokines. First observed in chronic LCMV infection, this observation has been extended to HIV, HCV, and HBV infections in humans and SIV in monkeys. The effects of blocking only PD-1, PD-L1, or PD-L2 or a combination thereof will need to be evaluated for efficacy and to identify the optimal timing and frequency of therapy to minimize the risk of immunopathology or autoimmunity

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ROLE OF THE CELL-MEDIATED IMMUNE RESPONSE IN HCV PATHOGENESIS

Carlo Ferrari

Laboratory of Viral Immunopathology, Unit of Infectious Disease and Hepatology,
Azienda Ospedaliero Universitaria di Parma, Italy

A high rate of chronic virus persistence is the hallmark of HCV infection. The type of immune response that the infected host is able to mount early following infection is believed to strongly dictate the outcome towards control or persistence of HCV.

Despite the rapid onset of HCV replication, HCV-specific T cell responses seem to be induced after an unexpectedly long interval of time from exposure, compared to other virus infections. These delayed responses appear also to be functionally impaired at variable degrees based on different studies. Breadth and intensity of CD4-mediated responses have unanimously been reported to be greater in patients with a self-limited infection compared to patients unable to control HCV spontaneously, who generally mount weaker and more narrowly focused responses.

In contrast, a variable degree of functional impairment has been reported for HCV-specific CD8 responses in the acute stage of HCV infection. By tetramer staining ex vivo, CD8 cells specific for pre-selected and widely recognized epitopes can be visible but dysfunctional for some weeks after their induction, since they can be unable to produce IFN-g and IL2 upon ex vivo peptide stimulation. CD8 responses appear to be of limited breadth and vigor in both self-limited and evolving infections also when they are analyzed with comprehensive panels of peptides covering the whole HCV sequence, which can give a more global representation of the anti-viral CD8 response. Different mechanisms have been suggested to be involved in the pathogenesis of the early HCV-specific T cell dysfunction. These include i. T cell exhaustion due to the rapid viral spread in the infected host causing an early exposure of T cells to high virus and antigen loads, as suggested by the possibility to partially restore the T cell function by blockade of the PD-1/PD-L1 pathway; ii. the intrinsic properties of the different HCV proteins which can modulate the antiviral T cell response, either by suppressing the T cell function directly or by interfering with NK and dendritic cell activity, making these cells unable to adequately support priming and activation of virus-specific adaptive responses; iii. a hyperactivity of suppressive CD4+CD25+FoxP3 positive cells; iv. mutational escape from CD8 responses .

Notes

"IMMUNITY AND AGING"

Beatrix Grubeck-Loebenstein,

Institute for Biomedical Aging Research of the Austrian Academy of Sciences,
Innsbruck, Austria.

The function of the immune system changes with age which leads to a frequent occurance and severity of infectious diseases and a decreased responsiveness to vaccines. This is due to early degenerative changes that take place in the thymus. As the thymus gradually loses its ability to replenish the populations of naïve T cells, memory and effector T cells increase in number and dominate the repertoire. This can lead to the loss of certain T cell specificities and changes in the polarization of the immune system.

It is the goal of this talk to illustrate changes in the naïve and memory CD8 T cell pool that occur with aging in humans. Analysis of $CD45RACD28^{+}$ and $CD45RACD28CD62^{+}$ cells, which are generally considered as naïve, reveals that these populations are not only extremely small, but have an impaired homing receptor expression, a restricted diversity and shortened telomeres in comparison to young controls. The data demonstrate that these cells, even if antigen-inexperienced, have divided a lot, lost their diversity and are therefore unlikely to guarantee full immunological protection following exposure to neoantigens. Lack of fully functioning naïve T cells can still, at least partly, be compensated by an increase in the number of $CD8CD45RO^{+}$ cells, in particular a subset within this population that expresses CD25 constitutively without being regulatory. $CD45ROCD25^{+}$ T cells have lymph node homing receptors, contain $CD4^{+}CD8^{+}$CD40L-expressing cells, produce large amounts of IL-2 and IL-4, display a polyclonal T cell repertoire and contain a variety of cells of different antigenic specificity. Gene array analysis, however, shows that $CD45ROCD25^{+}$ cells from elderly persons greatly differ from CD45RO cells from young persons. We therefore conclude that memory T cells have age-specific properties in elderly persons, but may still protect this age group in the absence of fully functioning naïve T cells.

Notes

THE CHALLENGE OF EARLY LIFE VACCINATION

Paul-Henri Lambert

Centre of Vaccinology, Department of Immunology and Pathology,
University of Geneva, Switzerland

Neonatal vaccination has a considerable potential global public health value However, early life vaccination has to face challenges related to immunological immaturity, persistence of maternal antibodies and safety concerns. Can new immunization approaches help to circumvent such obstacles?

1. T cell responses are often suboptimal during the neonatal period, largely due to DC immaturity. Experimental data indicate that formulations that combine specific DC targeting with appropriate adjuvant effects can ensure the generation of effective T cell responses in neonates.

2. Neonatal immunization leads to low primary antibody responses. Germinal Center reactions are hampered by slow FDC maturation. However, B cell priming can be achieved soon after birth, with efficient induction of B cell memory, allowing for strategies that combine neonatal priming with early boosting.

3. Persistent maternal antibodies can inhibit the replication of live vaccines but also affect infant response to of subunit vaccines. Maternal antibodies much more efficiently inhibit antibody responses than T cell responses largely through competing with epitope-specific B cell receptors. Simple approaches based on the increase of the antigen doses have occasionally been successful.

4. Infant immunization is associated with short duration of antibody production. This reflects the short survival of antibody secreting plasma cells (ASC) in bone marrow niches that reflects a slow maturation of molecular signaling at the stromal cell level. A redistribution of vaccine doses given during the first 15 months of life should ensure the persistence of protective level of antibodies throughout the period of highest risk. Immunization strategies that are based on the efficient induction of B cell memory have often been successful but can also lead to occasional vaccine failures in the context of rapidly invading bacterial infections.

Our present understanding of limitations of neonatal and infant immune responses should lead us to new immunization strategies and novel vaccine design that should help to achieve optimal protective efficacy.

Notes

DILEMMA OF THE ORAL ADMINISTRATION. A CASE STUDY OF A *SHIGELLA* VACCINE.

Philippe J. Sansonetti[1], Armelle Phalipon[1], Laurence Mulard[2], Christine Sadorge[3], David Lewis[4].

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[4] St George's Vaccine Institute, University of London, London, UK.

Shigellosis, or bacillary dysentery is a complex disease in the course of which the etiological bacterium, *Shigella*, cause the rupture, invasion, and inflammatory destruction of the intestinal epithelium. Increasing incidence of antibiotic-resistant strains and stability of the incidence of cases, due to persistence of poor hygiene conditions in the most impoverished areas of the planet, justify persisting efforts to develop a vaccine. Vaccination against *Shigella* may benefit from two major approaches :

- The development of orally-administered, live attenuated, vaccine candidates based on rational attenuation. They are expected to induce strong secretory IgA-based mucosal protection, straight where *Shigella*, an enteroinvasive microorganism, is infecting the intestinal epithelium.
- The development of subunit vaccines, mainly conjugate vaccines, parenterally administered based on the use of polysaccharides corresponding to or mimicking the protective O-antigens. Such vaccines are expected to elicit strong, IgG-based seric response in the course of which antibodies may either transudate through the epithelial lining, or protect against the tissular phase of infection.

Our laboratory, at Institut Pasteur, has been following these two lines of *Shigella* vaccine research and development, thus one can now try to evaluate the pros and cons of these approaches.

The live attenuated vaccine approach has led to two series of phaseI/II clinical trials. The first series was carried out with SC602, a *S.flexneri* 2a vaccine candidate under the auspices of the USAMRIID, the second series was carried out with SC599, a *S.dysenteriae* 1 vaccine candidate, under the auspices of Institut Pasteur, the French Department of Defense, and the St George's Vaccine Institute in London. These trials have shown reasonable attenuation and local and systemic immunogenicity, at least in western volunteers, thus encouraging to further develop this approach. Two major issues emerge at this point. (i) One is serotype specificity of protection which will require testing a combination of attenuated strains belonging to the major relevant serotypes. So far phaseI/II trials have dealt with single serotypes, testing a multivalent combination for issues like interference will be essential. (ii) The other one is the lack of fully reliable correlates of protection in the case of mucosal vaccines. This will require phase III efficacy trials in endemic areas to be able to evaluate the performance of such vaccines.

The conjugate subunit vaccine. We have set out to develop a new generation of parenteral conjugate vaccines based on the chemical synthesis of complex O-polysaccharides in *S.flexneri*. This approach has proven very successful regarding the strong immunogenicity of these

conjugates in mice, in comparison to the « classical » detoxified LPS conjugates. It is likely that the optimization of O-antigen chain length and density of conjugation to carrier toxoid molecule participates in optimized performance. The proof of concept is now required in humans, thus the need for a phase I/II trial in human volunteers for the first available serotype, *S.flexneri* 2a. Then the issue of need for a multivalent vaccine will be addressed by adding other relevant *S.flexneri* serotypes and trying to identify and develop a cross reactive protein carrier.

Notes

BACTERIAL SNEAK ATTACK: SUBVERSION OF THE HOST CELL CYCLE

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Our gastrointestinal tract is colonized by a vast community of symbionts and commensals that have important effects on immune function, nutrient processing, and a broad range of other host activities. *Escherichia coli* is one of these commensal bacteria, which live peacefully in our digestive tract. However, certain strains are pathogenic and are frequently incriminated in a broad spectrum of infections, affecting both farmed animals and humans. We have recently shown that both commensal and pathogenic *E. coli* can produce a potent hybrid compound that we called Colibactin and which is genotoxic in mammalian cells. Production of this toxin is linked to a genomic island that encodes giant modular nonribosomal peptide and polyketide synthases. Polyketides and nonribosomal peptides are extremely large classes of natural products that are assembled from simple acyl-coenzyme A or amino acid monomers. Contact with *E. coli* expressing this gene cluster causes DNA double-strand breaks and activation of the DNA damage checkpoint pathway, leading to cell cycle arrest and eventually to cell death. This functional nonribosomal peptide polyketide synthase gene cluster is widely distributed in commensal *E. coli* strains and is even found in a strain used as a probiotic agent. Although it is not yet clear how the peptide–polyketide compound functions at the molecular level, it is possible that it contributes to bacterial pathogenesis and bacterially induced carcinogenesis. The genotoxic effect may be exploited by the bacteria to modulate the host immune responses or slow the rate of renewal of the intestinal epithelium by blocking the cell cycle. In addition, the ability of bacteria to affect signaling pathways linked to cell cycle regulation and genome integrity suggests also that prolonged bacterial infection could contribute to carcinogenesis. Indeed, DNA double strand breaks are dangerous lesions affecting eukaryotic cells; if these are not repaired, they give rise to a high level of mutations, which are the principal triggers of cancer in man. These findings may provide clues about the role of microorganisms in the development of colonic cancers. Thus, the relation between pathogenicity and commensalism may be more complicated than has been assumed. The bacterial flora may participate in the development, differentiation and homeostasis of mucosa and hence the development of certain types of cancer, or protection against them.

Notes

DYNAMIC IMMUNE RESPONSES IN TUBERCULOSIS

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Tuberculosis kills 2 million people every year, even though the vaccine BCG is given to newborns in many countries, and in all countries with high tuberculosis risk. An incomplete understanding of the protective immune responses against tuberculosis is a limitation in designing an effective vaccine against this infection or disease. T cells are known to be important in control of the infection, and our data in both mouse and monkey animal models support this. We have investigated the functions of T cells that contribute to protection and pathology. IFN-g production from CD4 T cells appears to be necessary to the control of infection. Although other cells produce IFN-g during infection, the early production of this cytokine from the CD4 T cell population is necessary. Our data also support that CD8 T cells produce very37little IFN-g early in infection, but instead are cytotoxic. At later time points, the CD8 T cells begin to produce IFN-g. Finally, we have investigated the regulatory T cell populations (Treg) in tuberculosis, and have determined that peripheral levels of Treg cells correlate with specific disease states in the non-human primate model.

Notes

THE POPULATION-LEVEL IMPACT OF ROUTINE INFANT IMMUNIZATION WITH PNEUMOCOCCAL CONJUGATE VACCINE IN THE U.S.

Arthur L. Reingold

Professor and Head, Division of Epidemiology,
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A conjugate pneumococcal vaccine containing seven serotypes of S. pneumoniae was introduced into the routine infant immunization schedule in the U.S. in 2000 and high levels of vaccine coverage were quickly achieved. Population-based surveillance and observational epidemiologic studies have demonstrated a wide range of effects of the conjugate pneumococcal vaccine, including a marked decline in the incidence of invasive infections caused by the serotypes in the vaccine in age groups targeted for vaccination (direct vaccine effect) and in age groups not targeted for vaccination (indirect or herd effects); a reduction in the proportion of invasive pneumococcal infections caused by anti-biotic resistant strains of S. pneumoniae ; and an increase in invasive S. pneumoniae infections caused by at least one serotype not included in the vaccine (serotype replacement). In addition, a study of vaccine effectiveness showed that a three dose regimen of the vaccine, with one of the doses given after the first birthday, may be as effective as the four dose regimen currently used in the U.S. The results will be presented and their implications discussed.

Notes

PRIMARY PREVENTION OF CERVICAL CANCER WITH GARDSIL® (QUADRIVALENT HUMAN PAPILLOMAVIRUS [HPV] VACCINE).

Mark Feinberg, MD, PhD

Merck Vaccine Division/ Merck & Co., Inc.

Objective: Cervical cancer affects an estimated 490,000 women each year—resulting in more than 270,000 deaths worldwide annually. Cervical cancer is caused by human papillomavirus (HPV). HPV is also responsible for other anogenital cancers and lesions occurring sooner and more frequently after infection, such as cervical dysplasia and genital warts. The lifetime risk of HPV infection exceeds 50%. HPV 16/18 cause 70% of cervical pre-cancers and cancers, HPV 6/11 cause 90% of genital warts, and together HPV 6/11/16/18 cause 35-50% of low grade but clinically important cervical dysplasias. A vaccine targeting these types will substantially reduce the burden of HPV disease. This presentation will review of the efficacy, immunogenicity and safety of the quadrivalent HPV vaccine, Gardasil®, as well as opportunities and challenges facing implementation of effective HPV vaccination programs in developed and developing countries.

Methods: GARDASIL® is the only quadrivalent HPV (Types 6/11/16/18) L1 virus-like particle (VLP)-based vaccine. Efficacy trials were conducted in 20,845 16-26 year old women, with primary efficacy analyses in per-protocol populations (subjects received 3 doses; were HPV seronegative at Day 1 and HPV DNA negative through completion of vaccination). Immunogenicity studies were also conducted in 2,794 boys and girls, aged 9-15 years. For all studies, serum anti-HPV levels were measured by type-specific immunoassays and summarized as anti-HPV-6,11,16, and 18 neutralizing antibody geometric mean titers (GMT) and seroconversion rates.

Results: The prophylactic efficacy of a 3-dose regimen of GARDASIL® against HPV 16/18-related moderate/high grade cervical precancer and noninvasive cervical cancer was 100% (95% confidence intervals [CI]: 93%, 100%), and efficacy specifically against in situ cancers and immediate precursors of cancer remained at this level (100% with 95% CI: 88%, 100%). Prophylactic efficacy against HPV 6/11/16/18-related external genital lesions (vulvar/vaginal precancers and warts) was 99% (95% CI: 95%, 100%). In adult women, vaccine-induced anti-HPV responses were detected in 99.5% of subjects one month post-dose 3. Through up to 5 years of follow-up, anti-HPV GMTs remained at or above those measured following clearance of HPV infection and efficacy was maintained. A robust immune memory was evidenced by the rapid and strong increase in GMTs triggered by the administration of an immune challenge at 4.5 years post-dose 3. In adolescents aged 9-15 years, GARDASIL® was highly immunogenic. GMTs in girls and boys were 1.7-2.7 fold higher than those observed in young adult women. In all studies,

vaccine was generally well-tolerated, though a slightly higher proportion of subjects reported one or more injection site adverse experiences than the placebo group. Based on these safety and efficacy data, GARDASIL® was licensed in the United States and Mexico in June 2006, and is now licensed in over 50 countries worldwide. In June 2006, the US Advisory Committee on Immunization Practices (ACIP) recommended universal vaccination with the quadrivalent HPV vaccine for all females aged 11-26 (extending to age 9 with a physician's recommendation). Through the combination of HPV vaccination and effective cervical cancer screening programs, cervical cancer should now be a largely preventable disease. However, in developing countries where access to health services is limited, and cervical cancer mortality is greatest, innovative methods to deliver the vaccine must be defined and effectively implemented.

Conclusion: Vaccination of adolescents and young adults with GARDASIL® is expected to greatly reduce the burden of cervical and other genital cancers, dysplasia, and genital warts. Concerted efforts by global health partners will be needed to ensure the benefits afforded by HPV vaccination can be realized worldwide.

Notes

CASE STUDY: *ROTARIX*™, A HUMAN ATTENUATED ORAL ROTAVIRUS VACCINE FOR GLOBAL USE

Beatrice De Vos

GSK Biologicals, Belgium

GSK's pipeline, composed of novel vaccines for all ages, is prioritized by the unmet global medical needs. Targeting the improvement of health worldwide, each new vaccine has a specific development, registration and distribution strategy. GSK's Rotavirus (RV) vaccine *Rotarix*™ is a pertinent illustration of this approach.

In developed countries, the toll of the RV disease is largely measured in doctor bills, emergency room visits, hospital stays and parental days of work lost. Costs are high, but death is minimal. In developing countries, the toll is measured foremost in life lost, estimated at more than half a million deaths each year in children under 5 years of age.

Different vaccine development strategies have been applied over the last decade (use of animal and human strains, LVP, DNA). *Rotarix*™ is an attenuated human G1P[8] strain vaccine. The rationale for selection of a single human RV strain was based on evidence that natural RV infection is not associated with intussusception, and confers protection against subsequent severe disease regardless of the circulating serotype.

Rotarix™ is a safe and effective two-dose, oral live attenuated human G1P[8] RV vaccine indicated from the age of 6 weeks for prevention of RV gastroenteritis and has been approved in 90 countries, 11 million doses distributed and awaiting approval in many more countries. Children in about 50 countries in Latin-America, Europe, Middle-East, Asia and Africa are already benefiting from the vaccine. It is part of national immunization programs in Brazil, El Salvador, Mexico, Panama and Venezuela and imminent in Belgium and Luxembourg.

GSK has adopted a regulatory "South First" strategy for *Rotarix*™ to provide this new vaccine first to those who need it most. After a worldwide development program involving >100,000 infants and costs over 500 million €, Rotarix™ received his first licensure in Mexico in July 2004.This innovative regulatory approach was further rewarded by a Prequalification' (PQ) status granted by WHO in January 2007. The WHO PQ endorses the vaccine's quality, safety and efficacy, and its ability to fulfill tender specifications. This allows UN agencies and others to make large purchases and to use the vaccine in mass vaccination programs. This means that in less than 3 years, this strategy enables both developed and developing countries to have access to the vaccine.

The next challenge will be to ensure immunization policies are established and implemented globally to ensure the benefit of rotavirus vaccination is fully realized.

Notes

DEVELOPMENT OF A CELL-CULTURE DERIVED H5N1 PANDEMIC INFLUENZA VACCINE

Noel Barrett

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The rapid spread of avian influenza (H5N1) and the transmission to humans has induced world-wide fears of a new pandemic. Vaccines are considered the most effective means to control influenza outbreaks. The favoured strategy for vaccine production involves use of genetically attenuated reassortants to manufacture vaccine in embryonated eggs. A disadvantage of this strategy is the extended time required for generation and safety testing of such reassortants. In addition, egg supplies could be endangered by H5N1 infections of chicken flocks. Also clinical trials to date with H5N1 split vaccine formulations have demonstrated that very high antigen doses are required to induce seroconversion in immunized subjects. We report here on an alternative strategy which involves use of wild-type virus grown in a continuous cell culture (Vero) system to derive an inactivated whole virus vaccine. Candidate vaccines based on clade 1 (Vietnam/1203/2004/H5N1) and clade 2 (Indonesia/05/2005/H5N1) strains have been developed and demonstrated to be highly immunogenic in animal models. The vaccines induce both cross-neutralising antibodies and highly cross-reactive T-cell responses and induces protection in a mouse challenge model not only against the homologous virus but against other H5N1 strains including those from another clade.

Preliminary clinical data demonstrate (i) the vaccine is safe and has an excellent tolerability profile (ii) vaccine doses as low as 3.75 or 7.5 µg are highly immunogenic against the homologous H5N1 strain (iii) the non-adjuvanted formulation is more immunogenic than an alum adjuvanted formulation (iv) the vaccine induces antibodies which are capable of neutralizing not only clade 1 strains but also a widely divergent clade 2 strain (A/Indonesia/05/2005).

These data indicate that this cell culture strategy allows the high yield production of a pandemic vaccine and the whole virus vaccine based on the wild-type virus has the potential to induce broadly protective immune responses.

Notes

JAPANESE ENCEPHALITIS: A GLOBAL VACCINES APPROACH FOR GLOBAL IMMUNIZATION NEED

Erich Tauber

Intercell AG, Vienna, Austria

Japanese Encephalitis (JE) is the most important cause of viral encephalitis in Asia. It is estimated that the JE virus causes at least 50,000 cases of clinical disease each year, mostly among children under 10 years of age. The disease is endemic in South-East Asia, a region with more than 3 billion inhabitants. JE will continue to be a significant public health problem to both persons who live in endemic countries, as well as civilian travelers and military personnel who travel or are deployed to endemic countries.

Several first-generation, inactivated JE vaccines, using mouse brain as a substrate for growth of the virus, have been produced by Japanese, Korean, Vietnamese and other national manufacturers for decades.

There is only one JE vaccine which is licensed in the U.S., Canada and Australia, but not in Europe. Although effective, the use of JE-VAX® has been troubled by safety concerns.

Intercell AG has developed a second generation, purified, inactivated JE virus vaccine using a certified Vero cell culture substrate for virus propagation. Phase 3 trials have been completed recently, and will be discussed at the conference.

Notes

THE INDUCTION OF IMMUNITY OR TOLERANCE - A CHALLENGE FOR MUCOSAL VACCINE DEVELOPMENT

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There is currently great interest in developing mucosal vaccines against a variety of microbial pathogens. Mucosally induced tolerance also appears to be a promising form of immunomodulation for treating certain autoimmune diseases and allergies. An overriding question for the development of vaccines or immunotherapies is how to steer, for the different purposes, the immune response towards immunity or tolerance. Mucosal immune responses are tightly regulated, and the default response to most harmless antigens is tolerance induction. Pathogens characteristically generate ”danger signals” often through PAMP-TLR interactions: this avoids the default tolerance response and stimulates immunity rather than tolerance. Effective mucosal vaccines need to provide similar or alternative immunostimulatory signals: especially with inactivated or subunit vaccines, it is often critical to present the vaccine antigen together with an effective mucosal adjuvant. Conversely, agents for immunotherapy need to be constructed so as to enhance the physiological mechanisms to actively suppress harmful immune reactions to ingested or inhaled antigens. The most potent mucosal adjuvants are cholera toxin (CT) and the closely related Escherichia coli heat-labile enterotoxin (LT). While these toxins may be clinically useful as adjuvants for ex vivo dendritic cell vaccination, they are too toxic for in vivo administration to humans. Significant progress has been made recently to generate detoxified derivatives of these toxins with retained adjuvant activity. Other classes of mucosal adjuvants described include e.g. CpG and other TLR ligands and vitamins A and D. The clinically most advanced among the immunomodulating toxin derivatives is recombinantly prepared CT B-subunit (rCTB). rCTB serves as an important protective antigen in a widely registered oral vaccine against cholera. In preclinical studies rCTB has also proved to be a promising vector for either inducing local anti-infective immunity or, more surprisingly, peripheral anti-inflammatory tolerance to chemically or genetically linked foreign antigens administered mucosally. The outcome - immunity or tolerance induction - appears to be determined largely by the nature of the antigen. In a recent clinical study, oral treatment with a uveitis-inducing heat-shock-protein peptide linked to rCTB was found to control uveitis in patients with Behcet´s disease. We will discuss recent advances in the development of mucosal vaccines for protection against infections and for treatment of various allergic or inflammatory disorders and also draw attention to promising recent findings using sublingual antigen presentation for efficient induction of either immunity or tolerance.

Notes

TOLL-LIKE RECEPTOR (TLR) AGONISTS AND ZOT-DERIVED PEPTIDES AS VACCINE ADJUVANTS

Sefik S. Alkan

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Adjuvants are pivotal part of successful vaccines along with the antigens and the formulation /delivery methods. In this study we have investigated two different classes of molecules as potential adjuvants. In the first study we evaluated several Toll like receptors agonists. We focused on a subfamily of endosomal TLRs consisting of TLR7, TLR8 and TLR9. It is known that natural agonists consisting of nucleic acid sequences such as ssRNA or DNA with CpG motifs activate the innate immune cells through these TLRs to produce inflammatory cytokines. 3M's Imidazoquinoline class compounds 3M-001, -2 and -3, (also called immune response modifiers, IRMs) have been shown to activate the innate immune system via TLR7, 8 and 7/8, respectively. These compounds exhibit anti-tumor effects. While studying the effect of the agonists of the TLR7, 8 and 9 on the activation of NFkB transfected HEK cells, we have discovered that certain deoxyoligonucleotides (ODNs) could modulate imidazoquinoline effects. Thus, certain ODNs inhibited TLR7 and enhanced TLR8 signaling events in HEK cells and cytokine production (IFN-a, TNF and IL-12) by human primary peripheral blood mononuclear cells. Since TLR7 deficient mice are unresponsive to TLR8 agonist IRMs and similarly, natural ssRNA can't activate murine TLR8, we then asked the question if the combinations of IRM and ODNs could activate so called "nonfunctional "mouse TLR8. We transfected HEK293 cells with murine TLR8 and NFkB reporter constructs and stimulated with a combination of PolyT ODN (PolyT) plus TLR agonists. We found that PolyT/TLR activated NFkB while PolyT plus TLR7 agonist did not activate. Primary mouse cells responded to the IRM/PolyT ODN by secreting TNF. Cells from TLR7 -/- and TLR9 -/- mice responded to IRM/PolyT combination whereas MyD88-/- cells did not respond. We concluded, for the first time, that mouse TLR8 is functional. We then performed a series of experiments on cross talk between TLR2 to TLR9. By testing ctytokine/chemokine response of huPMBC to all possible tandem combination of TLR agonists, we found combinations of TLR agonists which are inert, additive, synergistic or antagonistic. We further demonstrated that conditioned PBMC medium from a synergistic combination of TLR agonists killed tumor cells in vitro significantly better than individual TLR agonists could do. Implication of these findings on vaccination and tumor therapy will be discussed. Finally, although its relationship to TLRs is still under investigation, we evaluated the adjuvanicity of ALBA's novel molecule, AT-1002, a six-mer synthetic peptide, FCIGRL, derived from V. Cholera's second toxin, Zonula Occludens Toxin (ZOT). AT-1002 is shown to open the tight junctions between epithelial cells and was expected to enhance antigen passage through the mucosal/endothelial barriers and hence stimulate antigen-specific immune

responses. Indeed, it has been demonstrated that mice immunized intranasaly with antigens such tetanus toxoid in the presence of AT-1002 exhibit significantly higher antibody titers and cellular immune responses than those induced by immunization with antigen alone. Thus, these studies suggest that tight junction modulator AT-1002 might provide a novel approach to vaccination.

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Notes

MONOCLONAL ANTIBODIES: A NEW PARADIGM IN INFECTIOUS DISEASES

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New strategies are needed to control infectious diseases. Passive vaccination has a proven record in the treatment of infections and entered the medical arena more than 100 years ago. Progress in the identification of protective vaccine antigens in the context of subunit vaccines, as well as further development of the monoclonal antibody technology to produce specific antibodies in unlimited quantities at reasonable costs provide new opportunities to revive the old idea of treating and preventing infectious diseases with passive vaccination. The highest medical need is associated with life threatening infections occurring in hospitals especially when caused by multi-drug resistant nosocomial pathogens and also with individuals whose immune defense is compromised (elderly people, organ transplant and cancer patients).

The availability of the entire genome sequence of pathogens has strongly facilitated the identification of protein antigens that are targets for functional antibodies. Surface expressed proteins from extracellular pathogens can induce opsonophagocytic and bactericidal antibodies, as well as those with function neutralizing activity. Most of the current vaccines protect individuals through vaccine-induced antibodies.

We have developed a comprehensive technology using human serology and genomic display libraries to identify the ANTIGENome of human pathogens and through subsequent *in vitro* and *in vivo* validation to select vaccine candidates. The most conserved antigens that have been shown to induce protective antibody responses in animals are further studied for the definition of *in vitro* correlates of protection. *In vitro* functional antibody assays are extremely valuable in indicating appropriate immunogenicity of the vaccine in early clinical trials and can serve as surrogate markers during late stage clinical development. At the same time the same antigens and assays can be used to select and develop monoclonal antibodies to prevent and treat diseases in populations which can not be protected with prophylactic vaccines. Examples for the usefulness of the parallel use of vaccines and antibodies exploiting the same antigens will be presented in the context of Pneumococcus, Group B Streptococcus and nosocomial pathogens.

Notes

IL-15 AS A VACCINE ADJUVANT TO INDUCE LONG-LIVED, HIGH AVIDITY MEMORY CD8$^+$ T CELLS, EVEN IN CD4-DEFICIENT HOSTS

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One critical hurdle for therapeutic HIV vaccines is the deficiency of CD4$^+$ T cell help during HIV infection. CD4$^+$ T cell help has been shown to be necessary for induction of long-lived memory CD8$^+$ cytotoxic T lymphocytes (CTL), and when CD8$^+$ T cells are primed in its absence, they are susceptible to TRAIL-mediated death during secondary stimulation. We have found that IL-15 expression by a vaccine vector allowed induction of longer-lived, higher avidity memory CTL. We also observed that CD40L, a molecule by which helper T cells mediate help, induces dendritic cells to secrete IL-15. We therefore hypothesized that one mechanism by which CD4$^+$ T helper cells induce longer-lived memory CTL may be to stimulate IL-15 production by the dendritic cell presenting antigen, and that therefore, IL-15 might overcome the need for CD4$^+$ T cell help. We have now tested this hypothesis by demonstrating that immunization of CD4-depleted mice with a recombinant vaccinia-HIV vaccine vector expressing IL-15 induced long-lived memory CTL, whereas immunization of the depleted mice with a recombinant vaccinia-HIV vector not expressing IL-15 resulted in short-lived CTL that disappeared within two months. Further, CTL induced with the IL-15-expressing vaccine were resistant to TRAIL-mediated death on secondary stimulation, whereas those induced without IL-15 underwent apoptosis. Resistance was associated with upregulation by IL-15 of anti-apoptotic Bcl-XL and downregulation of Bax, a downstream transducer of the TRAIL death signal. Furthermore, IL-15 production by dendritic cells was necessary for CD4$^+$ helper T cells to induce long-lived CD8$^+$ T cell memory. Thus, IL-15 was both sufficient to substitute for and necessary for such help. These findings help explain the role of helper T cells in inducing long-lived memory CTL and provide a practical approach to overcome the deficiency of CD4$^+$ T cell help during HIV infection for induction of CTL with a therapeutic vaccine for HIV, or with a vaccine for other opportunistic infections.

Notes

M2e-BASED UNIVERSAL INFLUENZA A VACCINE

Fiers, W., De Filette, M., Descamps, F., Birkett, A., Lycke, N., Ramne, A., Min Jou, W., & Saelens, X.,

VIB and University of Gent, Belgium, University of Göteborg, Sweden, Biovitrum AB, Sweden, & Acambis Inc, US.

Influenza virus escapes immunity in the population by drift and shift, phenomena responsible for epidemics and pandemics, respectively. The gradual changes (drift) and drastic changes (shift) occur in the dominant viral antigens, hemagglutinin and neuraminidase. M2-protein which also spans the membrane, forms a proton channel and is hardly present in virus particles, but highly expressed in infected cells. Its ectodomain, M2e, is 23 amino acids long, almost not immunogenic, and highly conserved among all human influenza A strains. There exist a human-type (hM2e), and an avian-type M2e-consensus sequence (avM2e) which differ at 5 positions. “Spanish flu” and most isolates from H5N1-infected patients have an intermediate sequence. The strong conservation and the limited changes allowed in the avM2e to hM2e adaptation, are mainly due to constraints imposed by the overlapping M1 (matrix) gene. M2e, expressed in multiple copies on the surface of HBc-particles, becomes highly immunogenic and induces protective antibodies. The vaccine is produced in high yields in E. coli. Immunization in mice protects against a severe, lethal challenge by various virus subtype strains, prevents or limits morbidity, reduces viral lung titer, and protection is long lasting. Several adjuvants, suitable for human use, enhance the immune response. Pre-existing antibodies against the carrier HBc do not significantly affect the immunogenicity. Vaccination also protects against a lethal challenge with a virus strain containing an avian type M2-protein. Indeed, hM2e-immune serum binds to a large panel of avM2e-sequences but the affinities vary from strong to fairly weak.

Nasal administration has several advantages, such as needle-free vaccination without risk of cross-contamination by blood-borne pathogens. Moreover, it induces mucosal immunity in the nasal and respiratory lymphoid tissues, an advantage for protection against a respiratory virus like flu. However, only few mucosal adjuvants are known. We used CTA1-DD which can bind only to immune system cells, and therefore completely lacks the toxicity of Cholera Toxin. Intranasal delivery of M2e-vaccine plus CTA1-DD induces as much M2e-antibodies in circulation as parenteral injection, provides complete protection against a lethal challenge, and reduces the morbidity more efficiently than in the parenterally administered control. The mechanism of protection is by antibody-dependent infected cell cytotoxicity, as will be shown at the meeting.

Notes

IT'S PRIME TIME FOR CLINICAL TRIALS OF PLASMID DNA VACCINE

Ron B. Moss

Vical Incorporated, San Diego, CA

Plasmid DNA (pDNA) vaccines to prevent and treat various infectious diseases are currently being studied in numerous clinical trials. To date, pDNA vaccines have been shown to have a tolerable safety profile while maintaining distinct advantages over conventional vaccine approaches, including the ability to combine plasmids and to focus the immune system on multiple epitopes of pathogens. In addition, and particularly for emerging pathogens, pDNA vaccines are simpler and more cost effective to manufacture. Thus for pathogens such as malaria, Ebola virus, B. anthracis, West Nile virus, HIV, seasonal and pandemic influenza virus, and cytomegalovirus, pDNA vaccines offer the opportunity to control disease on a global basis. Novel adjuvants, delivery devices, and prime-boost modalities have demonstrated enhanced immunogenicity of pDNA vaccines. Interestingly, immune responses elicited by pDNA vaccination may be qualitatively and quantitatively different than for conventional vaccines. The ability to prime the immune system with pDNA vaccines should result in long term memory, and prevention and control of infectious diseases. Additional trials which generate clinically relevant endpoints are warranted for pDNA vaccines to determine their true efficacy. A review of various clinical trials of pDNA vaccines will be presented.

Notes

THE FUTURE OF VACCINES IN THE LIGHT OF COMMERCIAL, SOCIAL AND DISEASE CHALLENGES

A. von Gabain

Panelists: J. Almond, M. Greco, A. von Gabain, J. Martin, R. Spier and R. Rabinovich
Interviewers: M. Ward and J. Hodgson
Moderator: G. Zettlmeissl

Every forth life is terminated by microbial infections. In less developed countries infectious diseases topped by the three major killers, AIDS, Tuberculosis and Malaria, provide a major barrier to escape the inferior social and economical conditions that trap a major part of the human population in an unacceptable low quality and standard of life. In countries with high medical standards infectious diseases have never been exterminated and lately returned into the medical arena; e.g. by the appearance of multi-antiobiotic resistant pathogens. The seasonal inter-pandemic flu wave is estimated to kill in average more than 107.000 citizens in Europe every year only. It is fair to state that the global community has presently no master plan in place that could prevent a pandemic influenza that has been estimated to kill 50 to 100 million people at the end of world war one. Increased population density, human mobility and changing life style have favored the comeback of known infectious diseases and the rise of novel, often caused by pathogens that were previously not recognized as medical problems or restricted to certain geographic areas. Furthermore, chronic microbial infections are increasingly discussed as triggers of certain forms of cancer, neurodegenerative and autoimmune diseases.

In spite of the explosion of genomic technologies, the pace in finding novel drug targets and cognate anti-infectives is frustratingly slow. Thus, the comeback of vaccines seems a quite logical response to the re-bouncing challenges of infectious diseases. This trend can be also attributed to the dramatic progress made by academic research during the last decades to understand the molecular biology of pathogens and the human immune system. Also the availability of cell culture techniques in combination with recombinant DNA technologies have facilitated the field. The progress in R & D has particularly enabled the field to move away from outdated manufacturing processes often yielding impure and ill-defined vaccines derived from whole microbes towards novel well-defined vaccines that comprise the minimal features needed to induce protective immunity against the target pathogen.

However, the development of novel vaccines is hampered by many factors. In developed countries, vaccines are still seen as a medical niche for which the society is seldom willing to pay a price that reflects the saved costs in health care and that makes the launch of novel vaccine economically attractive, considering high investment and risk involved in the development. Furthermore, established standard vaccinations and vaccines for novel applications with unmet medical need are facing in welfare societies increasingly the resistance of customers whose

legitimate fears are abused by vaccine opponents, some of them acting in the fashion of “charlatans camouflage”. Another dilemma in vaccine development is the regulatory hurdles during the registration procedure that often is benchmarking the next generation vaccines with rules invented to promote the launch of “classical vaccines”. Predictive animal models and immunological surrogate markers indicative for protective immunity in such animals and humans are rarely accepted as hallmarks for the definition of the endpoints of clinical trials. In contrast, the development and launch of vaccines suited for the need of less developed countries is impeded by the difficulties of distributing the vaccines in needed geographic areas and the limits of costs of goods. Lately, vaccine players from pharma and biotech industries have formed encouraging alliances with WHO, governments, NGOs and other organizations, mostly sponsored by the Melinda and Bill Gates foundation, to help delivering vaccines to populations that are not able to afford minimal heath care services. Some industrial vaccine players have even decided to move single vaccines into registration in endemic areas first, before re-launching the product into developed countries.

In the panel the two science journalists will expose the six panelists with questions regarding major aspects of vaccines in the light of novel disease targets, developmental challenges, underpinning basic research, market opportunities, customers’ acceptance, priorities in relation to the threats, chances to be financed by investors and/or sponsors, likelihood of infiltrating most exposed populations, new technologies for improved protection of elderly and neonates, latest trends and visions for the future. The discussion will be conducted by the Chair.

UNIQUE INTERACTION OF THE IMMUNOSTIMULATORY CATIONIC PEPTIDE $KLKL_5KLK$ WITH BIOLOGICAL MEMBRANES

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Cationic antibacterial peptides (CAPs) have recently been found to play key roles in innate immunity. IC31™ a novel adjuvant composed of a derivative of a natural CAP $KLKL_5KLK$, (KLK) and oligo-$d(IC)_{13}$ [ODN1a] has been shown to stimulate antigen-driven T and B cell responses and the maturation and activation of dendritic cells (DCs). KLK induces a sustained T_H2 dominant response against model antigens, enhances the association of antigens to DCs, and forms a depot at the injection site. KLK has also been suggested to facilitate the uptake and delivery of antigens and ODN1a into cellular compartments, but the nature of KLK´s interaction with the cell surface and other membrane-bordered compartments remains unknown. Here we monitored the effects of KLK on isolated membrane vesicles and investigated the partition of KLK within vesicular sub-fractions. KLK readily interacted with fluorescent dyes entrapped in the vesicles without apparent pore formation. Fractionation of vesicles revealed KLK predominantly in the membrane. None of these effects was seen with an immunologically inactive derivative of KLK (KLKLLPLLKLK; KPK). TEM revealed ragged-edged vesicles in KLK-treated samples with frequent widening of the intra-membrane space and general loosening-up of the bilayer structure. While no effect of KLK on osmotic resistance of human erythrocytes was seen, dramatic decrease in core and surface membrane fluidity was observed in KLK-treated erythrocyte ghosts as measured by fluorescence anisotropy. Finally, CD spectroscopy revealed lipid-induced random coil to β-sheet and β-sheet to α-helix conformational transitions of KLK. These results suggest a profound membrane interacting property of KLK that might contribute to the immunostimulatory activities of IC31™.

APPLICATION OF SOLID-STATE CIRCULAR DICHROISM TO PROBE CONFORMATIONAL STABILITY OF ANTIGENS ADSORBED ONTO ADJUVANTS

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Adsorption of antigens onto adjuvant is commonly used to enhance their immunological properties. However, our understanding of the influence of immobilisation on the secondary and in particular the tertiary structure of protein antigens has been limited due to the problems of applying solution based structural tools to particulate systems.

We have developed a novel technique based on circular dichroism (CD) to obtain high quality spectra of proteins and antigens bound to scattering particles. This technique was applied to model protein antigens bound to aluminium phosphate (Adju-Phos) and aluminium hydroxide (Alhydrogel). Raw CD spectra of antigen in solution and in suspension were found to be similar but a reduction in the intensity of bands below 240 nm was observed for the suspensions. Elimination of artefacts due to scattering and absorption flattening was achieved by optimisation of the optics and introduction of correction factors.

Using this approach it was possible to analyse the secondary and tertiary structure of the immobilised proteins. In freshly prepared samples it was found that the secondary structure of proteins adsorbed onto adjuvant were generally identical to in solution. The tertiary structure was also often similar but changes in band intensities, indicative of an increase in protein rigidity on binding, were observed. This novel CD technique is likely to be of major benefit for improving the analysis of solid-state formulations of therapeutic proteins and vaccines, and in particular for assessing their stability and or degradation in situ.

A NOVEL CD8$^+$ T CELL SUBSET THAT OCCURS IN HEALTHY ELDERLY PERSONS AND REPRESENTS A REPERTOIRE OF DIVERSITY

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Infectious diseases are frequent and severe in elderly persons and the efficacy of vaccinations is low. This is due to an age-related decline in the functions of the immune system referred to as immunosenescence. Specifically, the accumulation of highly differentiated CD8$^+$CD28$^-$ T cell clonal expansions has been shown to be associated with a lack of antibody production following influenza vaccination. Recently, we identified an IL-2/IL-4-producing CD8$^+$CD25$^+$ non-regulatory T cell population that occurs in a subgroup of healthy elderly persons who characteristically still have a good humoral immune response after influenza vaccination. We now demonstrate that CD8$^+$CD25$^+$ T cells have long telomeres, a highly diverse T cell receptor repertoire and a gene expression profile that is distinct from CD8$^+$CD25$^-$ T cells. Molecular tracking of specific clones revealed that the same clones occur in both, CD8$^+$CD25$^+$ and CD8$^+$CD25$^-$ T cells, demonstrating a lineage relationship between CD25$^+$ and CD25$^-$ CD8$^+$ T cells. Upon antigenic challenge, CD8$^+$CD25$^+$ cells can differentiate into functional effector cells. Altogether, our results suggest that CD8$^+$CD25$^+$ T cells represent an early stage in the differentiation of CD8$^+$ T cells. The accumulation of these cells in elderly persons appears to be a prerequisite of intact immune responsiveness in the absence of naive T cells in old age.

CHILDHOOD VACCINATION IN BANGLADESH: A DATA ANALYSIS OF THE 2004 BANGLADESH DEMOGRAPHIC AND HEALTH SURVEY

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Universal immunization of children under one year of age against the six vaccine preventable diseases (tuberculosis; diphtheria, pertussis, and tetanus [DPT]; poliomyelitis; and measles) is one of the most cost-effective programs in reducing infant and child morbidity and mortality. The Expanded Program on Immunization (EPI) is a priority program for the government of Bangladesh. It follows the international guidelines recommended by the World Health Organization (WHO). The guideline recommends that all children receive a BCG vaccination against tuberculosis; three doses of DPT vaccine for the prevention of diphtheria, pertussis (whooping cough), and tetanus; three doses of polio vaccine; and a vaccination against measles. WHO recommends that children receive all of these vaccines before their first birthday and that the vaccinations be recorded on a health card given to the parents.

In the 2004 Bangladesh Demographic and Health Survey (BDHS), data on childhood vaccinations were collected for all surviving children born during the five-year period before the survey. In Bangladesh, immunizations are routinely recorded on a child's health card. For each child, mother were asked whether they had the vaccination card for the child and, if so, to show the card to the interviewer. When the mother was able to show the vaccination card, the dates of vaccinations were transferred from the card to the questionnaire. If the vaccination card was not available (or a vaccination was not recorded), mothers were asked questions to determine whether the child had received each vaccine.

According to information from both the vaccination cards and mothers' report, 73 percent of Bangladeshi children aged 12-23 months are fully vaccinated. Although the levels of coverage for BCG and the first doses of DPT and polio are close to 90 percent or above, the proportions of children who go onto complete the third dose of DPT or polio vaccines fall to 81 and 82 percent, respectively. A much lower percentage (76 percent) receives the measles vaccine. Only 3 percent of children aged 12-23 months have not received any childhood vaccinations. Vaccinations are most effective when given at the proper age; thus it is recommended that children complete the schedule of immunizations during their first year of life (i.e., by 12 months of age). Overall, 68 percent of children aged 12-23 months had received all the recommended vaccinations before their first birthday.

There has been significant improvement in vaccination coverage in recent years. The proportion fully vaccinated among children aged 12-23 months has increased by 13 percentage points between 1999-2000 and 2004 (from 60 to 73 percent). Closer examination of the data by types of vaccines indicates that this trend is entirely due to reduction in dropout rates from the first to the third doses of polio and DPT vaccines.

Mother's education is strongly associated with children's vaccination coverage: only 60 percent of children of mothers with no education are fully vaccinated compared with 92 percent of children of highly educated mothers.

VACCINE THERAPY IN ASYMPTOMATIC HEPATITIS B VIRUS CARRIERS

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Previous our study demonstrated that, more than 20% of relatively healthy people of Mongolia have been infected with the Hepatitis B virus(HBV) and 8.8±1.1% of them are carriers of the HBsAg. These carriers are at high risk of serious illness and death from cirrhosis and primary liver cancer. Accordingly, HBV infection is still one of the main health problems in Mongolia.

Current therapeutic approaches to control hepatitis B virus such as Interferon and Lamivudine are not completely satisfactory. These outcomes emphasize the need for novel therapy. Consequently, it is important to study a therapeutic efficacy of Hepatitis B vaccine for HBsAg carriers.

The aim of this study was to investigate the antiviral potentiality of Vaccine therapy for asymptomatic HBV carriers by hepatitis B vaccine which developed in the Public Health Institute, Mongolia.

In this study, 30 symptomless HBV carriers have been enrolled. HBV carriers who have been selected for Vaccine therapy were given five intramuscular injections of the hepatitis B plasma vaccine with to 2ml (20μg/ml HBsAg) at a month intervals.

During the therapeutic vaccination, HBV carriers who selected for the therapy were tested biochemical parameters of liver function, quantity of HBV DNA, HBsAg titers, HBV markers and the vaccine-induced escape mutants of HBV.

After the vaccine therapy, the HBV carriers have shown complete clearance and reduction of HBV DNA in 43,3% of them and 10 people had undetectable HBsAg while 14 others showed significant decrease in HBsAg titers. Post-treatment, the average HBV DNA and HBsAg titer of serum was decreased significantly in comparison with the pre-treatment.

Our result demonstrated that the hepatitis B vaccine can be used for the treatment of HBV carriers and therefore can prevent the onset of chronic hepatitis and hepatocellular carcinoma and vaccine-induced escape mutants of HBV was not observed in the amino acid substitution of codon 126, 141 and 145 of the S gene, after Vaccine-therapy.

PHENOTYPIC PROPERTIES AND CYTOKINE SECRETION PROFILES OF PLASMACYTOID DENDRITIC CELLS INDUCED BY VARIOUS CpG-OLIGONUCLEOTIDES

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Introduction: Plasmacytoid dendritic cells (pDC) represent a rare but unique cell type of innate immunity. They are able to recognize nucleic acids of host and pathogens by their special set of pattern recognition Toll-like receptors represented by TLR7 and TLR9. Similar to conventional dendritic cells pDC can prime and polarize naive T-cells, while also having an important effector function in anti-viral immunity through the rapid and robust production of IFN-alpha. The known potential of pDC to exert direct anti-viral defense, together with their capability to activate T-lymphocytes to effector/memory or regulatory functions, has been suggested to be modulated by synthetic CpG-oligonucleotides.

Objectives: The aim of this study was to analyze the effect of two prototype TLR9-agonists on the phenotypic characteristics and cytokine secretion of isolated pDC manipulated in short term in vitro cultures.

Methods: Plasmacytoid dendritic cells were isolated from “buffy coat” of healthy volunteers by magnetic cell separation using negative selection. The expression of co-stimulatory molecules ICOS-L, CD80 and CD86, the chemokine receptor CCR7, the presenting molecule HLA-DQ, and the ectoenzyme and signaling receptor CD38 on the surface of pDC was monitored by three-colour flow cytometry 24 hours after treatment with type A (CpG2216) and type B (CpG2006) CpG oligonucleotides. The levels of secreted IL-6, TNF-alpha and IFN-alpha in the culture supernatants were determined by ELISA.

Results: Stimulation by type A CpG oligonucleotides induced high expression of CCR7 and CD38 as well as robust production of IFN-alpha and significant secretion of IL-6 and TNF-alpha. Interestingly, treatment of pDC with type B CpG resulted in pronounced expression of ICOS-L, CD80 and HLA-DQ, and induced only a low-level cytokine production.
Conclusion: On the basis of our data, it seems that type A TLR9-agonist increases the migratory potential and cytokine production of pDC. Conversely, type B CpG may enhance the antigen presenting function of these cells. Developing new strategies to expand these distinct functional activities *in vivo* and modulate pDC function associated with the specific regulation of host immunity may provide novel immune-based therapies.

INCREASE OF VIRUS VACCINES' IMMUNOGENICITY USING ADJUVANT OBTAINED FROM *GIPSOPHILA PANICULATA* PLANT INDIGENOUS TO KAZAKHSTAN

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Elaboration of highly immunogenic and safe vaccines is one of most important problem in preparedness for flu pandemics. One of attractive delivery system for elaboration of low toxicity, safe and highly immunogenic influenza subunit vaccine is immunostimulating complex formed by encapsulating antigens and saponins of plant origin. Accordingly our preliminary data *Cipsophila paniculata* plant indigenous to Kazakhstan have been shown to possess of immunostimulated saponins could be used for activation of immune response against to isolated viral antigens.

In the study presents immunostimulated saponins have been isolated from *Cipsophila paniculatata* plant indigenous to Kazakhstan by HPLC fractionation. Toxicity of saponin containing fractions was studied in chickens embryos, mice and chickens. Immunostimulating activity of saponin -containing preparations was studied using avian influenza virus model (strain A/FPV/Rostock/34). HA+NA external virus antigens were isolated from purified virus by non-ionic MESK detergent treatment. Subunit vaccine preparation was assembled with purified saponins isolated from *Cipsophila paniculatata* plant and immunostimulation activity of prepared immunostimulated complex was investigated in comparison with pure subunit vaccine and whole virus inactivated vaccine preparation.

It in the study presented was shown, that immunogenicity of subunit vaccine assembled with purified saponins isolated from *Gipsophila paniculata* plant is much higher in comparison with immunogenic activity of pure subunit vaccine or whole virus inactivated vaccine.

The results obtained have show that *Gipsophila paniculata* plant possess saponins with significant immunostimulating activity and this saponins could be use may be tor creation of highly immunogenic of influenza vaccine preparation.

"GENTLY ROUGH": OPTIMAL DOWN-REGULATION OF LPS IN *rfaH* MUTANTS FOR LIVE-ATTENUATED ENTEROBACTERIAL VACCINE CANDIDATES

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Enteric bacteria, such as *Escherichia coli*, *Salmonella enterica* and *Shigella* spp. consist of a very large number of serovariants due to the extreme heterogeneity of their surface antigens, particularly LPS. In order to raise cross-protective immunity among heterologous serovariants a tempting strategy would be to use mutants with defects in LPS synthesis (i.e. rough mutants). On the other hand, LPS is an important virulence factor contributing to several steps of the infectious process. Consequently, rough mutants tend to be overattenuated and are usually considered to be inappropriate as live vaccines.

Ideal live-attenuated vaccines find a balance between being attenuated (safe) and retaining immunogenicity (efficacy). We have tested several structural LPS mutants of *S. enterica* sv. Typhimurium and have shown that loss of the O-antigens itself (_*waaL*) did not elicit sufficient attenuation, whereas truncation at the depth of the inner core (_*waaG*) rendered the mutant overattenuated, i.e. unable to induce an efficient immune response.

RfaH is a transcriptional anti-terminator in enterobacteria required for the transcription of long operons including those encoding LPS synthesis. Mutants lacking RfaH were described before to exhibit deep rough-phenotype. However, in the current study we provide evidence that the truncation of LPS molecules in *rfaH* mutants is partial, only, i.e. different lengths of LPS chains are expressed. The majority of LPS molecules are truncated at the level of core oligosaccharides, indeed. A small part of LPS molecules, however, contain an intact core, some of which are capped by O-antigens. This was confirmed by the existing, although decreased potential of *S.* Typhimurium *rfaH* mutants to be transduced by the O-antigen-specific phage P22. Furthermore, an *rfaH* mutant of *Shigella flexneri,* was still agglutinated by O-specific serum and parts of the O-antigen ladder could still be detected by silver staining on the SDS-PAGE gels suggesting that the amount of the O-antigens retained in mutants of in this species could be higher than in *S.* Typhimurium.

This LPS structure - designated here as "gently rough" phenotype - sufficiently attenuates virulence while retaining immunogenicity of *rfaH* mutants. LD_{50} value of the *S.* Typhimurium mutant increased a million-fold in the murine typhoid model, while that of *S. flexneri* increased 100-fold in the mouse lung model. Vaccination with *rfaH* mutants induced protective immunity against the homologous serotypes in both species. Furthermore, in case of *Salmonella* cross-protective immunity was detected, likely to be mediated by conserved outer membrane proteins shared by various representatives of *Enterobacteriaceae*.

THE MYCOBACTERIUM ANTIGEN ESAT-6 AS THE DNA-VACCINE AND DIAGNOSTIC TARGETS

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Tuberculosis (TB) remains once of the leading cause of death due to infections and novel vaccines are required that can protect against disease. At present the only available vaccine against TB is *BCG*, which has been demonstrated to have variable efficacy in both humans and animals. Recently, protein ESAT-6 encoded by major genomic regions of difference (RD1) of *Mycobacterium tuberculosis* have been suggested as promising candidate for vaccine against tuberculosis and diagnosis of TB. The ESAT-6 antigen from *M. tuberculosis* is a dominant target for cell-mediated immunity in the early phase of disease in TB patients as well as in various animal models. Evaluation of this antigen in DNA-vaccine with polysaccharide conjugate could be a novel approach for the development of antituberculosis vaccine. With this aim, in the present study, plasmid pcDNA3.1mycHis(-)/lacZ/ESAT-6 encoded ESAT-6 antigen with spermidine-polyglucin conjugate was evaluated in mouse model of immunization. This experimental construction was assessed in mice for their ability to induce lymphoproliferation and IFN-g production by splenocytes. For it male *BALB/c* mice (6-8 week) were immunized intramuscularly three times with 50 _g of the DNA in experimental preparations. The ESAT-6 showed high stimulation index and IFN-g levels suggesting the induction of Th1 response. We have assessed toxicity of our experimental construction and showed it not toxic. We have also evaluated and reported that pcDNA3.1mycHis(-)/lacZ/ESAT-6 surrounded with polysaccharide matrix efficiently protect nucleic acids from degradation by nucleases in vitro.

The results obtained indicated that our experimental construction can induce specific T-cell responses (CTL and blast transformation) and be valuable vehicle for DNA-vaccine against tuberculosis.

In a future we plan to test protective properties of the experimental construction in mice model tuberculosis.

Also we have obtained recombinant protein GST-ESAT-6. We have used the expression system, based on carrier GST, for obtaining hybrid recombinant antigen of *M.tuberculosis*, which was fused with C-terminus of carrier. For expressing recombinant protein GST-ESAT-6 gene *esxA* from *M.tuberculosis H37Rv* was amplified by PCR and cloned into plasmid pGEX-2T. Antigenicity of protein was confirmed by Western blotting with monoclonal antibodies against GST and serum samples from TB patients. Now we evaluate the opportunity of application this antigen for diagnostics of the different form of tuberculosis, using IEA and IFN-g ELISA methods.

ENHANCEMENT OF ANTIGEN-SPECIFIC ANTIBODY AND CD8+ T CELL RESPONSES BY CODELIVERY OF IL-12-ENCAPSULATED MICROSPHERES IN PROTEIN AND PEPTIDE VACCINATION

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Although IL-12 has been widely accepted to play a central role in the control of pathogen infection, the use of recombinant IL-12 (rIL-12) as a vaccine adjuvant is known to be ineffective because of its rapid clearance in the body. To investigate the effect of sustained release of IL-12 *in vivo* in the peptide and protein vaccination models, rIL-12 was encapsulated into poly ($_{DL}$-lactic-co-glycolic acid) (PLGA). We found that codelivery of IL-12-encapsulated microspheres (IL-12EM) could dramatically increase not only antibody responses, but also antigen-specific CD4+ and CD8+ T cell responses. Enhanced immune responses were shown to be correlated with protective immunity against influenza and respiratory syncytial virus (RSV) virus challenge. Interestingly, the enhancement of CD8+ T cell response was not detectable when CD4+ T cell knockout mice were subjected to vaccination, indicating that the enhancement of the CD8+ T cell response by IL-12EM is dependent on CD4+ T cell “help”. Thus, IL-12EM could be applied as an adjuvant of protein and peptide vaccines to enhance protective immunity against virus infection.

THE ADJUVANT IC31™ REQUIRES TYPE I INTERFERONS TO STIMULATE CD8 MEDIATED IMMUNE RESPONSES

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The adjuvant IC31™ developed by Intercell AG is a very promising tool for elevating immune reactions. Therefore we were interested to elucidate the signal transduction pathway and function of IC31™, which consists of two components, a cationic peptide called KLK and ODN1a, which is a TLR9 ligand. We were not able to detect significant influence of IC31™ on the most common signalling cascades *in vitro*. Nevertheless the adjuvant activity *in vivo* was remarkably high if the mice were immunized with a peptide in combination with the adjuvant IC31™ measuring a $CD8^+$ dependent immune response. Therefore we used different knock out mice to investigate the dependency of this activity on known regulators of CD8+ cell development and function. Our results show that the adjuvant effect of IC31™ is strictly dependent on type I interferons as there was no detectable effect of IC31™ in mice lacking STAT1 or the type I interferon receptor. Furthermore a similar impairment was seen in STAT1-S727A mice. Phosphorylation of STAT at S727 was previously shown to increase IFN-g dependent, STAT1 mediated immunity. However type II interferons seem to play no role in this context as mice lacking interferon gamma showed an equal capacity to mount a $CD8^+$ T-cell response upon immunization with a peptide in combination with IC31™. To further address the mechanism of IC31™ action we studied in more detail the effect of IC31™ on conventional dendritic cells and $CD8^+$ T-cells. We found no detectable effect of IC31™ on the *in vitro* proliferation of $CD8^+$ T-cells. Whereas IC31™ alone had no effect on the expression of activation related surface markers on *in vitro* differentiated conventional dendritic cells, the combined treatment of these cells with a peptide and IC31™ was very effective in upregulating the expression of activation markers. While the signalling events responsible for the adjuvant activity of IC31™ are still poorly understood, we were able to demonstrate that IC31™ dependent enhancement of T-cell mediated immunity requires type I interferons and the signal transducer STAT1.

IDENTIFICATION OF NOVEL VACCINE CANDIDATES AGAINST LYME BORRELIOSIS

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Borrelia afzelii, *Borrelia garinii* and *Borrelia burgdorferi* sensu stricto (s.s.) are the etiologic agents of Lyme borreliosis, the most prevalent tick-borne disease in Europe, North America and Far Eastern countries. It is a multi-systemic infection that can involve multiple organs or tissues. If caught early the disease can be prevented with oral antibiotics. However, if left untreated, the bacteria can spread through the bloodstream, enter various tissues and cause severe diseases including meningitis, arthritis, carditis and severe skin disorder. The human immune system is capable of recognizing antigenic structures of intruding *Borrelia* and to eradicate the pathogen via antibody mediated immune responses. In order to identify novel cross-protective vaccine candidates that would protect against all three pathogenic *Borrelia* species, human sera were selected from individuals with high levels of anti-borrelial antibodies and bacterial surface display was employed to present genomic peptide libraries at the surface of *E. coli* cells. Approximately 100 antigenic proteins were selected using the antigen identification approach, among which also antigens previously reported as antigenic or protective, such as OspC and DbpA were identified. For further selection of the most promising and novel candidates for vaccine development, all antigens were subjected to a validation procedure consisting of gene distribution analysis in variety of pathogenic *B. burgdorferi* sensu lato (s.l.) strains, surface location with FACS, and immunogenicity studies to determine the reactivity with individual human sera. The gene distribution analysis shows that numerous of the identified antigenic proteins are conserved in all three *B. burgdorferi* s.l. genospecies causing Lyme borreliosis. As a result of the current validation analysis, 25 antigens have been selected for further functional studies including animal challenge experiments and generation of gene deletion mutants.

IC31™: AN ATTRACTIVE ADJUVANT FOR PANDEMIC INFLUENZA VACCINES

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IC31™ mediated cellular and humoral immune responses are extremely beneficial to induce protective responses against influenza viruses. Moreover, the dose of influenza vaccine antigen can be dramatically reduced and shortages in vaccine supplies avoided. In this study, BALB/c mice were injected i.m. once with a low dose of the vaccine plus IC31™. Importantly, at day 21 upon single co-vaccination with IC31™, higher levels of serum HI titer were induced compared to injection of the vaccine alone. Furthermore, the immune responses shifted towards more type 1 dominated humoral (IgG2a) and cellular responses (IFN-_ by murine $CD4^+$ T cells). Specific type 2 immune responses (serum IgG1 / IL-4 by murine $CD4^+$ T cells) remained nearly unaffected upon co-injection of IC31™. Analyses at day 200 upon single injection showed very sustained antigen-specific cellular and humoral responses demonstrating the powerful adjuvant capacity of IC31™

DENDRITIC CELL SUBSETS AS PRIMARY TARGETS OF VACCINES

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Dendrititc cells (DCs) are master regulators of both innate and acquired immunity and play a regulatory role in the induction and maintenance of self tolerance and in priming and polarizing immune responses. Dendritic cells develop from bone marrow-derived precursors to rare circulating subsets that give rise to tissue resident cells accumulating at antigenic portals. Tissue DCs act as molecular sensors of micro-environmental changes and the DCs' environment is able to modify the development, activation, mobility and effector functions of DCs. DCs communicate with epithelial, endothelial, stromal and almost all immune cells through direct or cytokine-mediated interactions and continuously receive and forward information to regulate and coordinate immune responses. Physiological tissue environment is translated to tolerance induction, whereas microbial or stress signals induce inflammatory and/or regulatory responses. Based on their unique characteristics DCs are considered as the most efficient antigen presenting cells (APC) and adjuvants of both preventive and therapeutic vaccines against pathogens and cancer.

Human myeloid DC subtypes are characterized by the unique expression pattern of CD1 molecules and are able to present self or microbial lipo-peptides, glycolipids and sulphatides to various T-lymphocyte subsets. We found that $CD14^{high}$ monocytes cultured in serum free medium with granulocyte-macrophage colony stimulatory factor (GM-CSF) and interleukin-4 (IL-4) differentiate simultaneously to both $CD14^{low}CD1a^{-}$ and $CD14^{-}CD1a^{+}$ DCs. Kinetic studies revealed that immature $CD1a^{-}$ DCs differentiate to more mature $CD1a^{+}$ cells, but activation signals permanently block this transition. The co-existing $CD1a^{-}$ and $CD1a^{+}$ DC subsets differ in their internalizing capacity as $CD1a^{-}$ cells are able to take up higher amounts of lipids, carbohydrates and various particles. Both DC subsets, activated by inflammatory stimuli, acquire a mature phenotype and similar migratory potential. However, $CD1a^{+}$ DCs – activated by CD40 ligand or by certain Toll-like receptor ligands – produce significantly higher amounts of biologically active IL-12p70 cytokine and CCL1 chemokine than their $CD1a^{-}$ counterparts. We also detected higher expression of RNA-helicases (RIG-1, Mda5) in activated $CD1a^{+}$ DCs associated with interferon-_ (IFN_) production, whereas both $CD1a^{-}$ and $CD1a^{+}$ cells expressed similar levels of NLR/Caterpiller family proteins and secreted similar amounts of biologically active IL-1_. These functional differences were translated to differentially polarized T-lymphocyte responses characterized by high or low IFN_ and IL-10 secretion, induced by $CD1a^{+}$ and CD1a– DCs, respectively.

Interestingly, the ratio of $CD1a^{-}$ and $CD1a^{+}$ cells could be modulated by internalized serum lipoproteins in a dose dependent manner indicating the importance of environmental factors in $CD1a^{-}$ and $CD1a^{+}$ dichotomy. The differentiation of CD1a– DCs was attributed to the expression of transcriptionally active PPAR_ and their target genes such as apolipoprotein E (ApoE) and retinoid acid receptor (Immunity 2004, J. Exp. Med. 2006, Blood 2007). These results suggest that the expression of CD1 molecules in DCs is associated with unique functional characteristics that can be modulated by exogenous and endogenous lipids. Programming DCs for high phagocytic activity by PPAR_ ligands followed by activation through synergistic signaling platforms offers a promising approach for optimizing the adjuvant and T-cell polarizing effect of DC.

IC31™: A NOVEL ADJUVANT THAT POTENTLY ACTIVATES TYPE I IMMUNE RESPONSES

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The novel adjuvant IC31™ consists of a combination of a negatively-charged synthetic oligodeoxynucleotide and a positively-charged peptide. IC31™ is characterized by a broad mechanism of action, as well as an excellent safety profile. Detailed analyses showed that the immunostimulatory effect of IC31™ is mediated via the TLR9/MyD88-dependent signaling pathway of the innate immune system. Additionally, both depot formation at the injection site as well as sustained activation of antigen presenting cells, followed by antigen uptake and processing, is likely responsible for the induction of the observed IC31™-mediated antigen-specific immune responses. It could be shown in mice that IC31™ induces strong T cell responses activating both $CD4^+$ helper T cells as well as $CD8^+$ cytotoxic T cells. In addition, strong humoral immune responses with increased antibody titers are generated by IC31™. Because of its activation of innate immune cell types, such as NK cells and DC, as well as its stimulation of Type I T cell responses, IC31™ may show broad applicability to the treatment of infectious diseases and tumors.

NKT CELL-MEDIATED NASAL VACCINE

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Mucosal vaccination, by which secreted form of antigen-specific IgA is produced, is one of good measures to protect from upper airway tract infection such as influenza. To induce sufficient immunity by mucosal vaccination, an appropriate adjuvant is generally needed. We found that intranasal injection of influenza antigen and a-galactosylceramide (a-GalCer) efficiently induced antigen-specific IgA and IgG responses, and also antigen-specific T cell response, in NKT cell-dependent manner. This nasal vaccine with a-GalCer effectively protected mice from live influenza virus (including H5 type) challenge. However, paradoxically, there are very few NKT cells in nasal mucosa. Therefore we further investigated the mechanism of NKT cell activation at nasal mucosa. We found that the intranasal administration of antigen and a-GalCer significantly induced NKT cell recruitment at nasal mucosa and regional lymphnodes that is mediated by CXCL16/CXCR6. Intraperitoneal administration of a-GalCer did not induce the upper airway tract IgA production, indicating that the nasal NKT cell activation is needed. Using cytokine knockout mice, we found that IL-4 is required for the NKT cell-mediated IgA production. These data elucidate the mechanism of mucosal NKT cell activation, which would support the development of nasal vaccination using NKT cell glycolipid ligands.

IMMUNO-ADJUVANT ACTIVITY OF INTERFERON ALPHA : MECHANISM(S) OF ACTION

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Recognition of conserved components of infectious agents by Toll-like receptors (TLR) on antigen-presenting cells (APCs) leads to the production of interferon alpha (IFNa) and other cytokines, that orchestrate the innate immune response. IFN a also plays a key role in the initiation of the adaptive, antigen-specific, immune response by inducing the maturation of myeloid dendritic cells (DCs) and enhancing antigen presentation. IFN a enhances T-cell dependent B-cell activation, antibody production, and acts as a powerful adjuvant when ad-mixed with influenza vaccine and injected intramuscularly (im). The use of transgenic mice expressing a green fluorescent protein reporter gene regulated by an IFN responsive chimeric promoter showed that IFN activated cells with a phenotype characteristic of myeloid DCs, are present in the peripheral circulation of mice as early as 4 hours after influenza vaccination. The number of circulating IFN-activated myeloid DCs increased markedly when IFN was ad-mixed with influenza vaccine and the mixture injected im. Administration of recombinant IFNa by either the oro-mucosal or intraperitoneal routes also enhanced the humoral response to concomitant im influenza vaccination even though IFN was administered independently of the vaccine. Oro-mucosal administration of IFN a markedly increased both virus-specific IgG1 and IgG2a characteristic of a mixed Th1/Th2 type response, and secretory IgA associated with resistance to infection. The number of IFN-activated myeloid DCs detected in the peripheral circulation, when IFNa was administered concomitantly but at a site distant to the vaccine, was comparable to that observed when IFNa was ad-mixed with influenza vaccine, and the mixture injected im. Expression analysis showed that numerous IFN responsive genes were induced in the lymphoid tissue of IFN treated animals including chemokines, and chemokine receptors, suggesting that trafficking of APCs to the site of vaccination may explain in part the mechanism(s) of the adjuvant activity of IFNa.

EFFICACY OF A POLYEPITOPE VACCINE DEPENDING UPON THE CONFORMATION OF THE CHIMERIC ANTIGEN

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Polyepitope vaccine, which contains B and Th cell epitopes from several antigens, can be a promising strategy to cope with the problem of pathogenic variation in vaccine development. However, to arrange the tandem linking pattern of the epitopes for the best efficacy is still a hurdle in process. For the purpose to develop vaccine against erythrocyte stage malaria, we selected 14 B and Th cell epitopes from 8 key Plasmodium falciparum antigens and constructed chimeric antigens with them by the technology of epitope shuffling, with which we obtained DNA libraries containing abundant of the polyepitope genes all in different tandem linking sequence. The polyepitope antigen genes selected from the library shown different immunogenicity when being expressed as recombinant proteins and being innovated into rabbits. Obviously differences in immunogenicity were shown in the chimeric antigens which contained almost the same epitopes but in different tandem sequences. Moreover, the IgGs from serum of rabbits immunized with three chimeric antigens, respectively, all eliciting high immunogenicity, but showed different efficacy in the assay of the in vitro growth inhibition of the malaria parasites. The most interesting phenomenon we found was that different immune efficacies were shown in the assays of growth inhibition by using different IgGs against the chimeric antigen, M.RC-Ag1, which was expressed either in different expressional system or in the vectors with different fusion fragments at the upstream of the ORF. Our work demonstrated that the conformation of the chimeric antigen, viz. both the tandem linking sequence of the epitopes and the expressional system for the recombinant protein, is the key point to be considered when to design a polyepitope vaccine targeting mainly to the humeral immune responses.

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HOW TYPE 1 INTERFERON PROMOTES MACROPHAGE DEATH UPON INFECTION WITH *LISTERIA MONOCYTOGENES* OR EXPOSURE TO THE TOXIN LISTERIOLYSIN O

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Intracellular pathogenic bacteria can cause the death of their host's cells. The death of infected cells can be controlled by both the host and the bacteria. The bacteria may benefit from host cell death or find that this enhances the immune response against them. Identifying how this control is exerted, and what are the consequences of death, is important for understanding the immune response to such infections.

Listeria monocytogenes is a bacterium which enters and replicate in the cytoplasm of host cells such as resting macrophages. This eventually causes the death of the cell. We have previously observed that the interferon beta signal, produced by the macrophage upon infection, increases death of infected cells [1]. This is particularly interesting as type 1 interferon was shown to be detrimental during murine *in vivo* infection by *L. monocytogenes* [2]. To find an explanation for this phenomenon, we have characterised the type of cell death induced, and looked at the consequences of the interferon beta response for potential death pathways in the cell. We now suggest interferon beta's influence on the reactive oxygen and nitrogen species present during infection leads to increased death. This death is necrotic; caspase activation is also triggered but is not necessary for death.

We also are investigating the influence of interferon beta on the macrophage's response to listeriolysin O, the pore-forming toxin secreted by *L. monocytogenes* to escape the phagolysosome. This is a member of the family of cholesterol-dependent cytolysins, which are virulence factors for many pathogenic bacteria. Type 1 interferon is reported to sensitise lymphocytes to die upon treatment with exogenous listeriolysin O by an unknown mechanism [3]: we observe interferon beta also increases the susceptibility of macrophages to death upon listeriolysin O treatment. In contrast, interferon beta is protective for macrophages against typical apoptotic stimuli. We are therefore investigating how this sensitisation occurs. It will be interesting to examine whether this plays a role during intracellular infection, or may instead be important in infectious foci containing extracellular bacteria.

L. monocytogenes provides a well-defined infection system both *in vitro* and *in vivo* with which to study both the response of the immune system to bacteria able to colonise the cytoplasm, and the action of specific cytokines during such an infection. Mechanisms defined for this may be relevant to other bacterial infections.

1. Stockinger, S., et al., *Production of Type I IFN Sensitizes Macrophages to Cell Death Induced by Listeria monocytogenes.* J Immunol, 2002. **169**(11): p. 6522-6529.

2. O'Connell, R.M., et al., *Type I Interferon Production Enhances Susceptibility to Listeria monocytogenes Infection.* J. Exp. Med., 2004. **200**(4): p. 437-445.

3. Carrero, J.A., B. Calderon, and E.R. Unanue, *Type I Interferon Sensitizes Lymphocytes to Apoptosis and Reduces Resistance to Listeria Infection.* J. Exp. Med., 2004. **200**(4): p. 535-540.

GENERAL INFORMATION

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Hotel Caruso
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Emergency Numbers:
Taxi (Mr. Hauhs, Mr. Nemeth): Tel: +43-2252-790-001
European Emergency Number: 112
Fire: 122
Police: 133
Medical: 144

Transport to Vienna:
Badner Bahn (train) departs from Josefsplatz in Baden approx. every 15 minutes and arrives in front of the Opera in Central Vienna (approx. 60 min.).

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THURSDAY APRIL 12

12:00-14:00	Check In	
14:00-14:20	Alexander v. Gabain	Opening Statement

SESSION I: New Vaccines in the Light of Novel and Old Targets

14:20- 14:50	Rudolf Valenta	Allergy Vaccines
14:50-15:20	Martin Bachmann	Therapeutic Vaccination Against Chronic Disease: Clinical Experience
15:20- 15:40	Break	
15:40-16:10	John Shiver	Vaccines Against Staph. aureus
16:10-16:40	Cornelis Melief	Therapeutic Vaccine Against HPV
16:40-17:00	Tom Ottenhoff	Results from a First Clinical Safety and Immunogenicity Phase I Trial with a New Subunit Vaccine Against Tuberculosis
17:00-17:30	Antonio Lanzavecchia	Human Monoclonal Antibodies and Analytic Vaccinology
18:30-19:00		Opening of Poster Session

19:00 Get Together

FRIDAY APRIL 13

7:00- 9:00	Breakfast	

SESSION IIA: Vaccines in the Light of Microbial Exposure, Life Stage and Immune Status

9:00-9:30	Gordon Freeman	The PD-1:PD-1 Ligand Pathway
9:30-10:00	Carlo Ferrari	Role of the Cell-Mediated Immune Response in HCV Pathogenesis
10:00-10:30	Beatrix Grubeck-Loebenstein	Immunity and Aging
10:30-11:00	Break	
11:00-11:30	Paul-Henri Lambert	The Challenge of Early Life Vaccination
11:30-12:00	Philippe Sansonetti	Dilemma of the Oral Administration. A Case Study of a Shigella Vaccine
12:00-13:30	Lunch Break	

SESSION IIB: Vaccines in the Light of Microbial Exposure, Life Stage and Immune Status

13:30-14:00	Eric Oswald	Bacterial Sneak Attack: Subversion of the Host Cell Cycle
14:00-14:30	JoAnne L. Flynn	Dynamic Immune Responses in Tuberculosis
14:30-15:00	Arthur Reingold	The Population-Level Impact of Routine Infant Immunization with Pneumococcal Conjugate Vaccine in the U.S

19:30 Austrian Evening

SATURDAY APRIL 14

7:00-8:30	Breakfast	

SESSION III: Novel Vaccines in the Light of Markets and Social Implications

8:30-9:00	Mark Feinberg	Primary Prevention of Cervical Cancer with GARDSIL® (Quadrivalent Human Papillomavirus [HPV] Vaccine)
9:00-9:30	Beatrice De Vos	Case Study: Rotarix™ a Human Attenuated Oral Rotavirus Vaccine for Global Use
9:30-10:00	Break	
10:00-10:30	Noel Barrett	Development of a Cell-Culture Derived H5N1 Pandemic Influenza Vaccine
10:30-11:00	Erich Tauber	Japanese Encephalitis: A global Vaccine Approach for a Global Immunization Need
11:00-12:30	Lunch Break	

SESSION IV: Novel Vaccine Strategies in the Light of Advancing Technologies

12:30-13:00	Jan Holmgren	The Induction of Immunity or Tolerance – A challenge for Mucosal Vaccine Development
13:00-13:30	Sefik S. Alkan	Toll-like Receptor (TLR) Agonists and ZOT-Derived Peptides as Vaccine Adjuvants
13:30-14:00	Eszter Nagy	Monoclonal Antibodies, a New Paradigm in Infectious Diseases
14:00-14:30	Break	
14:30-15:00	Jay A. Berzofsky	IL-15 as a Vaccine Adjuvant to Induce Long-Lived, High Avidity Memory CD8+ T Cells, Even in CD4-Deficient Hosts
15:00-15:30	Walter Fiers	M2e-based Universal Influenza A Vaccine
15:30-16:00	Ron B. Moss	It's Prime Time for Clinical Trials of Plasmid DNA Vaccine.
16:00-16:30	Break	

PANEL: The Future of Vaccines in the Light of Commercial, Social and Disease Challenges

16:30-18:30	Jeffrey Almond, Alexander v. Gabain, Michael Greco, John Hodgson, Jacques-François Martin, Regina Rabinovich, Ray Spier, Mike Ward

19:00 Gala Dinner