ABSTRACTS

Opening Lectures

1. CURRENT STATUS AND NEW TRENDS IN DEVELOPING ALTERNATIVE METHODS FOR QUALITY CONTROL OF VACCINES

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Traditionally, animal models have played a significant role in quality control of vaccines. However, there now is a tendency to move away from these models. Underlying reasons are: increasing concern about the ethical consequences of laboratory animal use, high costs related to animal testing and safety aspects. Another element is the continuing search for models that better fit their needs. We have to be clear about animal models. Although their contribution to safeguarding vaccine’s release criteria has to be acknowledged, we also have to face the fact that animal experiments are not always perfect, both in terms of relevance and reliability.

Most of the current alternative strategies have focussed on modifying existing protocols to best cover reduction and/or refinement in animal use. Eye-striking developments in terms of regulatory acceptance have been the introduction of humane endpoints in animal tests which are characterised by high levels of pain and suffering, and the replacement of challenge procedures by serology-based procedures. Furthermore, when possible the use of simplified tests (e.g. single dose instead of multi-dose potency tests) have been proposed. All together this has resulted and will result in significant savings in animal numbers and limiting severity levels.

New developments now focus on introducing in vitro methods, either as replacement of individual animal tests or as part of a more generic change, being a paradigm shift in vaccine quality control; de consistency approach. The range of in vitro methods varies from tissue culture (safety/potency testing) to antigenicity tests using monoclonal antibodies (potency tests). In consistency testing, which aims to demonstrate quality consistency in the consecutive vaccine lots/batches produced, emphasis is given to the use of physicochemical and immunochemical tests.

This presentation will provide an overview of current 3R’s achievements and future prospects.

2. THERAPEUTIC VACCINES: CHANGING THE DOMINANT PARADIGM IN ADVANCED CANCER

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Cancer Immunotherapy has been the main focus of Biotechnology for more than 25 years. Monoclonal antibodies have made their way to Registration and clinical practice, but therapeutic cancer vaccines not yet. There are two main reasons for this apparent failure, both related to dominant paradigms: one is rooted in basic Immunology and the other in Clinical Oncology. First wave of cancer vaccine candidates tried to imitate anti-infectious vaccines mobilizing long-lasting responses to non-self epitopes. We know now that most of the natural antitumor immune response is directed to “self” epitopes, and that the immune response has been naturally selected for short duration and contraction. Additionally, the clinical testing of cancer vaccines occurred under the dominant paradigm for evaluating cytotoxic antitumor drugs: Initial trials in advanced disease, looking for fast shrinkage of the tumors, in order to move then to the adjuvant setting with lower tumor burden, looking for cures. This is probably not the right strategy. Although there is a trend to increasing survival times in many cancers and mortality rates are showing plateau, roughly half of patients with a cancer diagnosis will reach the advanced cancer stage at some time.

Advanced cancer, as many other chronic non-communicable diseases of the post-reproductive life, can not be “cured”. Long term “control” is the attainable goal to seek, but chronic cancer control would imply chronic, relatively non-toxic, treatment. Some recent results of cancer immunotherapy suggest that this could be possible: Monoclonal antibodies are showing survival benefit in long term use, even after tumor progression. Cancer vaccines can be given for long periods without evidence of cumulative toxicities, and continue to expand specific immune response. Chronic immunotherapy will require some kind of combined intervention on the negative feedback loops that are activated after initial vaccination.

The cancer vaccine program of the Centre of Molecular Immunology (currently including one recently registered vaccine and three other in POC trials) is also accumulating evidence of chronic and combined immunotherapy. If these evidence continues to accumulate, they could drive a major switch in the therapeutic paradigm for advanced cancer.
Current potency and safety tests and alternatives for the evaluation of vaccines

1. ALTERNATIVE APPROACHES IN POTENCY TESTING OF DIPHTHERIA AND TETANUS VACCINES

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Diphtheria and tetanus vaccines, manufactured by traditional formaldehyde inactivated toxin, are key components in all current childhood combinations and provide a backbone for combinations intended for boosting of adults and adolescent. More than ten different combinations, produced by several manufactures are currently licensed in Europe, containing either high of reduced dose of toxoids. It is acknowledged that by far the largest proportion of laboratory animals are used for potency testing of these vaccines, in many countries still by direct challenge methods.

In this presentation I will summarise activities at NIBSC which contributed to validation studies supported by the Council of Europe and the European Commission and provide an example of successful introduction of serological potency method in house for a particular product. Ethical as well as economical and scientific benefits of the methodology transfer will be presented.

In addition to studies focusing on refinement of animal procedures for potency, it is clear that other entirely in vitro methods can also be used to provide valuable information on diphtheria and tetanus vaccines. In particular the antigen assay is an excellent tool to characterise vaccines, monitor trends in production process and provide data in support of consistency. Such an approach can also be used to monitor stability over time. For well established vaccines, the antigen assay could be used in support of reduced potency testing schemes encouraged within European OMCLs, without loss of important data on product consistency. Many animals could be saved if such an approach is considered and adopted more widely within National Control Laboratories.

2. SAFETY TESTING FOR BACTERIAL TOXINS: AN OVERVIEW

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Bacterial toxins such as botulinum neurotoxins are some of the most poisonous naturally occurring substances known to man. Although botulinum toxin is highly toxic it is administered safely in extremely small doses to treat painful muscle spasms and involuntary eye muscle contractions. It is also increasingly used for cosmetic purposes. Every batch of therapeutic product containing this toxin is tested in animals, usually by LD50 method. Traditional toxoid vaccines such as diphtheria and tetanus are produced by chemical inactivation process. Safety testing therefore forms an essential part of quality control, ensuring that product used in human vaccine is free from active toxin and cannot revere to toxicity. In this presentation I will summarise current state of knowledge and strategies applied and considered as replacement methods for testing of bacterial toxins. In particular how scientific understanding on the toxin mode of action, combined with desire to explore new technologies have led to development of an array of potential alternatives. However, scientific knowledge is still limited. It is acknowledged that all assays, including those using human cells and functional end points, will have some limitations, particularly in extrapolation to human use. In view of this, pragmatic solutions are adopted, whereby in considering a method for validation account should be taken of the strengths and weakness of the proposed approach as well as its relevance and suitability for the intended purpose and use. It is generally agreed that suitable batch release assays must have high precision, robustness and transferability to be useful in monitoring accurately active component in production lots and to confirm consistency. Examples of methods developed and adopted for several bacterial toxins will be presented.

3. CURRENT POTENCY AND SAFETY TESTS AND ALTERNATIVES FOR THE EVALUATION OF VACCINES: THE EUROPEAN PHARMACOPOEIA APPROACH

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Since the opening for signature of the European Convention for the Protection of Animals Used for Experimental and Other Scientific Purposes in 1986, the European Pharmacopoeia has carried out and is pursuing a programme of work committed to replacing, reducing and refining the use of animals for monograph requirements. This activity involves a large extent monographs on vaccines for human and veterinary use. The achievements of the last years and current activities are the consequence of the dedication of all the players in this exercise and the excellence of the relationship between EDQM and all its European or non-European partners. After a brief introduction on the regulatory environment for vaccines in Europe, the presentation will provide information on recent achievements and current European Pharmacopoeia activities in the field.

4. ALTERNATIVE METHODS FOR DETERMINING POTENCY OF HUMAN RABIES VACCINES

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Introduction

The potency of rabies virus vaccines is generally determined in vivo by the NIH challenge test as recommended by WHO Expert Committee on Rabies. The replacement of the in vivo potency test for Rabies vaccine by in vitro methods is at the moment a hot point discussed in many reports. Single radial immunodiffusion assay and enzyme-linked immunosorbent assay (ELISA) are some of the alternatives proposed. The aim of this work is to provide an overview on the state-of-the art of 3Rs alternatives for replacing the challenge test for human Rabies vaccines as well as to show some own experiences in this field. Materials and Methods

The overview will provide information of the current alternatives that are being evaluated worldwide and its regulatory acceptance. At the same time, we will share our experience with single radial immunodiffusion assay using neutralizing monoclonal antibodies and ELISA. Results

The results and suitability of the different methods for assessing the potency of rabies vaccine will be discussed.

5. 3RS: WOULD A VALIDATED IN VITRO ELISA ASSAY BE A SUITABLE ALTERNATIVE METHOD TO THE IN VIVO IMMUNOGENICITY ASSAY FOR HEPATITIS A VACCINES?

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The 3Rs rules for the replacement, reduction and refinement of the use of laboratory animal testing are now widely recognised as a need for ethical and economical reasons by the global scientific community including manufacturers and National Regulatory Authorities. Since many years Afssaps control strategy combines implementation of in vivo tests reducing strategies as well as the development of in vitro methods to monitor the consistency of production of vaccines batches. For Hepatitis A vaccines, in order to fulfil the 3Rs recommendations, a study was carried out to assess the feasibility of replacing the in vivo potency assay (mice immunogenicity assay) by the Afssaps’ validated in vitro assay (antigen content by ELISA) (Biologicals 2000, 28, 247-256) on routine testing.

A panel of vaccines (18 batches from three manufacturers) ranging from 200 to 24 IU/mL was included in this study as well as sub-potent (up to 4 IU/ml) vaccine batches (heated to +56°C). Hence, twenty two samples were tested both by the in vivo and the in vitro assays.

This study established that both loss of antigen content and neutralising antibodies detection were proportional to the heating incubation time of the vaccine samples. Moreover, a relatively strong correlation between both methods was demonstrated (r=0.895). Finally, all the batches declared compliant by the in vivo assay passed the in vitro test (in-house specification). In the same way, all the vaccine batches drastically altered by heating were declared non-compliant by both the in vivo and the in vitro assays. These results could be explained by the use of a monoclonal antibody in the in vitro assay which neutralises the immuno-dominant epitope involved in the induction of neutralising antibodies. Moreover, it was demonstrated that this antibody is able to compete with the human polyclonal neutralising antibodies used to detect the neutralising antibodies included in the immunogenicity assay.

In conclusion, this study allowed us to demonstrate that a correlation does exist between the two titration methods and encourage us to progressively switch from the in vivo to the in vitro assay.
6. WHOLE CELL PERTUSSIS VACCINE POTENCY TESTING BY ALTERNATIVE METHODS TO KENDRICK’S TEST

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Whole cell pertussis (wP) vaccines are widely used as combinations with diphtheria and tetanus toxoids, but also with inactivated polio viruses, hepatitis B and/or Haemophilus influenzae glycoconjugate. Most regulatory agencies require the mouse protection test (MPT) or Kendrick test for lot release potency testing. Due to the large variability in test results and the severe distress inflicted on the many animals involved, an alternative method is highly desirable. The pertussis serological potency test (PSPT) initially developed in mice was transferred to guinea pigs (gps) with the final goal to use the same animal series for diphtheria and tetanus potencies testing. The present study, financially supported by ECVAM, evaluated two features of the serological response to wP vaccination: 1) the overall antibody response as measured by whole cell ELISA (PSPT-wC-ELISA), 2) the functional neutralizing antibodies to pertussis toxin (PT, one of the main bacterial virulence factors) as measured by the Chinese hamster ovary (CHO) cell assay.

The results showed that 1) the gp model can be used for wP lot release potency testing, 2) comparable potencies were obtained in MPT and in the PSPT-wC-ELISA, 3) despite good repeatability and precision, the CHO cell assay did not generate results comparable to MPT and showed different ability of wP vaccines to induce neutralizing anti-PT antibodies. The same gps generated a good dose-response curve for diphtheria and tetanus components. To date, the PSPT-wC-ELISA appears as a promising approach for batch release potency testing of wP vaccines for which consistency in production has previously been demonstrated by the MPT. As a follow-up of this work, a collaborative study for the validation of the PSPT-ELISA will be run under the aegis of the Biological Standardisation Programme (Council of Europe/European Commission).

7. NOVEL IN VITRO TEST FOR TOXICITY OF PERTUSSIS TOXIN

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Introduction: Pertussis is caused by the Gram-negative bacterium Bordetella pertussis. One of the most eminent virulence factors is Pertussis Toxin (PTx), an upmost important antigen for immunisation via so called Acellular Pertussis Vaccines (ACV). To guarantee non-hazardous application for all recipients, detoxified pertussis toxin (PTd) has to be used. Successful detoxification of PTd has to be monitored for the absence of residual active toxin. The traditional procedure, demanded by regulatory authorities, to determine toxicity is animal testing. This lethal challenge test causes high resources of animal and provides inconsistencies, which make repetitions inevitable. Due to that an alternative testing method has to be developed.

Material and Method: Inside the cell PTx transfers ADP-Ribose onto inhibitory G-Protein and interferes thereby in the signal transduction pathway, which leads to an increase of cAMP on the one hand and decrease of ATP on the other hand. We implemented decreasing ATP levels caused by PTx as indicator for the activity of the latter. Results: We investigate a PTx-cell assay with human peripheral blood mononuclear cells (PBMC), which are incubated with PTx. After incubation for one, five and 24 hours the somatic ATP level of the cells is measured. PTx causes a dose and time related decrease of ATP level. In contrast, heat-inactivated PTx is not able to induce this kind of reaction. Conclusion: For the estimation of residual active PTx a cell assay involving the respective ATP status is to be developed. This test is similar to the in vivo situation of the PTx effect inside the cells of the human host and shows extremely encouraging results.

8. ALTERNATIVE PYROGEN TESTING – SCIENTIFIC, PRAGMATIC AND REGULATORY STATE

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Pyrogens, i.e. fever inducing substances, in parenteral drugs may affect patients up to life-threatening consequences. The Rabbit Pyrogen Test is the standard animal test regulated in the Pharmacopoeias since more than 50 years. Various efforts were made during the last decade to develop alternative pyrogen test principles. The common basis of these alternative tests
is the employment of human monocytes which become activated after contact with pyrogens (endotoxins as well as non-endotoxin pyrogens, NEPs). The chosen sources for monocytes are human whole blood, human peripheral blood mononuclear cells or monocytic cell lines. Nevertheless, the application in practice and, especially, commercialisation of these tests would have posed problems, mainly regarding the availability of standardised monocyte sources on an industrial level. The most practical as well as economical solution is the use of cryo-preserved human whole blood.

Despite the ethical achievement of replacement of the animal experiment Rabbit Pyrogen Test, the alternatives allow pyrogen testing of novel medicines which cannot be analysed by the established procedures, i.e. the Advanced Therapy Medicinal Products (ATMPs, cell therapeutics, gene therapeutics etc.). For illustration, injection of a cell therapeutic of human origin into a rabbit may lead to false positive results; endotoxin may be bound to the surface of the therapeutic cells and is not detectable by Limulus test leading to false negative results. So, novel Alternative Tests represent a new dimension in pyrogen testing of medicinal products.

9. NOVEL APPROACHES TO THE CHARACTERISATION OF BIOLOGICAL MEDICINES; FROM HUMAN ES CELL BASED ASSAYS TO STRUCTURAL TEM

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As biological medicines develop so do the challenges of ensuring their safety. Today’s emerging and postulated therapies present an ever moving target with greater demands on basic research, control and standardisation. NIBSC is developing cell based models and novel approaches to the characterisation of biological medicines. These include the use of differentiated human embryonic stem (hES) cells and advanced imaging technologies. Much of the current methodology for drug screening relies on animal modeling, or cultures of primary animal cells. Although primary human cells can be used as a source of functional cells for assays, it is generally agreed that using cells derived from cell lines as effector cells is more convenient, reproducible. In order to address these issues, two complex bioassay models, requiring differentiation and specific cell function, have been investigated [an opsonophagocytic killing assay (OPKA) and a neuronal response assay]. Research has focused on the differentiation of continuous cell lines to neutrophilic fate and the differentiation of pluripotent hES cells into a neuronal fate, potentially removing the need for animal testing and bringing the drug/disease screening one step closer to the intended recipient. Hurdles exist to the production of large numbers of functional neurons. These include; purity of the differentiated cells population and control over the specific neuronal cell fate from a pluripotent cell source. A further challenge is to the end point for bioassay [e.g., functional response (uptake and release of Noradrenaline) or change in electrophysiological response]. For characterisation of biological vaccines, we are developing our use of Cryo-TEM/structural-TEM and are reviewing its increasing role in biopharmaceutical product evaluation. The strengths and weaknesses of these techniques for routine analysis of biopharmaceuticals are not well described. In addition, the practicality of single particle analysis techniques and/or tomography to biological control requires careful evaluation. NIBSC is seeking to develop these novel methods initially in support of its research (e.g., application of tomography to 3D analysis of pleomorphic/irregular biological products and single particle analysis of regular proteins and viruses). This will allow NIBSC to develop the necessary skills and expertise to apply these techniques for quality control of new vaccines and other biologicals as well as being able to critically assess the data provided by industry.

10. COMBINED VACCINES – SINGLE ANIMAL APPROACH FOR SEVERAL ANTIGENS

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Due to ethical reasons, workload and costs by animal use OMCLs are interested in replacing the in vivo challenge potency assays for vaccines by refined methods and to reduce the use of animals by the replacement of the current multiple dose assay by a single dose assay whenever possible. A serological method may offer quantification of various vaccine components in the same animals, which would further reduce the number of animals needed. Before an alternative method for potency determination of multiple components can be introduced in Ph. Eur., it is necessary to demonstrate that the dose-response curves give a useful regression in the range of doses to be tested for each of the components simultaneously. This has been shown to be obtainable for a wide range of combined vaccines on the European market for the Diphtheria (BSP034), Tetanus (BSP035) and for the acellular Pertussis component (BSP083-to be published). Results from these studies together with preliminary results from hepatitis B vaccine serology will be presented. The condition of similarity of dose-response curves (test vs. standard) has to be fulfilled, even in the case of a single dose assay. Since the single dose assay does not allow for tests of linearity and/or parallelism, these properties have to be demonstrated in multiple dose assays. It may not always be
According to the European Pharmacopoeia the potency of diphtheria and tetanus vaccines for human use may be assayed by guinea pig serology for the routine test of batches. The suitability of the method has to be verified for the specific product. For combined vaccines serology can be performed using the same group of animals for diphtheria and tetanus components respectively. As various vaccine combinations are consisting of acellular pertussis (aP) antigens as well, investigations were done whether the immunogenicity of the aP components could be assayed in the same guinea pig sera as for the d and t components.

The practicability as well as pros and cons resulting from a guinea pig serological method for batch release testing of combined DTaP vaccines are content of the lecture.

11. SEROLOGICAL METHODS FOR TETANUS, DIPHTHERIA AND AP ANTIGENS IN A GUINEA PIG MODEL: 3 IN 1

Ute Roskopf-Streicher
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According to the European Pharmacopoeia the potency of diphtheria and tetanus vaccines for human use may be assayed by guinea pig serology for the routine test of batches. The suitability of the method has to be verified for the specific product. For combined vaccines serology can be performed using the same group of animals for diphtheria and tetanus components respectively. As various vaccine combinations are consisting of acellular pertussis (aP) antigens as well, investigations were done whether the immunogenicity of the aP components could be assayed in the same guinea pig sera as for the d and t components.

The practicability as well as pros and cons resulting from a guinea pig serological method for batch release testing of combined DTaP vaccines are content of the lecture.

12. DEVELOPMENT OF A SEROLOGICAL TEST FOR DIPHTHERIA VACCINES POTENCY BASED ON AN NIH/FDA APPROACH

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Introduction: Serological tests replacing the traditional challenge tests for Diphtheria and Tetanus vaccines Potency have been already accepted and introduced by WHO and the European Pharmacopoeia. However, developing countries from Latin America still use an in vivo neutralization test in which the anti - diphtheria antibodies are titrated on the skin of guinea-pigs. This method has demonstrated its consistency and correlation wit clinical results in United States; however it has many disadvantages in terms of variability, no use of a vaccine reference or control and ethical aspects. Besides, the availability of guinea-pigs is today a serious problem. That’s why a serological alternative becomes not only possible but necessary. Materials and methods: Groups of 10 mice (OF1) were immunized with 0.5 mL of vaccines containing Diphtheria Toxoid, bled after 28 days and sera were titrated against an in house anti-diphtheria hyperimmune serum by an ELISA. Test conditions (dose-response range, optimal concentrations of conjugate and coating) were determined. It was evaluated the precision of the whole assay. A correlation study was performed regarding the in vivo neutralization test. It was also estimated the behaviour of Diphtheria potency in presence of other antigens (Tetanus and whole-cell Pertussis). Results: All test conditions were established to assure a consistent performance of our method. The coefficient of variation intra and inter-assays was always lower than 20%. We obtained a good correlation between our serological method and the in vivo neutralisation test. The serological method was even able to discriminate much better between samples of similar biological activity than the in vivo test. There was no significant difference between Diphtheria potency in DT and DPT vaccines. Conclusions: This work is an important part of our strategy focused on replacing the NIH methods used in Latin America for the potency of Diphtheria and Tetanus by serological tests.

Veterinary Vaccines

13. ELISA FOR POTENCY TEST OF INACTIVATED ERYSIPelas VACCINE: PRACTICAL USE FOR BATCH RELEASE

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Inactivated erysipelas vaccine is one amongst 15 different products to be found on the “short” list of IVMPs for which a restricted test list for Official Medicinal Control Authorities has been agreed upon. With the revision of the European Pharmacopoeia monograph 0064 in 2004 the batch potency test of inactivated erysipelas vaccines can be performed by determining antibody levels using an immunochemical method like ELISA. The vaccine under examination is compared with an approved reference batch that yielded satisfactory results in the test described under potency.
14. MULTIDIMENSIONAL APPROACHES FOR GENERATION OF VACCINE CANDIDATES AGAINST A CUBAN ISOLATE OF RABBIT HEMORRHAGIC DISEASE VIRUS

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Rabbit Hemorrhagic Disease Virus (RHDV) is the etiological agent of a fatal disease that causes the decline of domestic and wild rabbit populations worldwide. The use of available inactivated vaccines for its prevention has inconveniences related to safety and existence of a highly pathogenic subtype (RHDVa). Here we describe the isolation and characterization of a Cuban isolate of RHDV obtained during the last epizootic. We developed different strategies for generation of vaccine candidates against RHDV (also against RHDVa), based on recombinant expression of the VP60 viral capsid protein. The first of these strategies consisted in the construction of a recombinant adenovirus that mediated in vivo and in vitro VP60 expression in mammalian cells. As second and third approaches we obtained two Pichia pastoris-derived VP60 multimeric structures formed by soluble and insoluble variants, differing in their antigenic profile. Expression levels were in the range of 100 mg to 1.5 grams per liter of culture. The recombinant protein variants were recognized by monoclonal antibodies directed to RHDV conformational epitopes. An immunization trial was conducted to investigate on the advantages or limitations of these antigens for vaccination. The earliest IgG response, titers and persistence of antibodies were determined by indirect ELISA, while their protective capacity against the two viral subtypes was evaluated by hemaglutination inhibition assay and competition ELISA. Rabbits vaccinated with the recombinant adenovirus or with the yeast-derived proteins developed an early and long lasting RHDV/RHDVa-specific immune response. However, the strongest IgG levels were found in the group immunized with the soluble variant of the antigen from P. pastoris, with HI titers up to 1/40960. The response in this group lasted up to two years after the primary immunization. The response in most cases was similar to that induced by VP60 from SF9 cells and superior to that obtained with inactivated RHDV.

15. IMMUNOGENICITY, SAFETY AND EFFICACY OF GOAT MILK DERIVED E2-MARKER VACCINE AGAINST CLASSICAL SWINE FEVER

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The last decades have been marked by the emergence and reemergence of severe swine diseases. In spite of this classical swine fever (CSF) remains either as the worst threat or sanitary problem affecting swine production at world wide level. CSF is a highly contagious and often fatal disease which control is based on either effective vaccination or rigorous stamping out policies, both with severe economic implications. In this work, the E2–CSF virus recombinant glycoprotein produced in the milk of goats was applied in a series of trials to assess immunogenicity, safety, transference of maternal immunity and protective efficacy. E2-vaccine applied in pigs intramuscularly challenged with 105 LD50 of a CSF pathogen strain, survived and only show mild clinical signs, while controls died or suffered severe disease. The immunization schedule, boosting at 21 days the prime vaccination, protected pigs for 9 month; which is higher than the growing period in finished pig. The vaccination of pregnant sows show not adverse effects and the 100% of its offspring had maternal derived immunity (MDI) over the levels of protection (>1:50) for 8 weeks. The progeny vaccinated in presence of MDI elicited a consistent antibody response. This fact is highly desired because it is an important limitation of live vaccines. Considering the early establishment of protection and the ability for inducing immune response in the presence of MDI, with the evaluated E2 vaccine, could be concluded that it is a well-built tool for controlling and eradicating CSF. In addition the structural nature of this vaccine constitutes a marker allowing differentiate vaccinated animals from infected one.
### 16. DEVELOPMENT AND CHARACTERIZATION OF A VACCINE CANDIDATE AGAINST CLASSICAL SWINE FEVER, BASED ON THE E2-CSFV GLYCOPROTEIN PRODUCED IN THE MILK OF NON TRANSGENIC GOATS

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Classical swine fever virus (CSFV) is the etiological agent of the most economically important highly contagious disease of swine worldwide. E2 is the major envelope glycoprotein present as a homodimer on the outer surface of the virus and represents an important target for the induction of neutralizing immune response against the viral infection. In this work the E2 extracellular domain was expressed in the milk of adenoviral transduced goats and its immunogenicity and protective capacity was assessed in a set of vaccination and challenge trials. We found that the vaccine candidate based on milk-derived E2 is able to induce high titers of neutralizing antibodies in pigs, and confer protection from both clinical signs and viral infection after challenge with 10^5 LD_{50} of the high pathogenic CSFV, since 15 up to at least 9 months post-immunization. Vaccinated pigs challenged only one week after a single immunization showed a partial protection level which was noticeably improved after the incorporation of an immunestimulating product in the vaccine formulation. Contrasting with vaccine based on C-strain, the water in oil formulation containing the milk-derived E2 supported conditions of thermal stress without evident alterations of its immunogenicity and protective capacity. Additionally, the vaccination of pregnant sows allowed conferring protective antibody levels to the newborn piglets up to about seven weeks after birth. Taken together, these results demonstrate the efficacy of the milk-derived E2 as vaccine antigen and highlight the advantages of using the mammary gland as bioreactor for the production of complex glycoproteins for veterinary purposes.

### 17. OFFICIAL CONTROL AUTHORITY BATCH RELEASE OF RABIES INACTIVATED VACCINES

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In France, rabies inactivated vaccines for veterinary use are controlled for potency following the recommendations of the European Pharmacopoeia monograph 0451. The method used directly derives from the NIH test (National Institutes of Health). This in-vivo technique consists in a single intra-peritoneal vaccination of groups of mice with several dilutions of test vaccine at D0 followed by an intra-cerebral administration of a single dose of CVS virus at D14. Rabies inactivated vaccines for veterinary use are registered on a short list of Immunological Veterinary Medicinal Products (IVMP’s) which are subjected to the Official Control Authorities Batch Release (OCABR) within the European Community as laid down in article 82 of directive 2001/82/EC amended by directive 2004/28/EC. These OCABR consist in the control of the IVMPs by an OMCL (Official Medicines Control Laboratory) before the product is commercialized. The result of this control shall be recognized by all member states, so that to avoid unnecessary duplicated tests. Since the 1st of January 2009, this OCABR system has been adopted by the French Authority for rabies vaccines.

### 18. IN SILICO PRIORIZATION OF VACCINE AND DIAGNOSTIC TARGETS IN MYCOPLASMA GALLISEPTICUM

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Mycoplasma gallisepticum (MG) is a major avian pathogen which causes chronic respiratory disease in chickens, leading to decreased productivity in both meat and egg. One crucial, initial step for the establishment of the disease is the adhesion of MG to its target cells that is achieved by lipoproteins localized on its surface. Data indicate that several cytadhesion or related molecules, that are anchored and/or exposed in mycoplasma surface, have yet to be identified. The identification of these antigens is of major importance to understand the mycoplasma pathogenic mechanisms and crucial for vaccine development, but experimentally it involves an extensive trial and error system. The new genomic technologies together with bioinformatics offer tremendous opportunities in vaccine developmental research. The sequencing of MG genome, opened the gates to extensively explore the whole genome derived proteomes of this mycoplasma. A computational algorithm that combine programs
or web resources for protein cellular localization based on different criteria (PROB, PsortDB, COG, SignalP), as well as its antigenicity (Antigenic), was used. After being normalized, the partial results were weighted according to the capacity of each program of identifying previously known MG surface proteins and then, all these values were added and a prioritization list was created. To define in each selected antigen the optimal region to be cloned, the most antigenic and with highest probability of being cell surface exposed segments were identified with the use of mentioned Antigenic and the programs Marcoil and TMHMM. Further studies have to be done to evaluate the validity of our predictions.

Poster Session

19. AN APPROACH TO QUALITY RISK MANAGEMENT BASED ON A FMECA (FAILURE MODE, EFFECTS AND CRITICALITY ANALYSIS): GENERAL CONCEPT AND PRACTICAL APPLICATION BY A NATIONAL CONTROL LABORATORY

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As a National Control Laboratory (NCL), the Agence Française de Sécurité Sanitaire des Produits de Santé (AFSSAPS) and especially the Laboratories and Control Directorate must assure the reliability of the controls and of the decision to release a lot of biological product. Therefore a methodology for risk management based on the ICH Q9 guideline and a FMECA (Failure Mode, Effects and Criticality Analysis) has been developed on the lot release process. This methodology is for routine testing and mainly to anticipate controls for particular medicines with public health issues or in emergency situation as a bioterrorism or pandemic context. Indeed, it will demonstrate the anticipation and the control of all the potential risks in testing samples during stressful circumstances.

Both the proposed methodology and a concrete application on the influenza vaccine will be presented. This vaccine was chosen because it is subject to batch release (routine testing) with specificities (seasonal composition, huge number of lots, short time between control and administration). Moreover, the context is quite comparable, in terms of controls, to an emergency situation.

From a detailed description of the batch release process, a small working group has identified 84 potential hazards. A wider group of people prioritized these hazards with the FMECA tool. No major risk was identified. Nevertheless, it was decided to implement 2 preventive actions on the 2 higher risks. Finally, it was demonstrated that all the potential inherent risks relative to the Afssaps' influenza vaccine batch release process were under control.

In conclusion, this methodology has many major interests for a NCL. As main example, it represents a formalization of risk management and can be regarded as a major asset during internal or external audits (National, European and/or International). Finally, it guarantees the reliability of control process and therefore the protection of the patient.

20. THE THREE Rs (REPLACEMENT, REDUCTION, REFINEMENT): INTEREST AND APPLICATION TO VACCINES RELEASED BY A NATIONAL CONTROL AUTHORITY

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The 3Rs concept (Replacement, Reduction, Refinement of animal experiments) launched in 1959 aims at reducing and optimizing in vivo assays performed in health domain, as well as in many other fields. Because of their specificity (biological nature and administered to healthy people and mainly children) regulatory batch release requirements pertaining to vaccines have been established at both European (Directive of the European Commission) and international level (World Health Organisation for UNICEF and other vaccination programmes). According to this procedure, both tests in laboratories and critical review of each Lot Summary Protocol must be performed by a National Control Authority (NCA) to check vaccines batch quality and safety.

As the French National Control Laboratory (NCL), the Agence Française de Sécurité Sanitaire des Produits de Santé (Afssaps, French Health Products Agency) and more specifically the Laboratories and Control Directorate (DLC) is involved in this quality control system. As part of vaccines batch release process, the use of animals is sometimes essential for the vaccines tests in spite of efforts made to reduce animal experimentation.

Since many years, Afssaps (DLC) has taken different measures to set up the 3Rs concept. First the DLC has developed a lot of
alternative methods to animals (Replacement) and particularly an *in vitro* ELISA assay as alternative method to the *in vivo* immunogenicity assay for Hepatitis A vaccines (Oral communication by B.Poirier, VaccinPharma 2009, May 2009, Cuba). DLC also performed many studies, based on European and WHO guidelines, in order to reduce and optimize the number of animals used for vaccines batch release (Reduction). These reducing procedures (for example switching from a multiple-dilution assay to a single-dilution assay) save more than 650 guinea-pigs and 3500 mice per year. Finally many measures have been taken in the laboratories to apply the concept of “Refinement”.

21. SEROLOGICAL ASSAY FOR DIPHTHERIA AND TETANUS POTENCY IN DTaP-IPV-HIB VACCINE

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**Introduction:** Sanofi Pasteur Limited has developed and implemented in collaboration with NIBSC and BGTD, a serological assay for batch release potency testing of diphtheria and tetanus toxoids in DTaP-IPV-HIB pediatric vaccine formulation as per the Ph. Eur. Requirements. **Materials and Methods:** Guinea pigs were immunized with multiple doses of either an In-house homologous Reference vaccine or a Production lot. The resulting sera were tested in validated ELISA methods for antibody to diphtheria and tetanus toxoids. Results were entered into a multi-dose parallel-line model calculation program or a t-test based single dose potency calculation program to obtain a potency estimate. **Results:** Six routinely manufactured lots of DTaP-IPV-HIB combination vaccine were included in the verification studies. A three-dilution (1/1.67, 1/5 and 1/15) serological assay for diphtheria and tetanus potency provided similar results to the lethal challenge assay and showed good linearity and parallelism. A single dose potency assay was shown to be suitable for tetanus potency determination as an alternative to the multi-dose assay. **Conclusion:** The serological assay allows for the use of the same group of animals for testing the potency of both diphtheria and tetanus components, thereby significantly reducing the number of animals required for testing and resulting in a more humane endpoint. Additional benefits of this assay include reduction in the invalidity rate, reduction in cycle time and significant cost savings.

22. THE EFFECT OF RECOMBINANT LAMININ-BINDING PROTEIN AS CANDIDATE VACCINE AGAINST CLEARANCE OF STREPTOCOCCUS PYOGENES M1+ 90226 FROM INTRANASALLY INFECTED BALB/C MICE

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**Background:** Laminin-binding protein (Lbp) is a surface protein of *Streptococcus pyogenes* that plays a role in adhesion and specifically interacts with human laminin. Interaction between Lbp and laminin can be used as a target for vaccine development against *S. pyogenes* infection. This research was intended to evaluate the ability of purified recombinant Lbp of M12 strain for enhancing clearance of M1+ 90226 *S. pyogenes* from intranasally infected mice. **Materials and Method:** Overproduction of Lbp was done in *Escherichia coli* BL21, purification was performed using affinity chromatography, characterization of Lbp was conducted using Sodium Dodecyl Sulphate Polyacrylamid Gel Electrophoresis (SDS-PAGE), Dot blot and Western blot. The ability of recombinant Lbp of M12 on the clearance of *S. pyogenes* from BALB/c mice infected intranasally was determined by immunization of Lbp intraperitoneally. As a negative control, BALB/c mice was immunized with tetanus toxoid (TT) intraperitoneally. The clearance ability of Lbp was determined by counting the number of á hemolytic colony in nasal secrete at 5 h (T5), 10 h (T10), 24 h (T24) and 30 h (T30) after challenging with M1+ 90226 *S. pyogenes* at a dose of 9.01 x 10^8 CFU/mL. The presence of anti Lbp sIgA on nasal swab and anti Lbp IgG in mices sera were determined by Dot blot and Western blot analysis. **Results:** Overproduction and purification of Lbp were successfully done and Lbp was characterized as a protein of 32.93 kDa and recognized by rabbit antibody anti Lbp. On clearance test, result showed that at T5 the á hemolytic colony decreased significantly (p=0.003) from intranasal secrete of Lbp immunized mice (48.3±17.6) CFU/mL compared to that of non-immunized mice (581.7±45.4) CFU/mL and TT immunized mice (483.3±59.7) CFU/mL. The similar result, obtained for T10 h after challenging. However, at T24 and T30 h after challenging the á hemolytic colony was not found in nasal secrete. Anti Lbp sIgA in nasal swab was not detected by Dot blot analysis, however anti Lbp IgG in sera mice was recognized by purified Lbp with low intensity using Western blot analysis. **Conclusion:** This research indicated that intraperitoneally immunization of 5 µg of recombinant Lbp from M12 strain could eliminate significantly M1+ 90226 *S. pyogenes* from nasal secrete. This preliminary results can be used as a basis to develop Lbp as a universal candidate vaccine.
23. IN VITRO NEUTRALIZATION ASSAY TO EVALUATE NEW HAV VACCINE CANDIDATES

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Introduction: The availability of cheaper vaccines against hepatitis A virus (HAV) could help to control this disease in developing countries. Neutralization assays are essential to evaluate new candidates since neutralizing antibodies are of primary importance in protection against HAV. In this work, was developed an in vitro neutralization assay to evaluate HAV vaccine candidates, obtained in our laboratory, using phage–display technology. Material and Methods: A viral stock of VHA cytopathic clone was prepared and titrated. 7E7 anti-VHA Monoclonal antibody (UI/mg) and anti-VHA human serum (314000 mUI/mL) were the positive controls. A human serum (<15 mUI/mL) and a Negative Ascitic Fluid, the negative controls. Pre-immune and sera from mice immunised with wild type phage and phages displaying VHA mimotopes were evaluated too. An HAV cytopathic inhibition assay for anti-HAV neutralizing antibodies was followed. 1130 mUI/mL of Monoclonal antibody and 2 fold dilutions of sera were incubated with 10³ and 10² TCID50 of HAV and inoculated in 96 well plaques with FRhK4 cells. After 7 days of incubation neutralizing titre was determined as reciprocal of the highest serum dilution reducing HAV growth (inhibition of CPE) by 50%. 10³, 10², 10, 1 and 0 TCID50 were included as viral controls. Results: 1130 mUI/mL of Monoclonal antibody and human sera positive (until 1226.5 mUI/mL) neutralized 10³ and 10² TCID50. Sera from mice immunized with phages displaying VHA mimotopes had neutralizing titres between 4 – 16. Neither negative controls nor pre-immune and sera from mice immunized with wild type phage neutralized HAV. Conclusions: An in vitro neutralization assay was developed to evaluate new HAV vaccine candidates.

24. VALIDATION OF ANALYTICAL METHODS FOR POLYSACCHARIDES A AND C FROM MENINGOCOCCAL VACCINES


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Introduction: In the biopharmaceutical industry the validation of the analytical methods has vital importance for the safety and reliability of the medications. Validation is a process that ensures the quality designed for a product. The aim of this work was to validate physico-chemical tests used for lot release of Polysaccharides A and C from Meningococcal vaccines, specifically the determination of Phosphorus, syalic acid O-acetyl and protein. Materials and Methods: The validation parameters evaluated were Precision, Accuracy, Specificity, Linearity, Limit of Detection and Quantification, depending on the purpose of each test under study. The results obtained were analyzed by using the traditional statistical approaches (program Statgraphics plus for Window 5.1). Results: It was demonstrated that all the evaluated methods fulfilled the parameters required according to the foreseen results. Conclusions: Validated quality control tests are a guarantee for assuring a reliable performance of lot release assays, thus contributing to a better quality and commercialization of vaccines produced at Finlay Institute.

25. VALIDATION OF AN ELISA FOR THE QUANTITATIVE DETERMINATION OF THE ANTITETANIC ACTIVITY IN THE GUINEA- PIG SERUM


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Introduction: Tetanus toxoid vaccines have been able to reduce the mortality and morbidity caused by Clostridium Tetani worldwide, reaching high standards of quality, efficacy and security. During decades the vaccine potency for these vaccines has been based on its immunogenicity whose final stage is a challenge test known by L+/10/50. This method has some limitations in terms of animal ethics and variability. That’s why it is necessary to develop alternatives for Tetanus Potency. One of the most relevant approaches is a serological evaluation with an ELISA for determining anti-Tetanus antibodies in guinea-pig serum. The aim of this work was to validate an ELISA to be used as an alternative for Potency testing of tetanus vaccines. Materials and Methods: Validation parameters like accuracy, precision, linearity and specificity were determined according to the current international and national guidelines. Likewise, 42 guinea pig pools were analysed by in vivo and ELISA methods in parallel and the results were compared by linear regression analysis. Results: The present ELISA showed a good accuracy, with recovery values between 90-110 %, intra and inter- assay imprecision ranged around 10%. Parallelism deviations were below 10%. Appropriate correlation was found between ELISA and the neutralization test (R²=0.89). Conclusions: All parameters yielded satisfactory results according to reported for this kind of methods.
26. EVALUATION OF THE IN VITRO VERO CELL ASSAY AS ALTERNATIVE TO THE IN VIVO TOXIN NEUTRALIZATION TEST FOR DETERMINING POTENCY OF DIPHTHERIA VACCINES

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Introduction: The diphtheria toxin causes a dermonecrotic effect on rabbit and guinea pig skin and it has cytotoxic activity on Vero cells. Both properties have been used for titration of diphtheria antitoxin in immunized guinea pig serum by in vivo and in vitro neutralization methods. The in vivo toxin neutralization is used as antibody test for quality control testing of diphtheria potency in vaccines. This test has some disadvantages related with its variability and ethical aspects. The Vero cell assay based on the cytotoxic effect of the residual diphtheria toxin was evaluated as alternative of the in vivo assay. Materials and Methods: A half of a single human dose of DT and DPT samples was inoculated to groups of 4-6 Hartley guinea pigs. 28 days later the animals were bled to estimate the antitoxin titre in vitro (Lr method) or in vitro (Vero cells). Typical cell culture parameters and others were evaluated to standardise the in vitro test. Results: A linear relationship was observed between toxin dose levels and concentrations of antitoxin (1, 0.1, 0.001, 0.0001 UI/ml). At highest antitoxin dilutions (0.001 and 0.0001 UI/ml) the toxin dose levels were 2-fold lower than the expected ones. These results indicated that the equine antitoxin standard doesn’t have enough avidity to completely neutralize toxin at low reactant concentrations. For Vero cell method we obtained coefficients of variations lower than 20 %, showing a high reproducibility. Parallel titration of serum samples from guinea pig immunized with 23 lots of DT and DTP vaccines showed a significant correlation (Spearman’s correlation coefficient 0.827) between in vivo and in vitro tests.

27. ESTABLISHMENT OF A TESTING METHODOLOGY FOR THE DETERMINATION OF METHYLPENTOSE FROM PURIFIED POLYSACCHARIDES OF STREPTOCOCCUS PNEUMONIAE

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Introduction: At the present time, the pneumonia continues being a lash for the humanity in spite of the effort of many investigators. The Streptococcus Pneumoniae is the main responsible for pulmonary illnesses in many regions of the planet. Although this microorganism affects people of the third age fundamentally, the incidence in children in early ages is high with severe clinical manifestations including death. Finlay Institute develops a combined project with the Synthetic Antigens Laboratory from University of Havana for obtaining a conjugated vaccine that protects against this illness in our country and others in our continent. In the following work we aim to establish a method for determining methylpentoses in pneumococcal polysaccharides. Materials and Methods: For that, we compared two warming alternatives in order to verify if there was any significant difference between both alternatives. Parameters like Specificity, Precision and Accuracy were also determined. Results: We found that there were no significant differences between the two heating methods. Parameters like Accuracy and Precision fulfilled broadly the criteria defined for them. Regarding specificity, although the method is not specific as reported, the method has the advantage of producing a color change if an undesired saccharide was present. Conclusions: This work constitutes the first step for the implementation of a future analytical background that will be used in the quantification of the active pharmaceutical ingredients in conjugated vaccines against pneumonia.

28. COMPARISON OF PRP CONTENT IN Quimi-Hib VACCINE SAMPLES BY TWO DIFFERENT METHODS


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Introduction: The quantification of PRP in Hib vaccines is one of the most relevant parameter to be tested. The values of PRP play an important role in the effectiveness of the vaccine. European Pharmacopeia (2008) has established the Bial Method in...
29. PRELIMINARY EVALUATION OF LETHAL POTENCY TEST FOR TETANUS VACCINES AT FINLAY INSTITUTE

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Introduction: WHO lethal challenge method is still the golden standard for determining potency of vaccines containing Tetanus Toxoid. The aim of this work was the standardization of the Lethal Test for the evaluation of potency of the Tetanus vaccines vax-TET y VADIFTET produced in our institute. Materials and Methods: We used our own working reference materials (WRM): one in-house DT vaccine calibrated against the 3rd International Standard of Tetanus Toxoid adsorbed (98/552) and a Tetanus Toxin evaluated in LD50. They were prepared four dilutions of reference and tested vaccines. The WRM values obtained during the respective characterizations were confirmed. The relationship between dilution and protection was also evaluated. All results were processed by using Probit analysis. Results: It was obtained a good dose-response curve in the evaluated range (3, 1.5, 0.75, 0.37) mg/mL. It could be established the working dilutions for vax-TET and VADIFTET assuring the fulfillment of the acceptance criteria (linearity and parallelism). All tested vaccines were in agreement with the specifications. The values were processed by using Probit analysis. Results: It was obtained a good dose-response curve in the evaluated range (3, 1.5, 0.75, 0.37) mg/mL. It could be established the working dilutions for vax-TET and VADIFTET assuring the fulfillment of the acceptance criteria (linearity and parallelism). All tested vaccines were in agreement with the specifications. The values corresponding to the characterized WRM were confirmed as demonstrated by the death caused by the challenge dose of Tetanus toxin (50 DL50) in the control group (100 % of control mice died in each test) Conclusions: It was demonstrated that the lethal challenge test can be implemented for determining Potency of Tetanus vaccines produced at Finlay Institute.

30. DEVELOPMENT AND VALIDATION OF A WESTERN BLOT ASSAY FOR MEN B AND VA MENGOC-BC® MENINGOCOCCAL VACCINES

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Introduction: identity tests are included in official guidelines to be carried out mainly in final products. it could be considered as a measure of manufacturing consistency. as vaccines are immunological products, identity tests may be based on the antigen-antibody interaction. the present work aims to show the development and validation of a western blot assay applied to the meningococcal vaccines (VA-MENGOC-BC® y Men B) produced at Finlay Institute in order to have criteria of identity for licensing and market releasing. Materials and methods: they were identified the proteins of immunological relevance for VA-MENGOC-BC® (p1, p3, p5 and 70 k) and men b (p1.15 and p1.4), both present in outer vesicles membranes and final lot respectively. it was evaluated the feasibility of implementing this technique using monoclonal antibodies for the identification of antigenic proteins. the validation parameters studied were specificity, detection limit, repeatability, intermediate precision, reproducibility and robustness. Results: all parameters fulfilled broadly the criteria defined. Conclusions: western blot can be used as an alternative for identity test of the meningococcal vaccines produced at Finlay Institute.

31. PRELIMINARY EVALUATION OF A SINGLE RADIAL IMMUNODIFFUSION TEST AS A REPLACEMENT OF FLOCCULATION ASSAY FOR DETERMINING ANTIGEN CONTENT IN DIPHTHERIA AND TETANUS TOXOID VACCINES

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Introduction: Flocculation assay is the traditional method for identity and quantification of antigen content in Tetanus and Diphtheria toxoids vaccines. Nevertheless, its visual end-point and variability have motivated the searching of new alternatives. Single radial immunodiffusion test has demonstrated its ability for being used as an alternative to flocculation test, although it depends on the product, quality of biological reagents and other factors. The goal of this study was to develop a Single Radial Immunodiffusion test (SRD) to estimate the antigen concentration (in Lf / mL) in sterile purified diphtheria and tetanus toxoids...
Introduction: Traditionally, the biological activity of whole-cell Pertussis (wP) vaccines has been measured by the intracerebral mouse protection test (MPT or Kendrick test). Nevertheless, this test has been heavily criticised taking into consideration the animal welfare, technical difficulty and reproducibility. That’s why some laboratories have combined efforts to look for alternatives allowing replacing or refining the Kendrick test. One of the alternatives is the PSPT (Pertussis Serological Potency Test), based on an in vitro assessment of the humoral response against a wide range of surface-antigens of Bordetella pertussis in mice after the immunisation with wP vaccines. Although the PSPT model relies on the full-dilution WHO / EP method, we think it’s possible to carry out this test by using a single dose (high) as recommended by FDA following the NIH method. The aim of this work is to standardise an ELISA for determining anti-pertussis antibodies in order to set up a PSPT model following the NIH/FDA approach. Materials and methods: Groups of OF1 mice were immunized with 0.5 mL of DPT vaccines, bled after 28 days and the sera were titrated against an in house anti-pertussis hyperimmune serum by a wP-ELISA developed in our laboratory. The dose-response range, optimal concentrations of conjugate and coating and variation were determined. Results: A good dose—response curve was obtained for every assay. All test conditions were established to assure a consistent performance of our ELISA. The coefficient of variation intra and inter-assays was always lower than 20%. The ELISA results were in general in agreement with the MPT assay. Conclusions: This work is a step in our strategy focused on replacing at the same time the Kendrick test and the NIH methods used in Latin America for the potency of Diphtheria and Tetanus by serological tests.

Veterinary Vaccines

33. DETECTION OF ANTIBODIES HI AGAINST NEWCASTLE DISEASE BY THE APPLICATION OF VACCINE NEWCASTLE INACTIVATED FOR PIGEONS, STRAIN LA SOTA IN SUSCEPTIBLE PIGEONS

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Newcastle disease in pigeons appears with respiratory, digestive and nervous signs. Virus circulation represents a potential risk for the aviculture of the area that has a high economic value and that represents a source of food for the population. The objective of this work was to develop a vaccine inactivated with the strain La Sota and aluminium hydroxide gel as adjuvant, with an antigenic concentration superior to classical chicken vaccines and to determine if his application in susceptible pigeons promoted the production of antibodies HI. Antibodies HI were detected before vaccination, 28 days after the first doses and 43 days once time the scheme was concluded. An analysis of proportion of reactors with equal or upper titles to 1:16 was done; 100% of pigeons had antibodies HI titles = 1:16, 28 days after first doses and 43 days of the second doses. It was analyzed geometric means and the standard deviation of titles. Geometric mean was 6,7143 to the 28 days of first doses and 7,4667 to the 43 days of the second doses. The application of the obtained vaccine promotes the production of antibodies HI against Newcastle’s disease in susceptible pigeons.
34. INTEGRATED PROGRAM FOR THE CONTROL OF RHIPICEPHALUS (BOOPHILUS) MICROPLUS TICK POPULATIONS IN CUBA BASED ON THE USE OF THE VACCINE GAVAC. ENVIRONMENT AND ANIMAL HEALTH IMPACT

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*Rhipicephalus (Boophilus) microplus* has been developing resistance against the chemicals commonly used for controlling the infestations. Vaccination has been proposed as alternative control method. The effect of the vaccination using the so-called hidden antigens, such as Bm86 has been studied in several countries. In Cuba, the previous program (before the 90s) for controlling tick infestations and the associated diseases required 5 to 10 millions dollars each year and was not able to fully control the problem. During the last fourteen years, the Agriculture Ministry has implemented an Integrated Tick Control Program based on the use of a vaccine (Gavac™) developed and commercialized locally. Here we show the results of the application of such a program to several hundred thousand dairy cattle. The aim of this method was to control, rather that eradicate, the level of infestations, favoring the establishment of an enzootic stability for tick borne diseases. Over a period of ten years (1995 – 2005) the program resulted on a reduction of more 85% on the annual spending on acaricides; 98% reduction in the number of animals sick of hemoparasitic diseases and an almost complete elimination of imports for therapeutics. The total savings for the cattle production due to the program are estimated to be around 15 millions dollars per year. Commercial tick vaccine for cattle based on the Bm86 gut antigen have proven to be a feasible tick control methods that offers a cost-effective, environmentally friendly alternative to the use of acaricides.

35. DNA VACCINE AGAINST INFECTIOUS BURSAL DISEASE VIRUS: IMMUNE RESPONSE IN VACCINATED CHICKENS

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Infectious bursal disease virus (IBDV), also known as Gumboro disease, is an acute, contagious viral disease of poultry. IBDV causes severe immunosuppression and mortality in young chickens. The present vaccines in the market are not totally effective. One of the most important drawbacks of live attenuated vaccines is determining the optimal time for vaccination of young chicks because maternal antibodies can interfere with vaccine efficacy and there is always a risk of reversion from attenuated to virulent forms of IBDV in the field. The objective of this study was development a DNA vaccine capable of protecting young flocks from IBDV. A genetic construction carrying the genes for the protective epitope against Gumboro disease was developed with the aim to evaluate its usage as a protective immunogen against this disease. To achieve this experiment, a fragment of gene codifying for VP2 protein variable region was amplified by RT –PCR. Following this procedure the fragment was cloned into an expression vector for mammalian cells. The final construction (pGCVI) was characterized by Molecular Biology techniques. The ability of this DNA vaccine to produce an immune protective response was analyzed by virus neutralization and animal challenge. The influence of maternal antibodies, the production of neutralizing antibodies using several concentrations of plasmid and the role of liposomes as adjuvants were determined. It was demonstrated that when using the plasmid adjuvated on liposomes, higher levels of neutralizing antibodies with less concentration of immunogen are obtained. In this group the protection rate obtained was of 90%. With these experiments we demonstrated that the (pGCVI) immunogen was able to express the VP2 conformational epitope; it also was able to produce high levels of neutralizing antibodies and confer an adequate level of protection to the chicks tested.
36. YEAST-DERIVED VIRUS LIKE PARTICLES FOR IMMUNIZATION AGAINST RABBIT HEMORRHAGIC DISEASE VIRUS: BIOCHEMICAL CHARACTERIZATION AND FINAL DEFINITION OF A RECOMBINANT VACCINE

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Various approaches have been conducted to date in order to select a feasible candidate for the large-scale production of a recombinant vaccine against Rabbit Hemorrhagic Disease Virus (RHDV). In this work, we describe an extensive biochemical and structural characterization of the most relevant candidate assessed, which was obtained through the intracellular expression at high levels of the VP60 capsid protein from RHDV in the yeast Pichia pastoris. The analyses with conformational-sensitive monoclonal antibodies, size-exclusion HPLC, electron microscopy and ultracentrifugation gradients confirmed the presence of high molecular weight structures similar in mass, size, and buoyant density to native RHDV. Virus-like particles (VLP) purification was then conducted by a single chromatographic step, in which different conditions were tested until reaching physical stabilization of VLPs through inhibition of aggregation. At pH 4.0 or 7.0 a maximum recovery of the protein was achieved without distorting the conformational structure expected. Moreover, the thermal stability of an oil-based formulation containing these VLPs was assessed at 4°C, 37°C, 48°C or 60°C. Physicochemical properties from the stressed and unstressed vaccines showed differences in viscosity although the capsid-like structure was maintained in all samples, except with treatment at 60°C, as evidenced by antigenic analysis and electron microscopy. Rabbits immunized with the yeast-derived VLPs developed a strong RHDV-specific response as evidenced by competition ELISA and hemagglutination inhibition assays. The protective capability against a lethal challenge with 100LD50 of RHDV was also demonstrated, thus ensuring the practical value of this candidate and providing the basis for the final definition on the use of a scalable and low-cost recombinant vaccine against the disease.

37. REFORMULATION STUDY OF GAVAC IMMUNOGEN


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In the year 2007, the firm Seppic replaced the adjuvant Montanide 888 (from animal source), which is one of the main components of the vaccine Gavac, by another one called Montanide 888 VG (from vegetal source). In this work, a study of the vaccine reformulation was performed. Twenty litters of the present formulation used as control, and 20 L with the new adjuvant were produced; both formulations were subdivided in three sub-batches and the analyses of thermal stability, mechanical stability, drop size, viscosity, potency, and innocuousness were made. The results obtained with both formulations were compared statistically by using the software Statgraphics Plus 5.1. An increase in the viscosity up to 1.7-fold was observed in the new formulation respect to standard one, the obtained values were lower than the specified standards. An accelerated stability test showed that the new formulation is mechanically stable during 5 days at 55°C and 15 days at 37°C. In addition, the cost of the vaccine Gavac did not change when Montanide 888 was substituted by Montanide 888 VG; thus, it is economically feasible its use in the new formulation. The new formulation accomplishes the quality parameters established for the vaccine Gavac.

38. DEVELOPMENT OF TRANSFER RECOMBINANT VECTORS EXPRESSING THE GB GLYCOPROTEIN FROM MAREK’S DISEASE VIRUS AND THE POLYPROTEIN FROM INFECTION BURSAL DISEASE VIRUS

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Vaccines using live viral vectors are very promising due to advantages they have as long-term responses which involve cell-mediated response and favor the presentation to the immune system similar to what occurs in vivo. Folwlpox virus is a vector...
with range of restricted host to avian species, allowing its extension to other species as vaccines. It is have the ability to accept large-size foreigner fragments and to induce both, humoral and cellular response. The avian viral diseases Marek’s (MDV) is a lymphoproliferative disease produced in chickens, characterized by the formation of lymphomas in T cells and demyelination of peripheral nerves. The disease has been controlled by vaccination with serotype 3 turkey herpesvirus (HVT) but also has been used attenuated serotype 1. The B glycoprotein from MDV induces neutralizing antibodies. The infectious bursal disease virus (IBDV) is an important pathogen of chickens. The IBDV multiplies rapidly in developing B cells in the Fabricius bursal, to induce immunsuppression. The traditional vaccines not protect against outbreaks of highly virulent strains. In the present work have been obtained different recombinants plasmid which contain a expression cassette flanked by Fowlpox virus no essential regions. These preliminary results will be used to obtain recombinant virus for the vaccine development.

Preclinical studies and characterization of vaccines

39. METHODOLOGY FOR PREPARATION OF IMMUNOGENIC GLYCOCONJUGATES OF TWO LINEAR POLYSACCHARIDES OF STREPTOCOCCUS PNEUMONIAE TO TETANUS TOXOID

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Introduction: Streptococcus pneumoniae is a major cause of morbidity and mortality worldwide and in Cuba, especially in infant population. Serotypes 1, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F are responsible of about 80% of the infections in developed countries and 70% in developing countries. Two types of vaccines exist for the prevention of the pneumococcal diseases: the polysaccharide and the conjugated vaccine. Only the last one have proved to be efficient in protect infants under two years old. Cuba is involved in a project to obtain a multivalent conjugated vaccine for the protection of our children. For this propose we obtain the monovalents conjugates of 6B and 19F to the carrier protein tetanus toxoid. Materials and Methods: The methodology has three main steps: i) fragmentation by acid hydrolisis, ii) activation by periodic oxidation and finally iii) conjugation to the carrier protein by reductive amination. Each stage was followed by NMR and HPSEC. The epitopic conservation of each intermediary was evaluated by ELISA. The conjugated products were characterized by HPSEC, SDS-PAGE and NMR. The immunogenicity and specificity of these conjugates were tested in Balb/C mice and white New Zealand rabbits. Results: Three doses of the glycoconjugates elicited a rise in immunoglobulin G antibody response statistically different to response against to capsular polysaccharide. Conclusions: The methodology used demonstrated to be efficient in obtain immunogenic conjugates in mice and rabbits.

40. GLYCOCONJUGATES FROM STREPTOCOCCUS PNEUMONIAE SEROTYPE 1 TO TETANUS TOXOID. PREPARATION AND IMMUNOGENICITY IN MICE

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Introduction: Streptococcus pneumoniae is a leading cause of meningitis, pneumonia, and severe invasive disease in infants and young children in Cuba and throughout the world. Pneumococcal conjugated vaccines enhance immune responses in high-risk population compared to polysaccharide vaccines, due to their T-cell dependent mode of action. Multivalent pneumococcal conjugated vaccine is a high priority National Project in our country. Thus, we developed a procedure to generate serotype 1 glycoconjugate as vaccine component. Materials and Methods: It involves activation of polysaccharide by peryodic oxidation and conjugation to tetanus toxoid as carrier protein, using reductive amination. All modifications of polysaccharide were followed by NMR. The conjugate was characterized by SDS-PAGE, HPSEC and protein: polysaccharide (w:w) ratio. The antibody responses to this conjugated was evaluated in a mouse model developed for pre-clinical evaluation of pneumococcal conjugated vaccines. Results: The immunization with three doses of 2 µg of serotype 1 conjugated elicited high titers of total IgG, mainly IgG1 and IgG2a, able to recognize the PS. Conclusions: Our results demonstrate the conjugates obtained using this methodology are immunogenic in mice.
41. OBTENTION AND PRELIMINARY CHARACTERIZATION OF PROTEOLIPOSOMES DERIVED FROM SEROGRUP A AND W135 NEISSERIA MENINGITIDIS

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**Introduction:** Serogroups A and W135 of *Neisseria meningitidis* are causing recurrent meningitis epidemics mainly in Africa. Despite the use of polysaccharide (PS) vaccine, periodic epidemics occur every 8–12 years with high attack rates of 100–500/100,000. The development of a vaccine based on outer membrane protein (OMP) could be a promissory alternative. **Materials and Methods:** We have obtained Deoxycholate-extracted Proteliposomes (PLs) from subgroup III serogroup A and W135 meningococcal strain Mk 686/02 and Mk 222/02, respectively, by the use of two different methodologies: (i) methodology used to obtain PLs from serogroup B *N. meningitidis* to produce VA-MENGOC-BC® vaccine and (ii) a new methodology introduced in our laboratory to obtain PLs from other bacteria. PLs were analyzed by Protein and LPS determination, chromatography on Sephacryl S-1000 and SDS-PAGE Coomassie and silver stain. **Results and Discussion:** Using either serogroup A or serogroup W135, the purification process yield with the method (ii) was 10-times higher than method (i) and the relation Protein/LPS was similar in both cases; chromatographic profile on Sephacryl S-1000 supported the OMP-detergent assembling forming nanoparticulated structure like PL or outer membrane vesicles (OMVs); SDS-PAGE showed the presence of protein bands with described molecular weights to *Neisseria meningitidis* major outer membrane proteins, such as PorA, PorB, RmpM and OpCa, as well as small amounts of Omp85 and NspA. **Conclusions:** A PLs-based vaccine from serogroup A and W135 meningococci may be an alternative to polysaccharide and conjugate polysaccharide vaccines for Africa.

42. IMMUNOGENIC RESPONSE OF CONJUGATED OF SEROTYPES 14 AND 18C FROM STREPTOCOCCUS PNEUMONIAE

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**Introduction:** *Streptococcus pneumoniae* is a leading cause of bacterial pneumonia, bacteremia, meningitis, and otitis media. More than 90 serotypes have been identified. The vaccination with capsular polysaccharides (PS) is ineffective in elderly people and young children, due to the T-independent nature of these antigens. However, conjugating PS to protein carriers have become them immunogenic for these groups of high risk. Currently, the Center for Biomolecular Chemistry is working on conjugated vaccines, in order to obtain a polyvalent vaccine against pneumococci. **Materials and Methods:** In this respect, the PS 18C and 14 were conjugated to tetanus toxoid (TT), by periodic oxidation and reductive amination method. The immunogenic properties of both conjugates were evaluated in rabbits, using a model developed for pre-clinical evaluation of pneumococcal conjugated vaccines. The schedule was 3 doses with 2 μg of each PS-TT conjugated every 14 days, one booster dose with unconjugated PsC at 4 months, and blood extraction 7 days after each immunization. The titers of total IgG were measured in the serum of rabbits by ELISA and the avidity and specificity of antibodies were also evaluated by immunoassays. **Results:** The immunization with 18C-TT and 14-TT conjugates elicited high titers of total IgG against natural PS and carrier protein. The total IgG against each PS were avid and specific for natural PS. Also, we found evidences of immunological memory in rabbits because they responded to PS immunization with high titers of antibodies. **Conclusions:** In conclusion, the serotypes specific polysaccharide 18C and 14 conjugated to TT are immunogenic in rabbits.

43. OBTENTION AND PRELIMINARY CHARACTERIZATION OF CELL WALL EXTRACT OF MYCOBACTERIUM SMEGMATIS

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**Introduction:** *Mycobacterium smegmatis* is a fast-growing nonpathogenic mycobacteria that nonetheless shares many features with the pathogenic *Mycobacterium tuberculosis*. The genome sequence of *M. smegmatis* has shown a high homology with *M. tuberculosis* genome and have been identified a protein homologous series between both species that are involved in diferents pathways like enzymes responsible for creating of different components of the cell wall. In the present study we have
characterized preliminary an extract of *M. smegmatis* cell wall with the purpose of studying its use as a potential vaccine candidate against tuberculosis. **Materials and Methods**: The extract was obtained by the use of sodium deoxycholate as detergent. The protein concentration of the extract was estimated by Lowry technique and the immunogenicity was evaluated in Balb/c mice by ELISA. **Results**: The extract obtained showed a protein concentration of 0.55 mg/mL. The ELISA results indicated the presence of antibodies against cell wall extract of *M. smegmatis* which can recognize *M. tuberculosis* antigens. **Conclusions**: The results of this work indicated the antigenic similarities between both micobacterial species and the feasibility of their use as potential new vaccine candidate against tuberculosis.

44. APPLICATION OF THE INTRATRACHEAL MODEL OF PROGRESSIVE PULMONARY INFECTION FOR PRECLINICAL EVALUATION OF NEW THERAPEUTIC AND PROPHYLACTIC FORMULATIONS AGAINST MYCOBACTERIUM TUBERCULOSIS


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**Introduction**: Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis*. Many vaccine candidates have been tested for their potential use. However, there is an increasing realization of the need for animal models to test vaccine efficacy in more realistic scenarios than in current models that test the vaccines ability to protect animals against challenge with *M. tuberculosis* virulent strains. In the present work we tested the feasibility of the intratracheal model of progressive pulmonary infection to evaluate bacterial load and morphometric and histological changes in the lungs of mice treated with different vaccine candidates. **Materials and Methods**: In a first approach, we evaluated the protective activity of two monoclonal antibodies (TBA61 and TBA84) administered by intratracheal route against challenge with virulent H37Rv strain inoculated by the same route. Data obtained revealed the reduction of bacterial load and milder morphometric and histopathological changes in mice treated with TBA61 at 21 days post-infection compared to those treated with TBA84 and control mice. In a second approach, we constructed a *M. tuberculosis* genomic expression library composed by nearly 8000 recombinant clones. BALB/c mice were immunized with this library three times at 21 days interval each other and later were challenged intratraehelically with *M. bovis*. Sacrificed were performed 21 days post infection and lungs were removed to assess bacterial load and to evaluate histopathological changes. **Results**: As a result, a protective response was induced in mice immunized with the genomic library, compared to those inoculated either with PBS or empty vector. **Conclusions**: Results presented here suggest that the intratracheal model of progressive pulmonary infection constitutes a reproducible, inexpensive and powerful alternative for the preclinical evaluation of therapeutic and prophylactic formulations against *M. tuberculosis* infection.

45. CHARACTERIZATION OF ANTIBODY RESPONSES TO COMBINATIONS OF A DENGUE 2 RECOMBINANT PROTEIN AND DENGUE 2 INACTIVATED VIRUS


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**Introduction**: Our group has previously obtained a dengue 2 (Den) recombinant protein containing the envelope domain III fused to the P64K protein from *Neisseria meningitides* (PD5). The immunization of mice and monkeys with PD5 protein has induced anti-Den antibodies and partial protection after challenge assays. Currently, prime-boosting schedules have been applied to improve the immune response induced by recombinant proteins. Here, we evaluated an immunization strategy in mice that combines the PD5 recombinant protein with a Den 2 inactivated virus (IV). **Materials and Methods**: Five groups of mice were designed: 1) Primed with IV and boosted with PD5 (IV-PD5), 2) Primed with PD5 and boosted with IV (PD5-IV), 3) PD5 alone, 4) IV alone, 5) Den 2. The humoral immune response was evaluated in the sera collected seven and 15 days after the last immunization in terms of total IgG, IgG subclass (IgG1/IgG2a) and hemaglutination-inhibiting (HAI) antibodies. **Results**: The IV-PD5 strategy elicited higher total IgG antibody titers than PD5 group (p<0.001), IV group (p<0.001) and PD5-VI group (p<0.01). The combination of PD5 and IV did not increase the cross-reactive antibody titers against serotypes 1, 3 and 4, which is one of the main advantages of candidates based on envelope domain III. Similar to IgG titers, the IV-PD5 group showed the highest functional antibody response as estimated by HAI test. The immunization with IV or PD5 alone induced IgG2a or IgG1 subclass, respectively. The IgG subclass pattern observed in the IV-PD5 and PD5-VI groups corresponded with the priming antigen. In the IV-PD5 group IgG2a was the predominant subclass while PD5-VI showed higher levels of IgG1. **Conclusions**: The use of immunization strategies that combine IV as priming antigen with recombinant protein increase the immunogenic capacity of PD5 in mice maintaining the serotype specificity of the antibody response.
46. PRELIMINARY CHARACTERIZATION OF AN ORAL MULTIVALENT VACCINAL CANDIDATE AGAINST CHOLERA-SHIGELLA-SALMONELLA

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Introduction: Diarrheic diseases remain a health problem and they are an important cause of morbidity and mortality worldwide. Approximately 2 million persons died every year, mainly under-five-years old children of developing countries. A great number of microorganisms are the etiological agents of these diseases, being Rotavirus, Escherichia coli, Shigella, Salmonella and Vibrio cholerae the most relevant. Presently, there are some monovalent vaccinal candidates that have shown short term effectiveness, but no positive results have been obtained yet with multivalent or combined vaccinal candidates. We are working in the development of a proteoliposome (PL) combination-based multivalent formulation, derived from the surface of different bacteria that cause diarrheas disease. This multivalent formulation will be orally administered in humans in an enteric-coated tablet or cochleates suspension. Materials and Methods: Purified PLs were partially characterized by protein determination and LPS, SDS-PAGE and chromatography on Sephacryl S-1000. In addition different formulations were assessed in immunogenicity studies in rats and mice, were measured by IgG serum and IgA saliva ELISAs against each of the different solid-phase antigens. Results: SDS-PAGE showed that PLs contain outer membrane proteins (OMP) with molecular weights ranging 12-96 kDa and that there are protein bands that might be common in the different monovalent preparations. Chromatographic profiles of each case support a successful assembly of OMP with the molecules of the detergent, forming nanoparticulated structures. IgG serum and IgA saliva ELISAs in both rats and mice showed a high and homogeneous response of specific antibodies for each antigen in the multivalent preparation. Conclusions: These are promising results and they stimulate the development of a PL combination-based multivalent vaccinal candidate to prevent the diarrhea.

47. DEMONSTRATION OF SAFETY BY MEANS OF NON CLINICAL TOXICOLOGICAL STUDIES OF THE CUBAN PENTAVALENT VACCINE (DPT-HB + QUIMIHIB)

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Introduction: In the established programs worldwide to combat illnesses the vaccines monovalents have prevailed. The increment of the foregone illnesses for vaccination at world level, together to the number of immunizations carried out simultaneously in short time to children, makes indispensable the development of combined formulations of vaccine existent offering immediate benefits. In our formulation vaccines anti-diphtheria-tetanus-pertussis, hepatitis B and Haemophilus influenzae type b are combined. Materials and Methods: Albino Sprague-Dawley rats were used to carry out the following studies: Acute toxicity, Local tolerance and Repeated dose. The measured parameters were clinical observations, food consumption, hematolological exams, biochemical, macroscopic and microscopic observations of the target organs and administration place. Results: Microscopic evidences showed the presence of granulomas of different grades in the administration place and stimulation antigenic in spleen and mesenteric ganglion. No significant variation was observed in the determinations performed. There was no adverse or toxic effect at local and systemic level either. It was demonstrated that the mixture of both vaccines (DPT-HB and Quimi-Hib) doesn’t interfere with the biological response of the five antigens involved in the formulation. Conclusions: We could demonstrate that the Cuban pentavalent vaccine (DPT-HB + QuimiHib) is a safety product.

48. PREDICTION OF B AND T EPITOPEs FROM DIFFERENT PROTEINS OF MYCOBACTERIUM TUBERCULOSIS, EXPRESSION IN BCG AND EVALUATION OF IMMUNOGENICITY IN BALB/C MICE

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**Introduction:** Among all infections diseases, tuberculosis remains being the number one killer in humans and an effective vaccine other than BCG, involving the identification and isolation of key components of the pathogen that can induce a protective immune response, it’s still needed. **Materials and Methods:** In this work, we used recombinant BCG strains expressing T and B epitopes of different proteins of *Mycobacterium tuberculosis* in the immunization of mice Balb/c. The T and B epitopes identified using bioinformatic tools was employed subsequently for design of multiepitopic constructions, which were inserted into vectors and transformed into strains of BCG. The humoral immune response against the T and B epitopes was evaluated by ELISA, while the cellular immune response was evaluated by testing lymphoproliferation and analysis of intracellular cytokines using flow cytometry. **Results:** We obtained IgG antibody response against specific B epitopes of ESAT-6 and CFP-10 in mice immunized with the strain BCGr-pNMN032. After studying the specific response of spleen cells by testing lymphoproliferation and detection of intracellular cytokines in CD4 + subpopulations and CD8 +, we obtained the recognition of T epitopes after administration of BCGr-pNMN032. The response showed a T helper type-1 pattern after immunization with two recombinant strains. **Conclusions:** This work has been able to accumulate substantial experimental data to emphasize on the need to combine different strategies for designing effective vaccines against tuberculosis, as well as the importance of context for the induction of an effective immune response.

**49. IMMUNOGENICITY IN MICE OF RECOMBINANT DENGUE 2 NS3 PROTEIN COINMUNIZED WITH DENGUE 2 RECOMBINANT SUBUNIT VACCINE**

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**Introduction:** Dengue is the most prevalent vector-borne viral disease. More than 100 millions people are infected by any of the four dengue viruses (DEN 1-4) every year. Its incidence, in terms of morbidity and mortality, has increased dramatically over the past 20 years. Although vaccines have been successfully developed against other flavivirus, there is no effective vaccine against dengue virus disease in the world. **Material and methods:** In this study the full length DEN-2 NS3 gene was cloned and expressed with N terminal histidine tag in Escherichia coli. In order to evaluate the immunogenicity of the NS3 recombinant protein , groups of ten BALB/c mice were coimmunized with a highly purified III domain E protein. Sera were analysed by Immunoblotting, Immunofluorescence techniques and the plaque-reduction neutralization test. **Results:** Immunized mice showed a strong induction of anti NS3 IgG antibodies by ELISA. All mice sera specifically reacted with the native DEN-2 virus NS3 protein by immunoblotting and positive immunofluorescence on DEN-2 infected C6/36 cells was also observed. On the other hand, sera from mice immunized with DEN-2 domain III subunit were neutralized in the plaque-reduction neutralization test. Comparatively, neutralizing antibody titers observed to DEN-2 serotype induced in recNS3-domain III coimmunized mice were approximately two fold greater than those induced by the recombinant subunit vaccine. **Conclusions:** Our results show a good immunogenicity of the recNS3 protein, so that further studies should be done to determine protection in mice in order to consider the NS3 protein as an immunogen in a possible dengue vaccine formulation.

**50. EXTRACTION AND CHARACTERIZATION OF THE BACTERIAL SURFACE COMPONENTS FROM VIBRIO CHOLERAE USING ZWITERIONIC, NON-IONIC AND ANIONIC DETERGENTS**

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With the objective to obtain antigens of the bacterial cellular surface of the wild strain of *Vibrio cholerae* C7258, and to study their potentiality as vaccine antigen source, three detergents were used; zwiterionionic, non-ionic and anionic, one of each type, in concentrations ranging between 5 and 15%. The extracts and their chromatographic fractions of Sephacryl S-1000 were characterized by electrophoretic and immunochemical techniques as well as studied by Scanning Electronic Microscopy. The zwiterionionic and non-ionic detergents in concentrations between 5-15% were of greater yield in the extraction of proteins and phospholipids of the bacterial surface in comparison with the anionic detergent used , DOC 5% to 10%. DOC was used in this study like a reference for being a well established detergent in the pharmaceutical industry.DOC and the nonionic detergent, resulted those of smaller capacity of extraction of LPS. When using the DOC to 10%, the zwiterionionic detergent to 15% and the non-ionic one between 5-15% we managed to obtain stable proteoliposomes of an average size between 82-93nm of diameter, formed by known proportions of proteins, phospholipids and LPS. We have been able to obtain when using some of the extraction conditions almost pure preparations with respect to some of the proteins of the external membrane of *Cholera* bacteria, like the OmpU, The fundamental components of proteoliposomes, proteins and LPS, conserved their molecular weights as it were possible to be verified against patterns and by processing its electrophoretic profiles with the help of computer programs. The conservation of the antigenicity of the main antigens without damages by the extractive process, was confirmed by immunoidentification techniques with monoclonal antibodies.
51. MUCOSAL AND SYSTEMIC IMMUNE RESPONSE AGAINST OVALBUMIN INDUCED BY SINGLE TIME VACCINATION STRATEGY


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Vaccination is considered by WHO as the most cost-effective strategy for controlling infectious diseases, but a successful programme of immunization can contribute much more than just vaccines. Epidemiologically targeted implementation of vaccines has diminished morbidity and mortality from infectious diseases that previously were scourges and economic burdens (such as measles, polio, diphtheria, invasive *Haemophilus influenzae* type b (Hib) and pneumococcal infections). In spite of this, the global coverage of many vaccines estimated at: DPT 89%, TT 69%, Hep B 60%, Hib 22%, Polio 80% and Yellow fever 48%, is still inefficient. Several factors have been largely responsible of a difficulty to attain immunization coverage and have been recognized as a problems of current vaccines, such as: the number of dose, excessive use of parenteral route over mucosal route, inadequate progress in the field of adjuvants for use in human vaccines, higher reactogenicity and unavailability against intracellular pathogens, infected or altered cells, scanty feasibility to combined more than one antigen in the same formulation and others. To bear in mind these principals problems of current vaccines, a novel protocol for vaccination named **Single Time Vaccination Strategy** (SinTimVaS) was developed. **Materials and Methods:** Using female BALB/c mice, we induce systemic and mucosal immune responses against Ovalbumin (Ova) with only one parenteral and one mucosal dose of Ova at the same time, using AFPL1 and AFCo1 adjuvants, respectively. **Results:** The feasibility of different mucosal routes and the induction of long-lasting memory were also shown. In addition, SinTimVaS was not restricted to our adjuvants but it also function with other mucosal adjuvant like cholera toxin. **Conclusions:** In conclusion, SinTimVaS could increase the vaccination coverage and reduce the time-cost of vaccine campaigns, adding the mucosal specific response induction.

52. PRECLINICAL TOXICITY TESTING FOR A NASAL ANTIMENINGOCOCCIC VACCINE (VAX CO B) IN SPRAGUE DAWLEY RATS


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**Introduction:** The meningococcal disease is a non-solved problem by the world science. In Finlay Institute a vacunal candidate constituted by obtained cochleates from vesicles of the external membrane of *Neisseria meningitidis* B has been developed. This membrane has demonstrated its capacity to stimulate a specific immune response at mucous level. Objectives: To evaluate potential toxicity of this vacunal candidate. **Materials and methods:** The following testings were carried out: a pilot study, single dose study, local tolerance and repeated dose in Sprague Dawley rats of both sexes. From the vacunal formulation proposed for clinical testing a volume of 50 µL was administered for each nostrils. A control group without treatment was included and there was a group with placebo containing all the vacunal components except the cochleate. Food intake, body weight, clinical behavior and anatomopathologic, hematologic, biochemical and immunologic studies were registered. The irritability rate in the nasal region was calculated starting from the observed histologic changes in this area. **Results:** It was demonstrated that there was no difference of local or systemic toxicological interest among the treatment groups and control group in any test performed. The histopathologic changes in the nostrils were light and the irritability rate classified the substance as non irritating. **Conclusions:** The vacunal candidate is potentially non toxic when being administered by intranasal route in Sprague Dawley rats.

53. IMMUNOGENICITY AND PROTECTIVE EFFICACY OF A RECOMBINANT FUSION PROTEIN CONTAINING THE DOMAIN III OF THE DENGUE 1 ENVELOPE PROTEIN IN NON-HUMAN PRIMATES


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**Introduction:** The Cuban project for developing a vaccine candidate against dengue viruses is focused on the evaluation of recombinant fusion proteins including the domain III of the dengue envelope protein fused to the P64k protein from *Neisseria*...
meningitidis. It is postulated that dengue envelope domain III is the main site for inducing neutralizing antibodies. Until date, only the dengue 2 recombinant protein has been evaluated in monkeys. Here we evaluated the immunogenicity and the protective capacity of the dengue 1 recombinant protein (PD10) in non-human primates. Material and Methods: Macaca fascicularis and Macaca mulatta monkeys were subcutaneously immunized with four doses of PD10 (100 µg) emulsified in Freund adjuvant on days 0, 30, 90 and 150. Similar groups were immunized with P64k as negative controls. All the animals were bled 15 days after last immunization dose and challenged 45 days later with 10^6 pfu of dengue 1 virus. The antibody response was monitored by ELISA and neutralization test. Viremia after challenge was followed by viral isolation and RT-PCR. Results: Sera from monkeys immunized with PD10 (M. fascicularis and M. mulatta) showed high total IgG and neutralizing antibody titers. The neutralizing antibody titers detected in PD10 immunized animals were similar to those induced after a dengue 1 virus infection in monkeys. In addition, monkeys immunized with PD10 did not show viremia estimated by viral isolation while the detection by RT-PCR demonstrated viremia reduction compared to the control animals immunized with P64k. Conclusions: Dengue 1 recombinant protein is immunogenic and protective in non-human primates using Freund adjuvant. Further studies including other adjuvants licensed for human use are required.

54. EFFECT OF DOSE AND BICARBONATE ON INTRAGASTRIC IMMUNIZATION WITH AFCo2

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Introduction: The Adjuvant Finlay Cochleate 2 (AFCo2) is a microtubular structure derived from Vibrio cholerae O1 proteoliposome (PL). It contains multiple antigens and immunopotentiator molecules like MSHA pilus, OmpU porin and lipopolisaccharide (LPS). Intranasal (IN) administration of AFCo2 (100 µg) induces high systemic and mucosal immune response comparable with attenuated 638 vaccine candidate on BALB/c mice and was used as positive control [1-2]. The aim of this work is to evaluate the effect of dose and pH gastric attenuation on mucosal and systemic immune response induced by AFCo2 intragastric (IG) immunization. Material and Methods: AFCo2 was obtained by rotary dialysis method and characterized using SDS/PAGE, Western BLOT:ing (monoclonal antibodies from Finlay Institute) and light microscopy. BALB/c mice were IG immunized with 100 µg or 200 µg of AFCo2 with or without sodium bicarbonate using a three doses one week apart schedule. Saliva and faeces samples were collected at seven and fourteen days after the last dose, respectively. Specific (anti PL) systemic and mucosal immune responses were evaluated using ELISA procedures to detect IgG (sera) or IgA (saliva and faeces) antibodies. Additionally, vibriocidal activity was determined in sera. Results: AFCo2 structures of ~16.45 µm were obtained; Main antigens and immunopotentiator molecules were not affected by the process; 200 µg of AFCo2 immunized by IG induced better specific mucosal and systemic immune responses than 100 µg, but similar than previous IN (100 µg) immunization and pH neutralization did not increased the specific immune responses induced by AFCo2. Conclusion: AFCo2 was obtained by dialysis rotary method; IG immunization required higher dose than IN route to induced comparable specific immune responses and Use of bicarbonate buffer is not required using AFCo2 on this animal model.

55. BORDETELLA PERTUSSIS DERIVED PROTEOLIPOSOME AS AN IMPROVED VACCINE CANDIDATE. PRELIMINARY BIOLOGICAL CHARACTERIZATION

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Introduction: Bordetella pertussis is the causative agent of whooping cough. The highest morbidity and mortality occurs in children, with over 40 million cases and 350000 deaths each year. In recent years, an increasing number of cases have been reported in adolescents and adults. These data support the argument that vaccination in infancy does not afford long-term protection and indicate a need for booster immunization in older age groups. There are two variants of vaccines against pertussis at present, inactivated whole cell and acellular vaccines. Both are protective against the disease but it’s known that the first one produces undesirable adverse reactions. Our group is working on the development of an improved vaccine candidate based on a proteoliposome derived from Bordetella pertussis. The aim of this work was to characterize it through physico–chemical and biological tests in order to evaluate its protective ability and reactogenicity. Materials and Methods: Protection assays in Balb/C mice using intranasal challenge model against local clinical isolations were done in Argentina. To
evaluate the potential toxicity, pirogenicity and LAL assays were developed. Proteoliposome antigenic composition was preliminarily determined through proteomic analyses and its conformational structure was observed using electronic microscopy. **Results:** The results indicated that the proteoliposome confers a high protective immune response against all the evaluated clinical strains. Pirogenicity and LAL assays showed that proteoliposome is apirogenic in the evaluated concentrations, while endotoxic units, measured by LAL, were comparable with the values reported for the constitutive proteoliposome of VA-MENGOC-BC® vaccine. Proteomic analyses indicated the presence of pertactin and fimbiae, two important antigens that normally are part of commercial acellular vaccines. Electronic microscopy showed spherical particles on the range of 25-70 nm. **Conclusions:** The results described in this work allow us to consider proteoliposome as a potential improved vaccine candidate against *Bordetella pertussis.*

### 56. SYNTHESIS OF GLICOCONJUGATES OF STREPTOCOCCUS PNEUMONIAE SEROYPES 14 AND 18C POLYSACCHARIDE TO SEVERAL CARRIER PROTEINS


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**Introduction:** *Streptococcus pneumoniae* is the major cause of mild and severe infections worldwide. The major clinical events associated with this pathogen are pneumonia, meningitis and acute otitis media. In 2005, WHO estimated that 1 million children less than 5 years old died from pneumococcal diseases. In 2000, Wyeth Vaccines licensed the first conjugated vaccine (Pneumovax) that proved to be immunogenic in infants due to a T cell dependent immune response, which leads to the development of immunological memory and maturation of immune response. The serotypes included in Pneumovax are 4, 6B, 9V, 14, 18C 19F and 23F, selected taking into account the prevalence of invasive disease and antibiotic resistance. Cuba is interested in the development a conjugated vaccine against this bacterium in order to protect the Cuban children. In this vaccine serotypes 14 and 18C glycoconjugated to carrier proteins will be included. **Materials and Methods:** To obtain the conjugates, the natural polysaccharides were sized by acid hydrolysis and activated by periodid oxidation. The conjugation to tetanus and diptheric toxoid procedure was carried out by reductive amination. Conjugates with both carrier proteins and different carbohydrate/protein ratios were obtained. The conjugates were evaluated by HPSEC, NMR and ELISA techniques. Only the conjugates 14-TT and 14-TD were evaluated in mice. The immunogenicity, antibodies avidity and specificity were evaluated by immunoassays. **Results:** Three doses of the 2 µg glycoconjugate every 14 days elicited high titers of total IgG specific for PsC 14. **Conclusions:** In conclusion, the conjugates 14-TT and 14-TD obtained following this methodology were immunogenic in mice.

### 57. FINLAY’S ADJUVANTS FOR MUCOSAL VACCINES


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**Introduction:** The mucosal surfaces of the respiratory, gastrointestinal (GI) and urogenital tract of humans and animals are the major portals of entry and/or sites of diseases caused by microbial pathogens. Thus, development of mucosal adjuvants/vaccines that elicit strong and prolonged local mucosal immune responses that would prevent pathogen attachment, replication and invasion, would be a significant advancement in the prevention and treatment of many infectious diseases. Most of the currently approved in human and veterinary vaccines are administered systemically, and these fail to elicit effective mucosal immunity. Hence, there are ongoing world-wide efforts for developing mucosal adjuvants and mucosal vaccine delivery systems. Proteoliposome (PL) derived from *Neisseria meningitidis* serogroup B (AF (Adjuvant Finlay) PL1) and its derived cochleate (Co, AFC01) contain multiple pathogen-associated molecular patterns as immunopotentiators, and can also serve as delivery system to elicit a Th1 type immune response. The purpose of these studies was to investigate the ability of AFC01 and AFPL1 as adjuvants to induce systemic and mucosal immune responses. **Materials and Methods:** Therefore, we used female mice immunized by intranasal or intramuscular with ovalbumin or glycoprotein (g) D2 from genital herpes simplex virus type 2 (HSV-2). Results High levels of specific IgG antibody were detected in all sera, but only specific IgA antibody in external secretions was only detected in mucosally immunized mice. The polarization to a Th1 pattern was documented by the induction of IgG2a/ IgG2c antibodies, positive delayed-type hypersensitivity and IFN-γ responses. Additionally, AFC01gD2 immunized mice showed practically no vaginal HSV-2 replication and 100% protection against a lethal vaginal HSV-2 challenge. **Conclusions:** These results support the use of AFC01 as potent Th1 inducing adjuvant particularly suitable for nasal immunizations.
58. STREPTOMYCES LIVIDANS POTENTIAL AS A VACCINE VEHICLE AGAINST TB

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**Introduction:** BCG protects against severe childhood forms of the disease, but fails to protect against adult pulmonary TB in countries where it is endemic. Also, there are 8 million people worldwide developing active TB annually and 3 million dying, therefore more effective vaccine is needed. The aim of this work was to evaluate the potential of Streptomyces lividans as vaccine candidate. **Methods:** 1 ml aliquots of Streptomyces mycelium suspension were lyophilized and kept at 4°C until use. Female Balb/c mice (18–20 g) were immunized by intraperitoneal route in 3 different experimental groups. Humoral response was evaluated by ELISA and Western blot assays. At the same time, Lymphocytes proliferation was assessed by MTT assay while the cytokine levels were determined by using double antibody system ELISA kits. **Results:** It was shown that mice immunized with S. lividans 1326 strain and BCG vaccine produced antibodies against the secreted proteins recovered from the culture supernatant of the respective strains. Proteins of different molecular weight present in the spent culture medium of the Streptomyces strains were recognized by the antibodies from immunized mice. We observed a detectable pattern of cross-reactivity between the sera of Streptomyces immunized group against proteins from the culture supernatant of the respective strains. Proteins of different molecular weight present in the spent culture medium of the Streptomyces strains were recognized by the antibodies from immunized mice. **Conclusion:** The S. lividans strain was sufficiently immunogenic in Balb/c mice in accordance with the schedule and the immunization pathway studied. In some cases the Streptomyces responses were stronger than the BCG as in the IFN-γ secreted by leukocytes.

**Therapeutic vaccines**

**Oral Session**

1. WATER IN OIL ADJUVANTS IN HUMAN THERAPEUTIC VACCINES

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Montanide ISA 51 VG and Montanide ISA 720 VG are adjuvants rendering stable W/O emulsions when mixed with antigenic media. In this review, chemical structures, optimised T connector emulsion preparation protocol will be presented. Induced immunological profiles in different experimental models are exposed on parameters such as antibodies patterns, cytokines induced and CTL response. Based on recently published clinical trials, safety of both adjuvants will be described, according to local and general adverse events scores. Development stages of the different therapeutic indications and regulatory status will also be discussed.

2. NANOGEL VACCINE DELIVERY VEHICLES DESIGNED TO TARGET DENDRITIC CELLS

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3. LIPID NANOPARTICLES AS DELIVERY SYSTEM FOR SUBUNIT VACCINES

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**Introduction.** With current gene and protein technology it is now possible to identify specific regions of some whole organisms or cells which are likely to be recognized by the immune system, and to reproduce them synthetically as subunit vaccines. These so called epitopes are very safe because they are non-living but they also tend to be only poorly immune stimulating. To improve the immunogenicity of a poorly immunogenic antigen, our approach is to use nanoparticles as delivery systems.

**Materials & Methods.** Liposomal delivery systems and related lipidic particles were prepared to enhance the immune response by more closely mimicking a virus or microorganism due to the possibility of multimeric antigen presentation and their large size compared to subunit antigens.

Results. Our group has developed and characterised the following colloidal delivery systems:
- functionalised liposomes (mannosylated or including adjuvants such as Quil A);
- immune stimulating complexes (ISCOMs);
- cationic ISCOMs (termed Pluscoms);
- ISCOM implants;
- cubosomes.

**Conclusions.** In this presentation an overview about the various nanoparticulate delivery systems our group has developed for the delivery of subunit vaccines will be given. New results in this field, both on physico-chemical characterisation and immunological activity of these nanosystems will be presented.

4. PROTEOMICS AND CANCER VACCINE DEVELOPMENT


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Proteomics is a new scientific field aimed at the large-scale characterization of the protein constituents of biologic systems. It facilitates comparisons between different protein preparations by searching for minute differences in their protein expression repertoires and the patterns of their post-translational modifications. These attributes make proteomics perfectly suited for searching for proteins and peptides exclusively or preferentially expressed in cancer cells in order to use them as candidates for therapeutic targets. The main proteomics technologies include 2D polyacrylamide gel electrophoresis, multidimensional high-performance liquid chromatography, mass spectrometry and protein arrays. Proteomics technologies are also used to analyze structural characteristics of vaccine candidates and to assess the relationship with their biological functions. The use of such technologies to identify primary structure, glycosilation patterns and other physical and chemical characteristics in one cancer vaccine is discussed here.

The example of the 1E10 mAb, a murine anti-idiotypic antibody that mimics N-glycolyl-GM3 gangliosides obtained at the Center of Molecular Immunology is discussed in this work. This antibody has been used adjuvated in Al(OH)₃, in several clinical trials including NSCLC, melanoma and breast cancer. The product used for these clinical trials was obtained initially from mice ascitis. Due to ethical, regulatory and scalability problems derived from the use of animals as bioreactors, it has developed a new pilot scale cGMP process based in stirred tank fermentation using protein-free medium. Also the bioequivalence between 1E10 obtained from ascitis (1E10A) and stirred tank (1E10ST) through a comparability exercise is demonstrated.

5. LIPID NANOPARTICLES AS DELIVERY SYSTEM FOR SUBUNIT VACCINES

**Rades T**, Hook S
The New Zealand National School of Pharmacy, University of Otago, PO Box 913, Dunedin, New Zealand.

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**Materials & Methods:** Liposomal delivery systems and related lipidic particles were prepared to enhance the immune response by more closely mimicking a virus or microorganism due to the possibility of multimeric antigen presentation and their large size compared to subunit antigens. **Results:** Our group has developed and characterised the following colloidal delivery systems:
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- cationic ISCOMs (termed Pluscoms);
- ISCOM implants;
- cubosomes. **Conclusions:** In this presentation an overview...
about the various nanoparticulate delivery systems our group has developed for the delivery of subunit vaccines will be given. New results in this field, both on physico-chemical characterisation and immunological activity of these nanosystems will be presented.

6. CIMAvax-EGF PROCESS DEVELOPMENT: TOOL FOR IN PROCESS CONTROL ESTABLISHMENT

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Specific active immunotherapy is becoming a promising approach for cancer treatment. Different cancer vaccines are under development, targeting tumor associated antigens. CIMAvax-EGF is a vaccine development at the Center of Molecular Immunology. This vaccine contain one of the most important ligands of the EGF-R coupled to the carrier protein and administered together with an adjuvant and has shown to be immunogenic and safe in previous phase I-II clinical trials. A manufacturing process at lab scale was developed early in the product development and it was used during the preclinical testing and initial clinical trials. Late clinical trials and commercial phase demanded further manufacturing process development for this vaccine. A new process design allowed solving the initial process limitations in scale-up, validation and GMP requirements. This work is focused in the use of statistical tools for the establishment of the acceptance range for the “in process control” defined during the manufacturing process development of CIMAvax-EGF. Additional the in-process control trend analysis, show that the process produce consecutive vaccine lots with consistent performance.

7. NEW STRATEGIES OF LIPOSOME-BASED VACCINES

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Liposomes are attractive protein antigen carriers enhancing humoral and cellular responses. Liposomal preparations, comprised of phosphatidylcholine (PC) and cholesterol (Cho), and containing different antigens such as human epidermal growth factor (hEGF), the major allergens from the mite Dermatophagoides siboney (maDers) or ovalbumin (OVA) induced in mice IgG pattern associated with a Th1/Th2 mixed response. Liposomes constituted by saturated PC (DPPC or DSPC) and Cho and carrying hEGF were more effective as immunoadjuvant than liposomes of unsaturated PC in mice, enhancing the IgG2a/IgG1 ratio and the ability of antibodies to inhibit hEGF-R/hEGF interaction. Mice immunized with DPPC:Cho liposomes/hEGF increased their survival after the challenge with EGF-dependent tumor cells in comparison with those immunized with Al(OH)3/hEGF. Furthermore, no allergic responses were detected in mice immunized with maDers encapsulated into DPPC:Cho liposomes. The presence of Cho in these liposomes was an important fact to achieve a high IgG2a/IgG1 ratio. Different strategies have been employed including the co-encapsulation of bacterial pore-forming toxins or peptides with antigens into liposomes in order to activate cytotoxic T CD8+ lymphocytes. Sticholyisins (Sts) are 20 kDa cysteinless isotoxins from the sea anemone Stichodactyla helianthus able to form tetrameric pores in membranes. Sts share functional homology with bacterial pore-forming toxins, therefore they could also modulate the antigen-specific immune response. Liposomes/ maDers+St II induced the highest IgG2a/IgG1 ratio suggesting the presence of a Th1 response pattern. Likewise, liposome/OVA+Sts promoted the activation of T CD8+ lymphocytes mediated response in vivo and conferred protection to mice from challenge with OVA-expressing tumor cells. Our results suggest the potentialities of these delivery systems for purposes of vaccination.

8. CATIONIC LIPOSOMES FOR VACCINE DELIVERY: A TALE OF TWO CHARGES

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Until recently cationic lipids and cationic liposomes were more frequently recognised for their use as gene delivery systems than for applications as vaccine adjuvants. However issues with biological recognition of cationic lipids, whilst a problem in gene therapy could indeed mean they offer a realistic opportunity for protein-based vaccines. The potential of cationic surfactant vesicle based formulations to electrostatically adsorb and deliver sub-unit antigens will be discussed within this presentation. A range of particulate vaccine delivery systems have been investigated by our group including liposomes, niosomes and microspheres. Polymeric microspheres were based on Poly-Lactic-Co-Glycolic Acid (PLGA) and vesicles were formulated with
Virus-like particles (VLP) are nanometer size particles from viral origin, purified or produced by genetic engineer techniques as recombinant antigens. Commercial anti-Hepatitis B vaccine is currently based on the hepatitis B surface antigen (HBsAg), a lipoprotein of 22 nm sizes and has shown to be highly effective in preventing the disease in most of vaccines. However, the current situation in the therapeutic field is disappointing, mainly based on reductionism of previous vaccine strategies and also in believing that commercial vaccine or the HBsAg alone could be enough to subvert the anti Hepatitis B immune tolerance and activate the immune system to control the viral load. Recent literature support that the cellular immune response to the Hepatitis B nucleocapsid antigen mediates the therapeutic effect after adoptive transfer providing a direct proof of concept in humans on the effect of the immune system in controlling the virus and produce therapeutic benefits. The present work will provide experimental data in normal and transgenic mice and also the results from clinical phase I study in humans, supporting the use of mucosal and parental administration of the combined formulation NASVAC, based on the mixture of HBsAg and HBcAg and the cross-potentiating effect of such combinations to improve the cellular immune response against both antigens.

9. A NEW IMMUNO-THERAPEUTIC STRATEGY BASED ON THE ADJUVANTEFFECT OF HEPATITIS B SURFACE AND NUCLEOCAPSID VIRUS-LIKE PARTICLES

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Virus-like particles (VLP) are nanometer size particles from viral origin, purified or produced by genetic engineer techniques as recombinant antigens. Commercial anti-Hepatitis B vaccine is currently based on the hepatitis B surface antigen (HBsAg), a lipoprotein of 22 nm sizes and has shown to be highly effective in preventing the disease in most of vaccines. However, the current situation in the therapeutic field is disappointing, mainly based on reductionism of previous vaccine strategies and also in believing that commercial vaccine or the HBsAg alone could be enough to subvert the anti Hepatitis B immune tolerance and activate the immune system to control the viral load. Recent literature support that the cellular immune response to the Hepatitis B nucleocapsid antigen mediates the therapeutic effect after adoptive transfer providing a direct proof of concept in humans on the effect of the immune system in controlling the virus and produce therapeutic benefits. The present work will provide experimental data in normal and transgenic mice and also the results from clinical phase I study in humans, supporting the use of mucosal and parental administration of the combined formulation NASVAC, based on the mixture of HBsAg and HBcAg and the cross-potentiating effect of such combinations to improve the cellular immune response against both antigens.

10. LENTIVIRAL VECTORS AS VACCINES

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Lentiviral vectors based on human immuno-deficiency virus type 1 (HIV-1) transduce dendritic cells in draining lymph nodes after subcutaneous injection. Using vectors encoding ovalbumin, or the tumour antigen NY-ESO-1 we have shown that lentiviral vector injection initiates potent antigen-specific CD4+ and CD8+ T cell responses. Protective and therapeutic anti-tumor immunity can be induced. To improve safety and efficacy, we have developed non-integrating lentiviral vectors, and also vectors with uptake and antigen expression targeted to antigen presenting cells. We have also expressed constitutive activators of NFkappaB or mitogen-activated protein kinase pathways. Triggering of NFkappaB or p38 led to activated DC, and substantially enhanced the anti-tumor immune response. Activation of ERK increased TGF-â expression, suppressed the immune response and stimulated expansion of regulatory T cells. These results provide a toolkit to regulate immune responses to immunization; vaccine responses to foreign or tumor antigens can be enhanced and harmful responses to self-antigens or introduced transgenes can be reduced.

11. THERAPEUTIC VACCINE FOR HIV

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Introduction: Human immunodeficiency virus (HIV)-1 infection continues to threaten human health worldwide. In addition to CD4 T-cell depletion, progression to AIDS is also associated with a chronic generalized immune activation. Antiretroviral therapy (ART) is successful in controlling viraemia and reducing immune hyperactivity but not eradicating HIV infection. Limitations of ART include the emergence and transmission of multidrug resistance, short and long-term side effects and high cost. A number of different approaches are being investigated towards the development of therapeutic vaccines to complement current and future antiretroviral therapies to counteract ART limitations. Materials and Methods: Therapeutic immunization targeting cell mediated immunity can complement (ART) for the removal infected cells (reservoirs) harbouring and drug resistant and drug sensitive HIV. The induction of antigen-specific immune responses may sustain immunological competence (preserve CD4+ T-cell numbers) and allow for prolonged safe ART-free periods. In addition to targeting infected cells and virus particles, immunotherapeutic approaches directed at curbing the generalized immune activation induced by HIV are also being pursued. Results: Successful therapeutic immunogens should improve reactivity to multiple HIV subtypes and accommodate diverse human populations (multiple human leukocyte antigens (HLA)). Ideal immunogens should also target restricted, relevant polyfunctional epitopes to regions of HIV-1 associated with control of infection. Reducing generalized immune activation will serve to restrict HIV production, reducing viraemia and slowing disease progression. Conclusion: Antigens showing a beneficial effect following therapeutic immunisation may provide the basis for a future preventative vaccine to avert infection or to slow disease progression should infection occur.

12. VIRUS LIKE PARTICLE BASED VACCINES FOR THE TREATMENT OF CHRONIC DISEASES

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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.

13. DEVELOPMENT OF STANDARDIZED ALLERGEN EXTRACTS OF TROPICAL HOUSE DUST MITES AS THERAPEUTIC VACCINES FOR ASTHMA

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House Dust Mites are the most relevant allergen sources in Cuba, associated to respiratory allergy. This work describes the development of standardized allergen extracts of three species: Dermatophagoides pteronyssinus (DP), Dermatophagoides siboney (DS) and Blomia tropicalis (BT) as therapeutic vaccines for asthma. Last two species are found only in tropical climates. Product standardization was based on development of analytical immunoassays for allergenic activity and composition (using IgE antibodies from allergic patients) and for quantification of Major Allergens with mono and polyclonal antibodies. The manufacturing process, starting from an original mite culture method, was able to achieve consistent results according to regulatory requirements. The stability of freeze-dried final products was evidenced during 60 months at 4°C. Acute and dose repeat toxicity studies in animal models supported the safety of injectable subcutaneous administration. Clinical trials for diagnostic use permitted to standardize the skin prick test, regarding optimal allergen concentration and cut-off value, maximizing sensitivity and specificity. Finally, efficacy and safety as therapeutic vaccines were assessed in 6 clinical trials of subcutaneous immunotherapy in allergic asthma. Treatment was effective for 76% of patients. Asthma symptoms and medication declined in 60% (CI95%: 51-69) as compared to placebo. No severe systemic reactions were reported. VALERGEN® vaccines were licensed for both indications: diagnostic and therapeutic, becoming the first registered allergenic products in Cuba, and in the case of DS and BT, the first worldwide. Availability of high quality standardized allergen vaccines becomes a valuable tool for expanding the etiological approach for asthma management.
We have evaluated whether V-5 Immunitor (V5) – a tableted therapeutic vaccine comprising heat-inactivated HBV antigens from pooled blood of HBV- and HCV-infected donors – may produce clinical benefit through induction of oral tolerance and reduction of immune-mediated liver injury. Once daily dose of V5 was administered per os to 10 patients with chronic hepatitis B and 30 patients with chronic hepatitis C in open label studies that lasted one month. Every patient who entered the study had elevated liver enzyme levels, which at the end of study have decreased in 100% of analyzed patients with hepatitis B. The reduction was highly significant, from 112.4 to 44.4 U/L (p=0.00009) and 118.8 to 46.1 U/L (p=0.0032), for ALT and AST respectively. In addition, half of intent-to-treat patients who were HBV surface antigen (HBsAg) positive at study entry, became negative after one month on V5 (p=0.0098). All patients, except one, reported complete recuperation from hepatitis-associated clinical symptoms present at baseline (p=0.0016). Total of 30 patients with chronic HCV were evaluated in 2 separate trials. In the first study involving 10 patients elevated liver enzyme levels have decreased from 157.7±73.4 to 49.9±43.8 U/L (p=0.0013) and 147.0±79.2 to 58.7±56.6 U/L (p=0.0132), for ALT and AST respectively. The AST/ALT ratio has improved from 0.93 to 1.18 (p=0.00058) indicating the reversion of progression to cirrhosis. All patients, except one, reported complete recuperation from hepatitis-associated clinical symptoms present at baseline (p=0.0016) with Mantel Haenszel’s odds ratio 9.4 (p=0.0021) at 95% confidence interval: 2.7<OR<476.3. Similar results were seen in another 20 HCV patients. Mean decrease in bilirubin levels was from 22.1 to 10.9 ìmol/L (p=0.0000000006) and ALT from 172.1 to 18.2 IU/L (p=0.000000000005). The positive response was seen in 19 of 20 patients (95%). No adverse events were observed at any time. Thus favorable biochemical and clinical responses were observed in both hepatitis B and C patients indicating that placebo-controlled, randomized Phase II study is now required to confirm preliminary findings.

15. PANFLUVAC: A EUROPEAN CONSORTIUM PROJECT DEVELOPING A VIROSOMAL PANDEMIC H5N1 VACCINE

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16. A NOVEL ACTIVE IMMUNOTHERAPEUTIC APPROACH FOR CANCER TREATMENT TARGETING AUTOLOGOUS VASCULAR ENDOTHELIAL FACTOR

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The antiangiogenic therapy of cancer has rapidly progress to the use of passive immunotherapy directed to the main growth factor involved in new vessel growth: the vascular endothelial growth factor (VEGF). Herein we present a summary of the preclinical results on the investigation of a new active immunotherapy strategy based on the targeting of autologous VEGF for the treatment of cancer. We first test our hypothesis using DNA based vaccines and evidence the potentiality of this approach for the establishment of an antitumoral, antiangiogenic and antimetastatic response related to the induction of a CTL response. Further exploration in this field revealed a similar antitumoral effect using a mutant variant of VEGF impaired for VEGFR2 binding, opening a new research line for the development of a protein based vaccine candidate. The administration of the protein in several adjuvants have resulted in an antitumoral response in C57BL6 mice challenge with MB16F10 melanoma, and in BALB/c mice challenge with the CT26 colon carcinoma. Immunization in these mice strains was associated with a sustained increase in antibody titers vs both human and murine VEGF, and with evidences of cellular response specific for tumor cells. Moreover, the immunization of large rodents and non human primates evidenced that is possible to break B cell tolerance to the autologous VEGF in this species in the absence of relevant side effects. Altogether these preclinical results indicate that a vaccine based on a mutant variant of VEGF could be safe, effective and offers potential for human clinical trials.
17. PRECLINICAL AND CLINICAL TRIALS USING HEBERPROVAC, A GONADOTROPIN BASED VACCINE TO TREAT PROSTATE CANCER

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Background and Aims: Previous studies with Gonadotrophin Realeasing Hormone (GnRH/LHRH) vaccines have shown the usefulness of immunization against this hormone in prostate cancer. To similar purpose, we generated a completely synthetic GnRH peptide mutated at position 6 and attached to the 830-844 tetanic toxoid (TT) helper T cell sequence which was specially formulated in oil adjuvant. Methods: To carry out preclinical setting we emulsified the mix of GnRHm1-TT peptide with Very Small Size Proteoliposomes (VSSP) and Montanide ISA 51 with mechanical adjuvation. On the other hand a Phase I clinical trial using Heberprovac was carry out in 8 patients diagnosed with advanced prostate cancer. Results: In animal models, important preclinical evidences were obtained in pigs, dogs, rats and in no human primates, where a significant decrease in the size of testes and prostate (p<0,01) and consequently, testosterone fall until castration was observed. In a murine model the immunization with Heberprovac produced a significant tumor growth inhibition of Dunning R3327-H androgen responsive prostate tumor and significant survival of immunized animals. The first clinical trial with this vaccine candidate was addressed mainly into demonstrate the safety of the candidate. The study was finished in 6/8 patients included. No important adverse effects were detected. The good immunogenicity of the vaccine candidate in all the patients was in relationship with the values of Testosterone and PSA obtained. As conclusions we considered that the good results obtained in preclinical experiments are correlated with the favourably results observed in the first clinical setting using Heberprovac.

18. THERAPEUTIC USE OF CIMAVAX EGF VACCINE IN THE TREATMENT OF NON SMALL CELL LUNG CANCER PATIENTS

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Non–small-cell lung cancer (NSCLC) is one of the most common malignant diseases with a high mortality rate worldwide. Over the last 20 years, elevated levels of the epidermal growth factor (EGF) receptor (EGFR) and its cognate ligands have been identified as a common component of numerous cancer types. In fact, EGFR is overexpressed in 40% to 80% of NSCLC, and this overexpression is associated with a poor prognosis and resistance to cytotoxic agents. An Epidermal Growth Factor (EGF)-vaccine (CIMAvax) have been use in combination with Chemotherapy to treat this kind of tumors for the last 10 years. Here we will discuss the clinical experience accumulated over the years with this vaccine in the treatment of stage III/IV NSCLC patients that leaded to the official register of this vaccine as a therapeutic option in Cuba.

19. GENETICALLY MODIFIED CELLULAR VACCINES FOR THERAPY OF HPV16-ASSOCIATED TUMOURS

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Genetically modified cellular vaccines were found to be efficient against cancer both in experimental models and in tumour-bearing patients. It was shown in various systems that the efficacy of conventional therapeutic modalities can be increased by their combination with relevant immunostimulatory vaccines as well as by depletion of immunosuppressive immunocytes. The aim of this communication is to demonstrate that depletion of immunoregulatory immunocytes (T reg cells and immature myeloid cells ) can enhance the efficacy of genetically (IL-12) modified cellular vaccines administered alone, in combination with low doses of the cyclophosphamide derivative, or with surgery, in the experimental model of HPV 16-induced murine tumours mimicking human HPV 16-associated neoplasms such as cervical carcinomas. Moreover, it is shown that the increased percentage of the immunosuppressive immature myeloid cells (Gr-1+CD11b+) observed after chemotherapy with the CBM-4A ifosfamide derivative can be reduced both in the tumour and in spleen of the tumour-bearing mice by administration of the IL-12-producing vaccine and that the proliferative and cytotoxic capacity of tumour infiltrating lymphocytes from the CBM-4A treated mice is enhanced by administration of the IL-12-producing tumour vaccine.
20. CLINICAL EXPERIENCE WITH THERAPEUTIC CANCER VACCINES TARGETING NEUGC-CONTAINING GANGLIOSIDES

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Gangliosides are one of the immunosuppressive molecules released by tumors to their microenvironment. These glycosphingolipids are differentially distributed in tumoral vs. normal tissues. One such ganglioside is N-glycolyl GM3 (NGcGM3), which has become an attractive target for antigen-specific antitumor therapy as they are not normally expressed in humans and therefore constitute foreign Ags. At CIM, two vaccine candidates had been developed against this antigen. The NGNglycylated vaccine composed by NeuGcGM3 in a proteoliposome of Neisseria meningitides with Montanide ISA 51 as adjuvant and the 1E10 vaccine (Racotumumab) featuring a murine anti-Idiotipic mAb related to the NeuGc-containing ganglioside. Here we will discuss the clinical experience accumulated over the years with both vaccines in the treatment of several malignancies as NSCLC, melanoma and breast cancer.

21. HER1 VACCINE: A NEW THERAPEUTIC APPROACH FOR CANCER

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The Epidermal Growth Factor Receptor (EGFR or Her1) plays a central role in regulating neoplastic processes. The EGFR over-expression in many human epithelial tumors has been correlated with disease progression and bad prognosis. Passive EGFR-directed immunotherapy but not active specific approaches have already been introduced in medical Oncology practice. We have developed an active specific immunotherapy based on Her1-extracellular domain. Immunization of mice with autologous Her1-ECD adjuvated in very small sized proteoliposomes (VSSP) and emulsified in Montanide ISA-51 could circumvent tolerance to self EGFR, by inducing specific humoral and cellular immune response. Vaccination induced high titers of anti-EGFR polyclonal antibodies (PAb) of IgG2a and IgG 2b isotypes, which bind EGFR+ tumor cells and abrogated ligands-dependent EGFR phosphorylation. These PAb arrested tumor cells in G0/G1 stage and provoked cells apoptosis. Besides, vaccination induced CD4+ T cells proliferation and specific DTH in mice. Noteworthy, vaccination of mice with autologous vaccine stimulated a potent anti-metastatic effect in the EGFR+ Lewis lung carcinoma model while reproduction associated side effect were absent. These results further encouraged the development of a phase I clinical trial with Her1-ECD/VSSP therapeutic approach in patients with EGFR+ tumors.

22. PROGRESS IN TRANSLATIONAL APPLICATION OF THERAPEUTIC INTRATUMORAL AUTOLOGOUS DENDRITIC CELL INJECTION COORDINATED WITH APOPTOSIS-INDUCING THERAPY

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Introduction: Immune evasion by cancer is complex. One aspect is that antigen presentation by dendritic cells may be immunogenic or tolerogenic, and cancers may promote tolerogenic presentation. In contrast, an involuting tumor or apoptosis-rich environment may be particularly immunogenic. In murine models in which tumor apoptosis is induced by local or systemic treatment, intratumoral injection of syngeneic dendritic cells can mediate acquisition of anti-tumor immunity. In patient care, apheresis product processed with culture of plastic-adherent myeloid cells with GM-CSF and IL-4 can be used to prepare clinical grade autologous, not-yet-loaded dendritic cells. The translational challenge is to demonstrate feasibility, immunogenicity and therapeutic relevance. Introduction: In a murine xenograft model, intratumoral dendritic cell injection or tumor irradiation was not curative, but the combination induced regression and immunity to rechallenge. Localized prostate cancer with high risk of later distant recurrence has features that make it a clinical setting with some advantages to test this in man: Acquisition of lymphocytes with specificity for specific cancer associated antigens can be detected, because HLA-A2/peptide combinations are defined for prostate-cancer associated antigens; metastatic disease, if present, is microscopic, and may have correspondingly less relative influence on dendritic cell phenotype than in cases of bulk visible metastatic disease; there is a standard therapy that involves definitive-intent irradiation of the entire gross tumor volume, and, on the other hand, a risk of recurrence that would justify addition of an invasive investigational therapy. Disadvantages from the clinical-development perspective is that the time-to-recurrence, among those having recurrence, may be long, the recurrence risks are estimated with statistical models, and the radiation and local-therapy techniques for clinically locally prostate cancer are evolving rapidly. Methods: Patients with clinically localized prostate cancer, with high recurrence risk defined by reference to a nomogram, accounting for PSA,
Clinical T-stage and biopsy Gleason score, who tested (+) for HLA-A2 were treated with combined androgen blockade (4 months), external beam radiation (45 cGy in 25 fractions), and interstitial seed brachytherapy, with the addition of intraprostatic injection of autologous dendritic cells prepared from a single apheresis, after the 5th, 15th, and 25th radiation fractions (on Fridays). Biopsies were collected to evaluate for intratumoral lymphocytic infiltration and for apoptosis. Results: The patient selection, DC preparation and injection were feasible, with all 15 injections on-schedule and well tolerated. Among the five treated men, there are no PSA recurrences at 28+ months follow-up. Immunologic monitoring shows at least one peptide with at least 2x SD increase of ELISPOT titer, for each patient, varying among the assayed HLA-A2 associated peptides, derived from PSMA, PSA, Her2/neu and p53. Immunohistochemistry showed minimal lymphocytic infiltrates, not suggestive of an indefinable induced infiltration of CD4+ or of CD8+ cells. Some apoptosis was seen on some of the biopsies. Conclusions: Therapeutic-intent intratumoral dendritic cell injection is feasible and immunogenic. The optimal translational-development context for a combination of induced apoptosis and minimal microscopic distant-disease remains to be defined. Further trials of the technique, in pancreatic cancer and in soft tissue sarcoma are in development at our center.

23. A RANDOMIZED TRIAL OF PERSONALIZED PEPTIDE VACCINE (PPV) PLUS LOW-DOSE ESTRAMUSTINE (EMP) VERSUS FULL DOSE EMP IN PATIENTS WITH HORMONE REFRACTORY PROSTATE CANCER (HRPC)

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Introduction: Personalized selection of the right peptides for each patient could be a novel peptide-based immunotherapy for boosting anti-cancer immunity in many patients (pts). Methods: This was a randomized (1:1), open labeled, cross-over study in pts with HRPC. Pts were randomized to arm A; PPV plus low-dose EMP (280 mg/day) or arm B; full dose EMP (560 mg/day) according to age and PSA levels. In arm A, pre-vaccination plasma were measured for their IgG levels for each of the 14 or 12 candidate peptides which can induce HLA-A2 or A24-restricted CTL activity against cancer cells followed by bi-weekly subcutaneous administration of the top four peptides (3mg each) showing the strongest IgG responses. Results: A total of 54 pts were enrolled between Jun 2006 and Dec 2008. The accurate into arms A and B was 27 and 27 pts, respectively. The main pts characteristics are (arm A/B): median Age 71/69 years, EOCG performance status 0/1 96%/4% and 100%/0%, HLA A2/A24/A2A24 40%/32%/28% and 54%/27%/19%, median PSA 27/25 ng/mL, and metastatic HRPC 96%/85%. The personalized peptide vaccination was well tolerated with no major adverse effects. The median PFS time was 246 days in the arm A group and 85 days in the arm B group, respectively. The PFS time in the arm A was statistically longer than that in the arm B (Log-rank test: p = 0.0007, Hazard Ratio: 0.27, 95%CI: 0.12 to 0.615). Conclusions: PPV plus low-dose EMP was associated with improvement in PSA based PFS compared to full dose EMP alone.

24. HER1-ECD VACCINATION DISPENSES WITH EMULSIFICATION TO ELICIT HER1-SPECIFIC ANTI-PROLIFERATIVE EFFECTS

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EGFR (HER1) highlights as one of the most relevant tumour associated antigen in epithelial malignant cells. Monoclonal antibodies and tyrosine kinase inhibitors against EGFR remain as the most advanced approaches in clinical trials. More recently, an active immunotherapy using the HER1 extracellular domain (ECD) adjuvated in very small sized proteoliposomes (VSSP) and emulsified in Montanide ISA-51 demonstrated its strength to inhibit tumour cell line proliferation by arresting cells in G0/G1 stage and induction of apoptosis. In this study, we present a simpler HER1-ECD-based formulation, which is lacking the oily component Montanide ISA-51. Generated antibodies following non-emulsive formulation immunization recognized membrane EGFR; avoid EGF and TGFα coupling to EGFR leading to a marked abrogation of EGFR phosphorylation levels. Non-emulsive formulation also arrests cell cycle in G0/G1 stage, demonstrating it preserves previous formulation quality in a newer and simpler formulation.
25. PRODUCTION AND LONG TERM STABILITY OF A POLYSACCHARIDED VACCINE CANDIDATE FOR NEISSERIA MENINGITIDIS SEROGROUP C


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The immunogenicity of bacterial CPS can be greatly improved by chemical conjugation to a carrier protein and ensures that a T-cell-dependent immune response is induced. Three batches of a conjugate vaccine candidate (MenC/P64k) were produced under Good Manufacturing Practices, comprising the polysaccharide from N. meningitidis serogroup C covalently linked to the P64k protein by the reductive amination method. The batches were analyzed for long-term stability. Vials were stored at 4°C and samples were taken at 3, 6, 12, 18 and 24 months of storage. The study confirmed that MenC/P64k conjugated vaccine candidate does not change its physical and chemical properties upon 24 months after manufacture under low temperature conditions as storage. Moreover, the product was highly immunogenic throughout this period as evidenced by 100% of seroconversion in Balb/c mice. The MenC/P64k conjugate vaccine can be conserved lyophilized at 2-8ºC storage for 24 months without appreciable change in physical and chemical properties. During this time the formulation preserved its immunogenicity capacity similar to freshly produced batches. Our results show that the recombinant P64k meningococcal protein is a good carrier candidate for future conjugate vaccines.

26. CHARACTERIZATION OF THE IMMUNE RESPONSES GENERATED IN MICE AGAINST HBSAG FOLLOWING CO-ADMINISTRATION THROUGH MUCOSAL AND SYSTEMIC ROUTES WITH NASVAC FORMULATION

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One goal on the development of therapeutic vaccines for chronic hepatitis B is to achieve a potent and multispecific cellular immune response. In order to elicit this kind of response we developed a formulation called Nasvac, containing the surface and nucleocapsid antigens of HBV (HBsAg and HBcAg, respectively) and designed for nasal administration. To enhance the immune response, we report here the results of the co-administration of this candidate in BalbC mice by intranasal (IN) and systemic routes. In the case of the systemic administrations we also explored the influence of the addition of alum in the generated immune response. After two doses all the combinations used in the study generated a similar HBs-specific IgG response in sera. Based on the IgG subclasses results, in two of the three systemic routes evaluated, the presence of alum in the formulation clearly favored a Th2 pattern. The HBs-specific IgA in vaginal washes did not differed among the groups. After the first dose we evaluated the HBsAg-specific secretion of gamma interferon by CD8+ spleen cells by ELISpot. In this assay we observed a higher response for the group immunized IN/ subcutaneous without alum. Based on the obtained results we selected that variant for future evaluation in the HBs- transgenic mice model.

27. SYNERGISTIC EFFECT OF LEUKOPENIA INDUCED BY CHEMOTHERAPY AND A GM3-CONTAINING ADJUVANT ON THE INDUCTION OF CTL RESPONSES

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The combination of chemotherapy and immunotherapy for advanced cancer treatment is a “hot point” nowadays. The chemotherapeutic agents commonly promote severe lymphopenia and myeloablation. Contrary to preventive vaccines, cancer vaccines demand special types of adjuvants able to enhance anti-tumor responses in an immunocompromised scenario caused, in part, by chemotherapy. Very small sized proteoliposomes (VSSP) is a nanoparticulated TLR2 and TLR4 agonists
Combination vaccines are imperative to reduce the number of injections and to increase vaccination’s coverage. MenC/P64k is a polysaccharide conjugate vaccine candidate for Neisseria meningitidis serogroup C using P64k as protein carrier. A phase I trial demonstrated that MenC/P64k is well tolerated, with a good safety profile. The candidate was also highly immunogenic towards the polysaccharidic component. MenC/P64k is a lyophilized formulation which is mixed with aluminium phosphate at the moment of administration. The adsorption of MenC/P64k onto alum salts has not yet been study and constitutes the aim of this work. Adju-Phos (2%) and Alhydrogel (3%) were diluted to 5 mg/mL in buffer phosphate. MenC/P64k was added to each gel and shaking for 5 hours at 25 °C. Samples of P64k, free oxidized polysaccharide (FPox) and P64k plus FPox were included as controls. Polysaccharide and protein adsorption were measured by the resorcinol-hydrochloride and biscinconic acid methods respectively. The resulted Alum MenC/P64k (10ìg) formulations were evaluated in New Zealand White rabbits. Aluminum hydroxide showed high capacity (99.9%) to adsorb either MenC/P64k or P64k. This contrasted with the low capacity of Aluminum phosphate: P64k (36%) and MenC/P64k (6%). Both adjuvants showed no capacity to adsorb FPox. Rabbits immunized with MenC/P64k in aluminium hydroxide seroconvert and showed a 3 fold titer increase respect to rabbit receiving MenC/P64k in aluminium phosphate. The P64K protein mediates MenC/P64k adsorption onto Aluminum hydroxide. Data support stability studies of MenC/P64k conjugate candidate as a liquid formulation, which could facilitate combination vaccines.

28. DIFFERENTIAL ADSORPTION OF MENC/P64K CONJUGATE VACCINE CANDIDATE ONTO DIFFERENT ALUM SALTS. INFLUENCE ON THE ANTIBODY RESPONSE

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Combination vaccines are imperative to reduce the number of injections and to increase vaccination’s coverage. MenC/P64k is a polysaccharide conjugate vaccine candidate for Neisseria meningitidis serogroup C using P64k as protein carrier. A phase I trial demonstrated that MenC/P64k is well tolerated, with a good safety profile. The candidate was also highly immunogenic towards the polysaccharidic component. MenC/P64k is a lyophilized formulation which is mixed with aluminium phosphate at the moment of administration. The adsorption of MenC/P64k onto alum salts has not yet been study and constitutes the aim of this work. Adju-Phos (2%) and Alhydrogel (3%) were diluted to 5 mg/mL in buffer phosphate. MenC/P64k was added to each gel and shaking for 5 hours at 25 °C. Samples of P64k, free oxidized polysaccharide (FPox) and P64k plus FPox were included as controls. Polysaccharide and protein adsorption were measured by the resorcinol-hydrochloride and biscinconic acid methods respectively. The resulted Alum MenC/P64k (10ìg) formulations were evaluated in New Zealand White rabbits. Aluminum hydroxide showed high capacity (99.9%) to adsorb either MenC/P64k or P64k. This contrasted with the low capacity of Aluminum phosphate: P64k (36%) and MenC/P64k (6%). Both adjuvants showed no capacity to adsorb FPox. Rabbits immunized with MenC/P64k in aluminium hydroxide seroconvert and showed a 3 fold titer increase respect to rabbit receiving MenC/P64k in aluminium phosphate. The P64K protein mediates MenC/P64k adsorption onto Aluminum hydroxide. Data support stability studies of MenC/P64k conjugate candidate as a liquid formulation, which could facilitate combination vaccines.

29. PROCESS VALIDATION FOR CIMAvax-EGF VACCINE MANUFACTURE


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The Center of Molecular Immunology has been working on a novel cancer immunotherapy targeting the epidermal growth factor (EGF). CIMAvax-EGF is a vaccine composed of a conjugated of rhEGF and a carrier protein (rP64k), designed to trigger an anti-EGF antibody response. Advanced clinical trials demanded the scale-up and validation of the process manufacturing. As the product development cycle advanced to late clinica trials, the manufacturing process needed to be improved to comply with the GMP requirements and undergo validation. The work presented is focused on the process validation of two step of the manufacture process the CIMAvax-EGF: the conjugation reaction (between rhEGF an rP64k) and the purification of the chemical conjugates using UF/DF membrane. For this purpose the tolerance analysis tool was applied.

30. INHIBITION OF TUMOR-INDUCED MYELOID-DERIVED SUPPRESSOR CELLS FUNCTION BY A NANOPARTICULATED GM3-CONTAINING ADJUVANT

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The interaction between cancer vaccines’ adjuvants and myeloid-derived suppressor cells (MDSCs) is currently poorly understood. Very small sized proteoliposomes (VSSP) is a nanoparticulated adjuvant under investigation in several clinical trials. Experiments to evaluate the relationship between VSSP and myeloid cells circuit showed the induction of splenic CD11b+ population with less capacity to suppress CTL responses than MDSCs isolated from C26GM and EL-4 tumor-bearing mice. Functional activity of VSSP-induced MDSCs involved nitric oxide reactive species, probably through the preferential use of NOS3 instead of NOS2. Phenotypic characterization of these two CD11b+ populations showed, in VSSP-derived MDSCs, a relative decrease in the more suppressive Gr1+low IL-4Rα+high subpopulation. Interestingly, when EL-4 and C26GM tumor-bearing mice were also treated with VSSP, the isolated CD11b+ cells exhibited a reduced ability to inhibit T cells proliferation and CTL responses. Actually, both phenotype and function of these CD11b+ cells resembled VSSP-derived and not tumor-induced MDSCs. These results were also confirmed by the adoptive transfer of MDSCs and transgenic CD8+ T cells into tumor-free immunized mice. VSSP treatment of both CD11b+ cells donors and recipient mice abrogated the MDSCs-mediated inhibition of IFNγ production by transferred T cells. Moreover, VSSP was also able to avoid the suppression induced by an implanted CD11b+ cells-promoting tumor, contrary of what was seen with DCs vaccination. This effect of VSSP might be related with a more swiftly differentiation of tumor-induced MDSCs into mature APCs, both in the spleen and tumor site. Thus, VSSP could influence tumor-induced immnosuppression by generating MDSCs with reduced suppressive capacity in tumor-bearing hosts.

31. FROM ASCITIS TO BIOREACTOR: 1E10 AS A CASE STUDY

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1E10 mAb is a murine anti-idiotypic antibody that mimics N-glycolyl-GM3 gangliosides. This antibody has been used as an anti-idiotypic vaccine, adjuvanted in Al(OH)3, in several clinical trials including NSCLC, melanoma and breast cancer. The product used for these clinical trials was obtained from mice ascitis. Due to ethical, regulatory and scalability problems derived from the use of animals as bioreactors, we have developed a new pilot scale cGMP process based in stirred tank fermentation using protein free medium. Here we demonstrated the bioequivalence between 1E10 obtained from ascitis (1E10A) and stirred tank 1E10 (1E10ST) through a comparability exercise. We characterized the glycosylation, primary sequence, purity, pI, aggregation, degradation, real time and accelerated stability, absorption to Al(OH)3 and biological activity “in vivo” and “in vitro”. The main differences are the improvement in the adsorption to Al(OH)3 in 1E10ST respect to 1E10A, an increase in sialylation of 1E10ST and a slightly lower pl for 1E10ST than 1E10A. There weren’t posttranslational modifications neither mutations on the aminoacid sequence reported from cDNA for this mAb. Both 1E10 have shown similar “in vivo” and “in vitro” biological activity.

32. THE PRODUCTIVE/REGULATORY STRATEGY OF THE CANCER VACCINE NGcGM3/VSSP

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The Center of Molecular Immunology has developed NGcGM3/VSSP cancer vaccine, which is generated by hydrophobic incorporation of NgcGM3 into the outer membrane protein complex (OMPC) of Neisseria meningitides bacteria. The clinical results in a Phase II, Proof of concept (POC) trial showed a significant increase of overall survival ratio of treatment in metastatic breast cancer patients. In consequence, the submission of the conditional registration in Cuba for NGcGM3/VSSP cancer vaccine could be done. However, this fact demanded a new productive/regulatory strategy for the product. The first step was the technology transfer of active pharmaceutical ingredients (API) production’s to Good Manufacturing Practice (GMP) facilities through the Contract Manufacturing Organization (CMO) with BioCen. Three different consistencies API’s batch were produced and released. Finally, the formulation step was transfer to the CIM’s formulation/filling GMP Plant. This work allowed the scale up and GMP status of vaccine’s productive process for covering the clinical trials supplies and establishes a redundant Quality Monitoring System for the whole steps of productive process. As a result of this strategy we show the relation between Pharmaceutical Development and Technology Transfer in the product life cycle, which could reduce time, cost and regulatory requirement for product licensure.
The use of Aluminum Hydroxide (AH) gel as adjuvant is very common in many vaccines. Particularly, in allergen therapeutic vaccines, AH induces a depot effect and enhances IgG antibody production. The adjuvant effect depends on the degree of antigen adsorption on to the gel. In order to develop a new allergen vaccine formulation, we evaluated the effect of phosphate ions in the buffer solution on the adsorption process of Dermatophagoides siboney (DS) House Dust Mite allergens to AH. For this purpose 4 pilot scale batches, were manufactured in aseptic conditions. Three of them contained DS allergen extract (VALERGEN-DS, BIOCEN, Cuba) in saline solution without phosphate ions, at adjusted neutral pH, and another with the same extract in phosphate buffered saline solution pH 6.8. All of them were adsorbed to AH. The adsorption degree was assessed by measuring the content of the Der s 1 allergen protein in the supernatant by MAb-ELISA. The adsorption values of the batches formulated without phosphate were higher than 98.2 %, with a mean value of 98.8 %. There was no significant difference between them (p<0.05), proving the process’ consistency. In contrast, the degree of adsorption of the batch prepared in phosphate solution was the lowest: 53.4 %. These results show the negative effect of phosphate ions on the adsorption process of mite allergens to AH, recommending the use of a buffer containing less or none phosphate ions.

Bacterial pore-forming proteins have been used to design cytosolic delivery systems of macromolecules, such as antigens. Different strategies have been employed including the co-encapsulation of cytolytic toxins or peptides with antigen into liposomes in order to improve the antigen-specific cytotoxic T CD8+ lymphocyte response. Sticholysins (St I and St II) are 20 kDa cysteinless isotoxins from the sea anemone Stichodactyla helianthus forming tetrameric pores in membranes with a 2 nm diameter. Considering that Sts share functional homology with bacterial pore-forming toxins, it was possible to presuppose that they could also exhibit the ability to modulate the antigen-specific immune response. Liposomal vesicles comprised of dipalmitoyl phosphatidylcholine (DPPC) and cholesterol (Cho), containing ovalbumin (OVA) as model antigen and St I or St II (liposomes/OVA+St), were used. The cytotoxic T lymphocyte assays in C57BL/6 mice demonstrated the capability of liposomes/OVA+St to promote activation of the T CD8+ lymphocytes mediated response in vivo, in comparison with liposomes without St or the positive control (P I+C). Liposomes containing St I W111C, a mutant able to form a reversible inactive dimer stabilized by disulphide bond, similarly enhanced the OVA- specific T CD8+ lymphocytes mediated response. Moreover, liposomes/OVA+St conferred protection to mice from challenge with OVA-expressing tumor cells. These immunomodulator properties of Sts are probably a result of their particular molecular structure and/or membranotropic properties and suggest their potentialities for the design of new cytosolic delivery systems of exogenous antigens.

A heterophilic ganglioside cancer vaccine was developed by combining NeuGcGM3 with the outer membrane protein complex of Neisseria meningitides to form very small size proteoliposomes (VSSP), a proven adjuvant agent in pre-
clinical studies. A phase I clinical trial was performed to determine safety and immunogenicity of this vaccine administered subcutaneously in metastatic breast cancer and melanoma patients. **Patients and Methods:** Two Phase Ib/IIa clinical trials were carried out in patients with advanced breast cancer and melanoma, to evaluate immunogenicity and toxicity of a subcutaneously administered NeuGcGM3-based cancer vaccine. Thirty-five metastatic breast cancer patients were included in six dose-level cohorts of 150, 300, 600, 900, 1200, and 1500 Ig. So were thirty-five metastatic melanoma patients. **Results:** Of 28 treated breast cancer patients, who completed the induction phase, developed anti-NeuGcGM3 antibody titers between 1:160 and 1:10240 immunoglobulin G (IgG), and 1:320 and 1:10240 IgM. Noteworthy, those patients vaccinated with 900 Ig of NeuGcGM3 elicited the highest maximum IgM median titer against the ganglioside. This value is comparable, although not statistically because of sample numbers disparities, to the median maximum IgM titer elicited by the same type of patients vaccinated intramuscularly with NeuGcGM3/VSSP/Montanide ISA51 (Phase II Study). Remarkably, even though the follow-up for melanoma patients hasn’t finished, the maximum IgG and IgM titer against NeuGcGM3 reach up to 1:2560 and 1:10240, respectively. **Conclusion:** NeuGcGM3/VSSP is an unusual immunogenic ganglioside vaccine administered subcutaneously without adjuvant but VSSP.

### 36. PROCESS DEVELOPMENT FOR PRODUCTION OF A THERAPEUTIC CANCER VACCINE BASED ON EXTRACELLULAR DOMAIN OF HER-1 RECEPTOR


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The epidermal growth factor receptor (EGFR) belongs to the erbB family of 4 closely related cell membrane receptors, also known as the Type I receptor tyrosine kinase family (HER-1). Although expressed in nonmalignant cells, the EGFR can be found overexpressed or mutated in many human epithelial tumors such as breast, lung, prostate, vulva and ovarian tumors. In our work we present the up- and down-stream process development in order to obtain a therapeutic vaccine based on the extracellular domain (ECD) of HER-1. In the present study a number of different protein-free media and supplements with various additives were screened in spinner flasks for cell growth, protein production and integral of viable cells. The development of a fed batch strategy employing the selected media formulation in 15 L bioreactor considerably enhanced process yields. We demonstrated for first time that HER1-extracellular domain can be purified by a downstream process based on immunoaffinity chromatography from bioreactor supernatant of HEK 293 cell line. Filtered supernatant was applied to CNBr- activated Sepharose CL-4B with monoclonal antibody anti human EGF immobilized, followed by three additional chromatographic polishing steps. ECD-HER1 was obtained with high purity (>95%), low DNA content, and biological activity, allowing the clinical use as cancer vaccine formulation.

### 37. BIOLOGICAL SAFETY DEMONSTRATION OF CIGB-440 VACCINE ADYUVATED WITH ALUMINUM HYDROXIDE

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Chronic infection originated by the virus of hepatitis B (HBV) supposes a problem of important public health. Nearly 350 million carriers of this virus exist in the world. The infection by HBV is responsible for 1.5 million annual deaths by cirrhosis and hepatocellular carcinoma. Immunotherapy with vaccines in the treatment of the chronic infection by the HBV not only includes prophylactic vaccines available in the market, but also the development of new vaccine candidates with therapeutic aims. In the present work the results of the toxicological studies performed to vaccine CIGB-440 adyuvated with aluminum hydroxide are exposed. The toxicological scheme to evaluate the security of this vaccine include: studies of acute toxicity, local tolerance and repeated doses of toxicity, with duration of 14, 40 and 60 days, respectively. The product was administered by intramuscular route using both sexes of Sprague- Dawley rats. Different levels of doses were explored, including the therapeutic dose, as well as placebo of the formulation. Obtained results showed that in the studied levels of doses of CIGB-440 vaccine is not toxic and does not shows systemic adverse effects in the species used. In macroscopic observations alterations were not observed. We can conclude that CIGB-440 vaccine is safe and tolerable by intramuscular route.
38. RECOMBINANT NUCLEOCAPSID-LIKE PARTICLES FROM DENGUE 2 VIRUS INDUCE PROTECTIVE CD4+ AND CD8+ CELLS AGAINST VIRAL ENCEPHALITIS IN MICE

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Virus-like particles (VLP) are an effective type of subunit vaccine that mimics the overall structure of the virus. The relatively large size of VLP often leads to efficient antigen processing and presentation by dendritic cells, thus promoting maturation and migration. The variety of molecular assemblies typical of viral structures offers several alternatives for the obtention of virus-like particles. The capsid proteins of enveloped viruses, in particular, have been widely used for this purpose; and the VLP obtained in these cases are often named nucleocapsid-like particles (NLPs). In the present work, the recombinant capsid protein of dengue-2, in a particulate form, was evaluated in mice to determine the level of protection against viral challenge and to measure the induced cell-mediated immunity. The NLPs adjuvated on alum did not induce antibodies against the whole virus whereas splenocytes of the immunized animals upon virus stimulation secreted high levels of IFN-$\gamma$. In addition, a significant protection was obtained upon challenge with lethal dengue-2 virus. Finally it was demonstrated the contribution of the memory CD4$^+$ and CD8$^+$ cells in the IFN-$\gamma$ secretion as well as in the protection rate. This study constitutes a proof of concept that cell-mediated immunity is able to protect against dengue virus infection without the contribution of the humoral immune response. On the other hand, it supports the NLPs as a suitable vaccine candidate against dengue virus.

39. CIGB-230, A DNA-BASED THERAPEUTIC VACCINE CANDIDATE AGAINST HEPATITIS C VIRUS INFECTION

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Hepatitis C virus is a worldwide health problem. No vaccine is available against this pathogen and therapeutic treatments currently in use are of limited efficacy. CIGB-230, a novel therapeutic vaccine candidate based on the mixture of pldKE2, a plasmid expressing HCV structural antigens, with a recombinant HCV core protein, Co.120, has rendered satisfactory results in pre-clinical studies. In addition, CIGB-230 has been already administered by intramuscular injection on weeks 0, 4, 8, 12, 16 and 20 to fifteen HCV-chronically infected individuals, non-responders to previous treatment with interferon plus ribavirin. No serious and no severe adverse events were observed at any moment. Interestingly, following the final immunization, neutralizing antibody responses against heterologous viral pseudoparticles were modified in 9 individuals, including 6 de novo responders. In addition, 73% of vaccinated patients exhibited T cell proliferative response and T cell IFN-gamma secretory response at 24 weeks after primary immunization with CIGB-230. Furthermore, 33.3% of individuals developed de novo cellular immune response against Core antigen and 46.7% diversified this cellular immune response against more than one antigen. In addition, despite persistent detection of HCV RNA, more than 40% percent of vaccinated individuals improved or stabilized liver histology, particularly reducing fibrosis. Therefore, CIGB-230 is a promising candidate for effective therapeutic interventions.

40. DEVELOPMENT OF AN ANTI IL-15 AUTO-VACCINE


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The IL-15 is an immune co stimulatory cytokine that is expressed on uncontrolled form in several autoimmune and inflammatory diseases as Rheumatoid arthritis and psoriasis. Inhibition of IL-15-induced signaling could be clinically beneficial. In our approach
to inhibit IL-15 we tested active immunization with structurally modified IL-15 in Aluminum, Montanide and Freund adjuvants in non human primates and we obtained high titers of neutralizing antibodies against native human IL-15. Recombinant IL-15 was obtained from E coli. After purification steps, the protein was obtaining with more than 95 purity percent and adjuvated with Aluminum hydroxide, Montanide and Freund. Monkeys were immunized by subcutaneous injections of adjuvated- recombinant IL-15 or control preparation. Sera were collected 15 days or 3 month after the third immunization. The antibodies titer was determinate by ELISA and CTLL-2 proliferation assays. Active immunization with adjuvanted recombinant modified IL-15 induces high titers of IgG neutralizing antibodies against human IL-15. Sera from immunized animals inhibit IL-15-dependent cell lines proliferation, suggesting that active immunization is capable of breaking immunological B cell tolerance. Aluminum and Montanide showed the similar levels of antibodies, but Aluminum induced the better neutralizing response. The immune response is regulate and decrease to baseline 2 months after immunization but the booster recovery high titer of antibodies. No chance in hematological and biochemical parameters was observed in immunized animals. Vaccination with recombinant modified IL-15 was able to induce neutralizing antibody response to native IL-15 in non human primate.

41. SUBLINGUAL IMMUNOTHERAPY WITH TROPICAL HOUSE DUST MITE ALLERGEN VACCINES: THERAPEUTIC EFFECT AND HIGHER SAFETY PROFILE FOR ALLERGIC ASTHMA IN A CUBAN POPULATION


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Subcutaneous allergen-specific immunotherapy is burdened with the risk of severe systemic reactions; therefore, sublingual administration route has been increasingly investigated worldwide. This study was conducted to assess the therapeutic effect and safety of allergen therapeutic vaccines of Dermatophagoides pteronyssinus, Dermatophagoides siboney and Blomia tropicalis House-Dust mites (VALERGEN, BIOCEN, Cuba) by sublingual route, in asthmatic patients. Three Double-Blind Placebo-Controlled clinical trials were performed in 40 patients each, showing asthmatic symptoms and positive predominant Skin Prick Test (SPT) to each mite, respectively. Half of subjects were randomized to placebo. Patients received the treatment consisting on sublingual drops with increasing daily doses for 3 weeks and maintenance doses (2000 BU) twice a week until 12 moths. Therapeutic effect was assessed after 6 and 12 months using symptoms/medication diary cards, peak expiratory flow (PEF) measures and skin sensitivity to investigated mites. Adverse reactions were classified using the World Allergy Organization scale. The treatment reduced significantly (p<0.01) clinical symptoms (38%, CI95%: 33-44) and medication intake (26%, CI95%:21-32) with respect to placebo. The skin sensitivity to the allergens decreased also significantly (p<0.01). The allergen amount needed to induce a positive SPT increased 52-fold. PEF variability decreased also significantly (p<0.05). The treatment was considered effective in 77% of patients. A major advantage as compared to subcutaneous route was a remarked lower frequency of adverse effects. Local reactions were noted only in 0.43% of administrations. No systemic reactions were observed. The results indicate that sublingual immunotherapy using VALERGEN vaccines is effective and safe in mite-sensitive asthmatic patients.

42. HETEROLOGOUS PRIME-BOOST STRATEGY IN NONHUMAN PRIMATES COMBINING THE INFECTIVE DENGUE VIRUS AND A RECOMBINANT PROTEIN IN A FORMULATION SUITABLE FOR HUMAN USE


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The present work has the main goal to proof the concept of the heterologous prime-boost strategy combining an infective dengue virus with a recombinant chimeric protein carrying the domain III of the Envelope protein. In a first study, monkeys received four doses of the protein PD5 (domain III of the Envelope protein from dengue-2 virus, fused to the protein carrier P64k) and were subsequently infected with one dose of dengue virus. In a second study, monkeys were infected with one dose of the infective virus and subsequently boosted with one dose of the recombinant protein. In the first study, the antibody response in vaccinated monkeys after challenge was high in terms of antiviral and neutralizing antibodies and similar to those developed in the group that received one or two doses of infective virus. The antibody titers were observed until one year after
Immediate allergic reactions are mediated by IgE antibodies specific to environment allergens. Induction of IgG4 allergen-specific antibodies can play a blocking role of the allergic response in patients subjected to allergen immunotherapy (therapeutic vaccination). These antibodies could be induced also by natural exposure to high allergen levels. The aim of this work was to determine allergen-specific IgE and IgG4 antibodies in a population of 120 adult allergic patients, with positive Skin-Prick-Test (SPT) responses to at least one of the 3 most relevant species of domestic mites in our country: Dermatophagoides pteronyssinus (Dp), D. siboney and Blomia tropicalis. SPT was performed with VALERGEN allergenic extracts (BIOCEN, Cuba) at a concentration of 20 000 BU/mL. Allergen-specific IgE and IgG4, as well as, total IgE levels were measured by ELISA. The IgE response was similar in intensity between mite species, whereas greater IgG4 response was observed to Dp. A significant correlation (p<0.05) was detected between the skin reaction size and total or allergen-specific IgE, as expected. On the other hand, the IgG4 response was inversely correlated to specific IgE, and, in the case of patients with high titers, IgG4 was even negatively correlated to the skin reaction size, suggesting to play an anti-allergic protecting role in these patients. Thus, allergen-specific IgG4 antibodies may become a relevant parameter for a full characterization of the patient’s clinical allergic status and an attractive target for monitoring the success of immunotherapy.

43. IgG4 ALLERGEN-SPECIFIC ANTIBODIES ARE INVERSELY CORRELATED TO IgE MEDIATED ALLERGIC RESPONSE IN ASTHMATIC PATIENTS ALLERGIC TO DOMESTIC MITES

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VSSP (very small sized proteoliposomes) is a new approach to enhance immune restoration and control HIV replication. Properties of VSSP as immunopotentiator have been reported. VSSP is a potent adjuvant for dendritic cells activation and Th1 differentiation. VSSP was obtained through the incorporation of NAcGM3 (a strong immunosuppressive ganglioside) into the outer membrane protein complex of Neisseria meningitides (Nm). On the other hand, certain experimental evidences support the view that NAcGM3 and CXCR4 are components of a functional multi-molecular complex critical for HIV-1 entry. 43 patients treated with intramuscular injections of VSSP, emulsified with Montanide ISA 51, were enrolled in a Phase I clinical trial. Two groups were conformed; patients in the first group initiated anti-retroviral therapy (lamivudine + stavudine + nevirapine) 3-6 months before starting VSSP treatment while in patients from the second group the retroviral therapy and VSSP were concomitantly administered. The first five doses were injected every two weeks (induction phase) and the other six doses, monthly. Primary outcome was the clinical tolerance. Main toxicities were: pain in the injection site, fever and cepahlea, which disappeared spontaneously or by antipyretic treatment. The frequency of events (classified according to WHO) was similar in both groups. All treated patients remained alive and/or free of new AIDS-defining events for eighteen months. The 96% of adverse events were classified as mild and moderate. After 18 months of VSSP+ anti-retroviral viral therapy treatment the increase of the median of CD4 were 54 cells/µL in the first group and 408 cells/µL in the second group. More than 42% of patients generated antibodies against the NAcGM3 ganglioside. Patients remain alive and free of opportunistic disease for 18 months. Our findings support the safety of VSSP + antiretroviral treatment in HIV/AIDs patients.

44. SAFETY OF VSSP AS IMMUNOPOTENTIATOR IN CUBAN HIV/AIDS PATIENTS TREATED WITH ANTIRETROVIRAL


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VSSP (very small sized proteoliposomes) is a new approach to enhance immune restoration and control HIV replication. Properties of VSSP as immunopotentiator have been reported. VSSP is a potent adjuvant for dendritic cells activation and Th1 differentiation. VSSP was obtained through the incorporation of NAcGM3 (a strong immunosuppressive ganglioside) into the outer membrane protein complex of Neisseria meningitides (Nm). On the other hand, certain experimental evidences support the view that NAcGM3 and CXCR4 are components of a functional multi-molecular complex critical for HIV-1 entry. 43 patients treated with intramuscular injections of VSSP, emulsified with Montanide ISA 51, were enrolled in a Phase I clinical trial. Two groups were conformed; patients in the first group initiated anti-retroviral therapy (lamivudine + stavudine + nevirapine) 3-6 months before starting VSSP treatment while in patients from the second group the retroviral therapy and VSSP were concomitantly administered. The first five doses were injected every two weeks (induction phase) and the other six doses, monthly. Primary outcome was the clinical tolerance. Main toxicities were: pain in the injection site, fever and cephalalgia, which disappeared spontaneously or by antipyretic treatment. The frequency of events (classified according to WHO) was similar in both groups. All treated patients remained alive and/or free of new AIDS-defining events for eighteen months. The 96% of adverse events were classified as mild and moderate. After 18 months of VSSP+ anti-retroviral viral therapy treatment the increase of the median of CD4 were 54 cells/µL in the first group and 408 cells/µL in the second group. More than 42% of patients generated antibodies against the NAcGM3 ganglioside. Patients remain alive and free of opportunistic disease for 18 months. Our findings support the safety of VSSP + antiretroviral treatment in HIV/AIDs patients.
45. SAFETY PROFILE OF A THERAPEUTIC DNA VACCINE PREPARATION, IN HCV-CHRONICALLY INFECTED INDIVIDUALS

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About 3% of the world’s total population is infected with the hepatitis C virus (HCV). Current therapies against HCV are effective in only half of the patients approximately and limited by side effects that often require discontinuation. In the present work we describe for the first time the safety profile of a new therapeutic vaccine candidate against HCV: CIGB-230, which is based on the mixture of a plasmid expressing HCV structural antigens, with a recombinant HCV core protein. A phase I clinical trial was conducted in accordance with good clinical practice principles. Six doses of CIGB-230 were administered by intramuscular injection in the deltoid region of the left arm each 4 weeks to fifteen HCV-chronically infected volunteers non-responders to previous treatment with interferon plus ribavirin. Blood samples were collected every four weeks. All patients completed the treatment. Adverse events were light or moderate and most of them were detected during the first 24 h after immunization. Local pain, headache, and asthenia were the most frequent adverse events. After the last immunization, HCV genotype 1b ARN was detected in all individuals. Hematological and biochemical parameters, including serum aminotransferases and parameters related with hepatic synthesis, remained generally stable during treatment. No anti-mitochondrial, anti-nuclear and extractable nuclear antigen autoantibodies were generated during immunization. In conclusion, vaccination with CIGB-230 in HCV-chronically infected individuals is safe and well tolerated.

46. NUCLEOCAPSID-LIKE PARTICLES OF DENGUE-2 VIRUS ENHANCED THE IMMUNE RESPONSE AGAINST A RECOMBINANT PROTEIN OF DENGUE-4 VIRUS

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Virus-like particles (VLP) are an effective type of subunit vaccine that mimics the overall structure of the virus. The relatively large size of VLP often leads to efficient antigen processing and presentation by dendritic cells, thus promoting maturation and migration. The variety of molecular assemblies typical of viral structures offers several alternatives for the obtention of virus-like particles. The capsid proteins of enveloped viruses, in particular, have been widely used for this purpose; and the VLP obtained in these cases are often named nucleocapsid-like particles (NLP). In this study we evaluate a novel formulation in mice containing nucleocapsid-like particles of dengue-2 (recNLP) co-immunized with a chimeric protein composed by the dengue-4 envelope domain III fused twice within the meningococcal P64k protein of Neisseria meningitidis (PD24). After four doses in balb/c mice, the animals receiving the PD24-recNLP mixture showed the highest levels of antiviral antibodies. Similar results were obtained for IFNα-secretion levels, indicating a functional Th1 cellular response. Consistently, survival percentages after viral challenge were significantly superior in mice immunized with the mixture than in those inoculated with PD24 protein alone (92% vs. 50% in the first immunization schedule and 100% vs. 50% in the second). In addition, in vivo-depletion experiments demonstrated the decisive role of the CD4+ and CD8+ cells in the protection conferred by the immunization with PD24-recNLP. This study demonstrates for the first time, the adjuvant capacity of dengue-2 virus recNLP. Additionally, the presented evidence highlights the potential of these particles for enhancing the immune response against heterologous recombinant proteins.
47. INFLUENCE OF A PROTEOLIPOSOME ADJUVANTED ALLERGEN VACCINE ON TO AN EARLIER RESPONSE AGAINST NEISSERIA MENINGITIDIS

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A novel anti-allergic therapeutic vaccine candidate is based on purified allergens of Dermatophagoides siboney House Dust Mite and proteoliposome (PL) of Neisseria meningitidis as immuno-stimulatory adjuvant. A major potential benefit provided by this vaccine would be enhancement of efficacy of allergen vaccination, reducing the number of injections required for that treatment. The PL is a component of the anti-meningococcal vaccine (VAMENGOC-BC, Finlay Institute, Havana), therefore, this study aimed at assessment of the influence of the anti-allergic vaccine on to an earlier response against N. meningitidis induced by prophylactic vaccination. It was measured the PL-specific IgG antibody response, including IgG1 and IgG2a subclasses, before and after the administration of three doses of the allergen vaccine (2 µg Der s 1, each) in Balb/C mice vaccinated previously with two doses of VAMENGOC-BC. The allergen specific IgG and subclass antibody response was also evaluated. The administration of the PL-containing allergen vaccine in these mice showed only a slight dose-dependent increase on PL-specific IgG, IgG1 and IgG2a antibodies. Unexpectedly, previous immunization with VAMENGOC was associated to a significant increase of the allergen-specific IgG, IgG1 and IgG2a antibody response induced by the later administration of the allergen vaccine (ANOVA, p<0.05). Current results confirmed that the highest IgG2a and IgG1 response to the allergen vaccine was obtained after the third dose. In conclusion, the results sustain the safety of this novel anti-allergic vaccine with regard to its lack of negative influence to anti-meningococcal response.

48. IMMUNOLOGICAL EVALUATION IN NONHUMAN PRIMATES OF FORMULATIONS BASED ON THE CHIMERIC PROTEIN P64K-DOMAIN III OF DENGUE 2 AND TWO COMPONENTS OF NEISSERIA MENINGITIDIS

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The main problem in the development of successful vaccines against dengue based on recombinant proteins is the necessity to use potent adjuvants to reach a proper functional immune response. Our group reported the expression, characterization and immunological evaluation of the recombinant protein PD5, which contains the domain III of the Envelope protein from dengue 2 virus fused to the carrier protein P64k. This construct completely protected monkeys against viral challenge when the Freund’s adjuvant was employed. The present work relies on the evaluation of PD5, produced with a high purity and under GMP conditions, when formulated either with outer membrane vesicles (OMV) or the serogroup A capsular polysaccharide (CPS-A) from Neisseria meningitidis, both adsorbed on aluminum hydroxide. The antibody response to the formulation containing the CPS-A was clearly superior to that of the formulation with OMV. The experiment of in vivo protection supported this evidence, since only the group immunized with PD5 and CPS-A was partially protected upon viral challenge. This is the first study in which the polysaccharide A of N. meningitidis is successfully employed as adjuvant for viral antigens.
Introduction: The use of vaccines for patients suffering chronic diseases like cancer and other severe infectious illnesses (AIDS, Hepatitis, Tuberculosis, Malaria) is increasing nowadays. Great interest is being put on developing therapeutic vaccines with the hope of reaching very good results in a short time of period. Biopharmaceutical industry shows very promising results in the development of this kind of products, requiring a refinement of regulatory mechanisms that assure the needed flexibility for reaching the market without compromising the quality, safety and efficacy of these products according to the international standards. The aim of this Paper is to perform the pharmacological clinical development evaluation for therapeutic vaccines for Cancer and AIDS in Cuba as well as to set up the points to consider for the clinical evaluation strategy. Methodology. For that we reviewed the current national and international regulations specific for therapeutic vaccines, carried out a quantitative-qualitative analysis of therapeutic vaccines already known in Cuba and identified the most common problems for implementing a consistent clinical evaluation strategy. Results: They were assessed 51 clinical trials authorisations and 7 from them classified as recombinant protein, ganglioside, antidiotypic, vector and peptide vaccine candidates. With this information was compiled a Web format document able to provide access to the regulatory framework for therapeutic vaccines. At the same time, a new document was proposed for implementing the clinical evaluation strategy of therapeutic vaccines for Cancer and AIDS taking into account relevant aspects like design, population target, effectiveness and toxicity evaluation as well as market authorisations. Conclusions: The present work enables the National Regulatory Agency to regulate in a better way the clinical evaluation of therapeutic vaccines for Cancer and AIDS.

50. EVALUATION OF THE PHARMACOLOGICAL CLINICAL DEVELOPMENT FOR THERAPEUTIC VACCINES IN CANCER AND AIDS

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Introduction: The use of vaccines for patients suffering chronic diseases like cancer and other severe infectious illnesses (AIDS, Hepatitis, Tuberculosis, Malaria) is increasing nowadays. Great interest is being put on developing therapeutic vaccines with the hope of reaching very good results in a short time of period. Biopharmaceutical industry shows very promising results in the development of this kind of products, requiring a refinement of regulatory mechanisms that assure the needed flexibility for reaching the market without compromising the quality, safety and efficacy of these products according to the international standards. The aim of this Paper is to perform the pharmacological clinical development evaluation for therapeutic vaccines for Cancer and AIDS in Cuba as well as to set up the points to consider for the clinical evaluation strategy. Methodology. For that we reviewed the current national and international regulations specific for therapeutic vaccines, carried out a quantitative-qualitative analysis of therapeutic vaccines already known in Cuba and identified the most common problems for implementing a consistent clinical evaluation strategy. Results: They were assessed 51 clinical trials authorisations and 7 from them classified as recombinant protein, ganglioside, antidiotypic, vector and peptide vaccine candidates. With this information was compiled a Web format document able to provide access to the regulatory framework for therapeutic vaccines. At the same time, a new document was proposed for implementing the clinical evaluation strategy of therapeutic vaccines for Cancer and AIDS taking into account relevant aspects like design, population target, effectiveness and toxicity evaluation as well as market authorisations. Conclusions: The present work enables the National Regulatory Agency to regulate in a better way the clinical evaluation of therapeutic vaccines for Cancer and AIDS.
51. ANTI-GANGLIOSIDE ANTI-IDIOTYPIC MONOCLONAL ANTIBODY-BASED CANCER VACCINE INDUCES APOPTOSIS AND ANTIANGIOGENIC EFFECT IN A METASTATIC LUNG CARCINOMA

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Anti-idiotype monoclonal antibody (mAb) 1E10 was generated by immunizing BALB/c mice with an Ab1 mAb which recognizes NeuGc-containing gangliosides, sulfatides and some tumor antigens. 1E10 mAb induces therapeutic effects in a primary breast carcinoma and a melanoma model. However, the tumor immunity mechanisms have not been elucidated. Here we show that aluminum hydroxide-precipitated 1E10 mAb immunization induced anti-metastatic effect in the 3LL-D122 Lewis Lung carcinoma, a poorly immunogenic and highly metastatic model in C57BL/6 mice. The therapeutic effect was associated to the increment of T cells infiltrating metastases, the reduction of new blood vessels formation and the increase of apoptotic tumor cells in lung nodules. Interestingly, active immunization does not induce measurable antibodies to the 1E10 mAb, the NeuGc-GM3 or tumor cells, which may suggest a different mechanism which has to be elucidated. These findings may support the relevance of this target for cancer biotherapy.

52. CELLULAR IMMUNE RESPONSE DEPENDENCE FOR ANTIMETASTATIC EFFECT OF A TUMOR ASSOCIATED GANGLIOSIDE-CONTAINING VACCINE

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Gangliosides are glycosphingolipids differentially expressed on normal and cancer cells. In humans, the N-glycolylated variant of GM3 ganglioside (NGcGM3) is a tumor-associated antigen. Furthermore, this ganglioside exhibit immunosuppressive properties becoming an attractive target for antigen specific cancer immunotherapy. We prepared an NGcGM3 ganglioside-based vaccine by combining Neisseria meningitides derived outer membrane proteins and the ganglioside, originating very small size proteoliposomes (VSSP). NGcGM3/VSSP vaccine has demonstrated to potentiate ganglioside specific humoral response in advanced cancer patients. The present work explores relevance of effector cellular immune response for the anti-tumoral effect of the NGcGM3/VSSP vaccine.

The vaccine efficacy was evaluated on a 3LL-D122 carcinoma tumor model of spontaneous metastatization. NGcGM3 expression was confirmed on primary tumors and in metastatic lung sections obtained from 3LL-D122 cells inoculated C57BL/6 mice. Animals challenged with tumor cells were subcutaneously treated with NGcGM3/VSSP vaccine to address anti-metastatic effect of the ganglioside preparation. Spontaneous lung metastases were evaluated three weeks after primary tumor resection. Therapeutic administration of NGcGM3/VSSP significantly reduced spontaneous lung metastases. Noteworthy, specific depletions of CD8+ and NK1.1+ T cells abrogate the NGcGM3/VSSP vaccine anti-metastatic effect, suggesting vaccine effectors mechanism involve cell mediated immune response.

Obtained results are the first evidence of effector cells recruitment by this vaccine preparation even when putative antigen targeted on tumor cells naturally induced a T cell independent immune response.
53. PHASE IB/IIA OF NGCGM3/ VSSP IN THE TREATMENT OF ADVANCED CUTANEOUS AND OCULAR MELANOMA PATIENTS

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Introduction: Active specific immunotherapy is an extensively explored choice in the last decade by the Center of Molecular Immunology, La Havana, Cuba, evaluating among others, a cancer vaccine composed by NGcGM3 in a proteoliposome of Neisseria meningitides (NGcGM3/VSSP). Material and methods: Fifty-seven patients were included in two clinical trials with NGcGM3/ VSSP with and without Montanide ISA 51 as adjuvant. Immunization schedule was fortnightly for the first five doses and then, monthly up to one year. Main objectives were Immunogenicity, toxicity and tumor response. Results: NGcGM3/ VSSP toxicity was local pain in the site of injection, flu-like symptoms, consisted of: fever, myalgia, chills, and headache, mainly grades I-II. Pre and post immune antibody titers against NGcGM3 ganglioside were evaluated in all assessable patients. AntiNGM3 IgG and IgM antibody responses were induced in all vaccinated patients. Additionally, IgA specific antibodies were detected. No statistical differences were found among different dose levels of NGGM3 and ganglioside antibody titers. Interestingly, antibody responses against NAcGM2 and NAcGM3 were present. Anti tumor activity with unexpected survival times despite the advanced stage of the patients was shown, mainly associated with the 900 µcg level subcutaneously. Vitiligo as evidence of autoimmunity was observed in some patients mainly in lower dose levels not directly associated with the use of adjuvant. Conclusions: NGcGM3/VSSP showed immunogenicity and safety with some evidences of antitumor activity in advanced melanoma patients, suggesting that NGcGM3 might be a target for the treatment of melanoma. Further studies with this type of formulation are recommended.

54. NGcGM3/VSSP VACCINE AND RACOTUMOMAB IN THE TREATMENT OF ADVANCED BREAST CANCER PATIENTS

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Background: Gangliosides are one of the immunosuppressive molecules released by tumors to their microenvironment. N-glycolyl GM3 (NGcGM3) has become an attractive target for antigen-specific antitumor therapy as it is not normally expressed in humans and therefore constitute foreign Ag. At CIM, two vaccine candidates had been developed against this antigen. The NGlcoylated vaccine composed by NeuGcGM3 in a proteoliposome of Neisseria meningitides with Montanide ISA 51 as adjuvant and the 1E10 vaccine (Racotumumab) featuring a murine anti-idiotipic mAb related to the NeuGc-containing ganglioside. Due to the results obtained with those vaccine in clinical trials a therapeutic combination was considered to be optimal for the treatment of patients with advanced breast cancer. An expanded used program with NGcGM3/VSSP vaccine and Racotumomab in the treatment of advanced breast cancer patients were carried out to evaluate their safety and effect on survival. Materials and Methods: Twenty patients with advanced breast cancer were enrolled in the treatment combination arm of the expanded used program. NGcGM3/VSSP and Racotumobab were administrated one dose every 14 days (induction period) and later every 28 days (consolidation period). Both drugs should be administrated one week delay in order to decrease toxicity. The response to the treatment and appearance of side effects were analyzed. The data were analyzed by SPSS version 16.0 for Windows. Results: There was a tendency to an increase in survival for vaccinated patients added to an acceptable safety profile with this concomitant treatment schedule.
55. COMBINATION OF PLATINUM FIRST-STANDARD FRONT LINE CHEMOTHERAPY AND ANTI-IDIOTYPE MAB 1E10/ALUMINUM VACCINE IN PATIENTS WITH ADVANCED NSCLC

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The combination of vaccines and chemotherapy holds promise for cancer therapy, but the effect of cytotoxic chemotherapy on vaccine-induced antitumor immunity is unknown. This study was conducted to assess the effects of systemic platinum first-standard front line chemotherapy on Anti-diotype Mabs 1E10/aluminum vaccine induced humoral immunity in patients with advanced NSCLC. Patients with advanced NSCLC stages III/IV were treated with cisplatin/vinblastine as standard first front line therapy given concomitant with the 1E10/aluminum vaccine. The 1E10/aluminum vaccination schedule consisted in the administration of the first five intradermally vaccine doses (induction phase) concomitantly with the cisplatinum/vinblastine first line. Re-immunization was administered at 28 day intervals, and vaccination was performance beyond progression, until unacceptable toxicity or patients worsening performance status. Humoral immune responses against 1E10 Mab and NeuGcGM3 antigens were measured by standard ELISA assays, and changes in lymphocyte cells subpopulations during combination treatment was measured by Flow cytometry analyses (FACS). 21 advanced stages III/IV NSCLC patients received platinum first line standard chemotherapy concomitantly with 1E10/aluminum vaccine. The combination was safe. Not serious adverse events (SAEs) were observed according to the CTC-NCI adverse events criteria version 3.00. All patients developed high antibody responses against 1E10 Mab and NeuGcGM3 antigens were measured by standard ELISA assays, and changes in lymphocyte cells subpopulations during combination treatment was measured by Flow cytometry analyses (FACS). 21 advanced stages III/IV NSCLC patients received platinum first line standard chemotherapy concomitantly with 1E10/aluminum vaccine. The combination was safe. Not serious adverse events (SAEs) were observed according to the CTC-NCI adverse events criteria version 3.00. All patients developed high antibody responses against 1E10 Mab and NeuGcGM3 antigens was obtained, as in the standard not concomitantly vaccination schedule used in others clinical trials protocols. Moreover, an early onset kinetics IgG isotype antibody response specifically for NeuGcGM3 was detected. The combination of 1E10/aluminum vaccine and systemic chemotherapy has an acceptable safety profile in patients with advanced NSCLC. Concurrent first line standard systemic chemotherapy did not affect the generation of specific humoral responses against Mab 1E10 and NeuGcGM3 antigen following vaccination.

56. ANTI-TUMORAL EFFECT OF ACTIVE IMMUNOTHERAPY IN C57BL/6 MICE USING A RECOMBINANT HUMAN VEGF PROTEIN AS ANTIGEN AND THREE CHEMICALLY UNRELATED ADJUVANTS

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Following the clinical success of Bevacizumab, a humanized monoclonal antibody that affects the interaction between vascular endothelial growth factor (VEGF) and its receptors, blocking tumor-induced angiogenesis has become one of the most important targets for the development of new cancer therapeutic drugs and procedures. Among the latter, therapeutic vaccination using VEGF as antigen presents itself as very attractive, with the potential of generating not only a growth factor blocking antibody response but also a cellular response against tumor cells and stromal elements, which appear to be a major source of tumor VEGF. In this paper, we report the development of a protein vaccine candidate, based on a human modified VEGF antigen that is expressed at high levels in E. coli. With respect to controls, immunization experiments in C57BL/6 mice using weekly doses of this antigen and three adjuvants of different chemical natures show that time for tumor development after subcutaneous injection of Melanoma B16-F10 cells increases, tumors that develop grow slower, and overall animal survival is higher. Immunization also prevents tumor development in some mice, making them resistant to second tumor challenges. Vaccination of mice with the human modified VEGF recombinant antigen produces antibodies against the human antigen and the homologous mouse VEGF molecule. We also show that sera from immunized mice block human VEGF-induced HUVEC proliferation. Finally, a possible contribution of T cell cytotoxicity to the overall anti-tumor effect is suggested from the results of vaccination experiments where CD8+ lymphocytes were impaired using neutralizing rat antibodies.
57. VACCINATION WITH A GNRH PEPTIDE ANALOGUE ASSOCIATED TO CASTRATE LEVELS OF TESTOSTERONE AND PROSTATE SPECIFIC ANTIGEN REDUCTION IN MEN WITH ADVANCED PROSTATE CANCER


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Introduction: Prostate cancer is one of the leading causes of death by malignancies among men. A phase I clinical trial was conducted at the Camagüey Oncology Hospital to establish the safety and tolerance of GnrHm1-TT, a variant of GnrH conjugated to tetanus toxoid. Production of anti-GnRH antibodies, serum testosterone suppression, and PSA normalization were also assessed. Methods: Eight men with advanced prostate cancer, suitable for hormonal treatment were recruited. Informed consent was obtained prior to inclusion. The Institutional Ethics and Review Committee and the National Regulatory Agency approved the study. All patients received seven doses of vaccine composed by 3 mg peptide, 245 μg VSSP and 500 μL Montanide ISA-51 each fifteen days for the firsts four injections and monthly for the remaining three. Blood samples were taken for general clinical laboratory parameters, anti-GnRH antibody titer (ELISA), serum PSA (UMELISA) and testosterone (RIA). Digital rectal examination, abdominal tomography and ultrasonography, and bone gammagrapy were made in order to determine disease evolution and individual response to vaccination. Results: The vaccine was well tolerated. Side effects and adverse reactions that agreed with the protocol safety hypothesis. After the fourth injection all patients developed a marked GnRH antibody titer that remained elevated at the end of a trial. Subsequently, testosterone was suppressed in all patients and it has remained at castrate level after 12 months follow-up without any booster. PSA fell to 0 in all patients and has continued as normal values. Interpretation: GnrHm1-TT vaccination was safe and showed preliminary efficacy results in the research study conditions.

58. COMBINING CHEMOTHERAPY AND EGF VACCINATION IN THE TREATMENT OF ADVANCED LUNG AND PROSTATE CANCER PATIENTS


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An Epidermal Growth Factor (EGF)-vaccine (CIMAvax) have been use in combination with Chemotherapy to treat Non Small Cell Lung Cancer (NSCLC) and Hormone-Refractory Prostate Cancer (HRPC). CIMAvax was given prior and after standard first line chemotherapy to patients with advanced (NSCLC), to show the immunological and clinical results in a phase 1 study. Twenty patients diagnosed of advanced NSCLC were recruited. Two vaccinations were given previous to the first line chemotherapy, following of monthly vaccination after concluding chemotherapy. Vaccination dose was increased as compared with previous trials. The primary endpoints were immunogenicity and safety. Anti-EGF antibody titers were more than 20 times higher than those previously obtained, without any increase in adverse events. Serum EGF concentration decreased to undetectable levels in all patients. Ninety-two patients showed an immunodominant antibody response against the central region on the EGF molecule. High percentages of EGF/EGFR binding inhibition which positively correlate to antibody response against the EGF immunodominant region were found. Survival correlates positively with antibody titers and was favorably compared with the outcome obtained in a Control Group from a previous randomized trial. Combination of EGF-vaccination at high dose, with chemotherapy is feasible and well tolerated. The high anti-EGF antibody titers and reduction of serum EGF concentration do not entail more severe adverse event. The correlation of survival with antibody titers warrants confirmation in a wider and randomized trial currently ongoing.

For HRPC, CIMAvax also was given prior and after chemotherapy (mitoxantrone10 cycles). In a very preliminar analysis of a randomized phase II trial, 36 of 212 patients to be included were evaluated. Very high titers of anti-EGF antibodies were obtained post immunization and the EGF concentrations in serum of decreased until very low levels as well as in NSCLC vaccinated patients described previously. Vaccine was safe and vaccinated patients showed a very prolonged time to symptomatic progression.
59. CLINICAL TRIAL WITH THE VACCINE NGGLICOLILGM3 IN THE TREATMENT OF BREAST CANCER PATIENTS IN STAGE II-III FREE OF DISEASE. PRELIMINARY EXPERIENCE IN THE INOR

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Introduction: In Cuba, the breast cancer constitutes the first cause of incidence and mortality for cancer in women, being notified every year approximately 2500 new cases and 1300 deaths. Both indicators (incidence and mortality) are growing in the time. Among the immunotherapy variants, we can find the active specific, a therapeutic modality which tries to go the immune answer of the host against the malignant cells. Is in this frame that we intend to show the preliminary results of the therapeutic vaccine Nglicolil-GM3 used with adjuvant intention in a series of patients with breast cancer in stage II-III, operated, and free of disease in the National Institute of Oncology and Radiobiology (INOR). Methods: In a clinical trial phase III where the therapeutic vaccine Nglicolil-GM3 is tested, a preliminary analysis is been made to the first 35 included patients that had already concluded the programmed immunization chronogram, being evaluated the toxicity profile and the presence of appearance of relapse of the disease in this group. Likewise the critical route of the patients is exposed from its inscription in the institution until its inclusion in the clinical trial. Results: The most frequent adverse events in the patients were migraine, fever and decline. In the analyzed patients adverse events of graveness (grade 3-4) were not reported. Relapse of the disease was presented in 3 of the studied patients. Conclusions: In an initial study to the first 35 patients included in the INOR, the use of the vaccine Nglicolil-GM3 in the adjuvant scenario in patients with breast cancer stage II-III, has shown to be well tolerated. In the course of a higher advance of the trial will have the possibility of valuing approach of effectiveness.

60. USE OF IMMUNOTHERAPEUTIC PRODUCTS IN CLINICAL ASSAYS PERFORMED IN SANTIAGO DE CUBA. AN OVERVIEW

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During last three decades the therapeutic treatment of patients with cancer has enhanced its possibilities with the incorporation of a fourth modality denominated Immunotherapy. This therapy can be used to complement some of conventional oncospecific treatments, and it is based on focusing the immune response against tumour cells to obtain a repair, stimulation or amplification of responsible immune mechanisms involved in tumour’s growth and dissemination. This work summarizes the results obtained during 15 years in the management of clinical trials performed with immunotherapeutic products in Santiago de Cuba. In these years, more than 100 investigators of 22 medical specialities and 4 of our institutions (Hospital Oncológico Conrado Benítez, Saturnino Lora, Juan Bruno Zayas and Infantil Sur) have participated in clinical trials. Additionally, we have executed 18 protocols in 15 localizations such as prostate, glyoma, lung, esophagus, colon, ovary, skin by using 8 immunotherapeutic products from the Molecular Immunology and Genetic Engineering Centres, so around 170 patients have been beneficiated. Overall, all investigations have been performed respecting the ethical principles and good clinical practices.

61. HUMORAL IMMUNE RESPONSE CHARACTERIZATION IN ADVANCED BREAST CANCER PATIENTS TREATED WITH ANTI-IDIOTYPE VACCINE 1E10

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Ganglioside GM3 (NeuGc) is a glicosphingolipid not is expressed in normal human tissues however is over-expressed in some tumors. Monoclonal antibody 1E10 is anti-idiotypic of P3 mAb, specific for n-glycolillated gangliosides and sulphated glycolipids, 1E10 mAb is an antigenic mimicry of ganglioside GM3(NeuGc). This work is focused in the serological characterization of the antibody response in twenty-four patients with advanced breast cancer treated with anti-idiotypic vaccine 1E10. The treatment schedule consisted in first five doses intradermally during an induction phase and follow up ten doses monthly during a re-immunization phase with mAb 1E10 precipitated in alum hydroxide. Vaccination generated high tilters of antibody response. The serological response versus mAb 1E10 is characterized by a long lasting response of antibodies and high tilters and predominantly anti-idiotypic. The immunological responses were not only versus 1E10mAb but also versus the nominal antigen, the ganglioside GM3(NeuGc). A strong and consistent serological response of antibodies IgG and IgM were generated over the time versus GM3(NeuGc). We found also evidence of the generation of an anti-idiotypic cascade, by the identification of Ab5 antibodies in patients sera.
62. EVALUATION OF SAFETY PROFILE OF THE EGF VACCINE IN PATIENT WITH ADVANCED LUNG CANCER

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In this work a meta-analysis of the data of 7 Clinical Trials with the EGF vaccine in patients with advanced non small cell lung cancer (NSCLC) was conducted. The objective of this meta-analysis was to increase the power of the study and to develop the hypothesis for the next study. The study included 471 patients with advanced non small cell lung cancer that were included in 7 Clinical Trials with the EGF vaccine. Patient’s distribution was: Pilot I (12 patients), Pilot II (34 patients), Pilot III (25 patients), Pilot IV (42 patients), Pilot V (28 patients), Phase II (80 patients), 226 patients of Phase III and PUCE (24 patients). The meta-analysis was designed to evaluate the immunogenicity and security of the vaccine in different formulations (Alumina and Montanide ISA 51), pre-treatment with cyclophosphamide or not and different doses of the EGF Vaccine. To evaluate the vaccine safety profile was analyzed the behavior of all hematologic parameters and laboratory variables as well as the appearance of adverse events during the time of the studies. Most of the reported adverse events were mild or moderate and they were not related with the immunization with the EGF vaccine during the study. The vaccination with the EGF vaccine was safe and it influences the overall survival of patient with advanced NSCLC. Consequently, an improvement in quality of life of these patients with advanced lung cancer was observed.

63. STRUCTURAL CHARACTERIZATION OF A NOVEL EGF TARGETED THERAPEUTIC CONJUGATED VACCINE (CIMAVAX): ITS ROLE IN QUALITY CONTROL

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The efficacy of rEGF-rp64k vaccine has been demonstrated in the clinics for the treatment of EGF dependent tumors. Nevertheless, since this product is the result of a chemical conjugation using glutaraldehyde as a crosslinker in a single step, its high degree of heterogeneity imposes a challenge for the Quality Control techniques to be used during release and also for comparability and stability studies. Several approaches have been used for the characterization of this product and its raw components and have comprised amino acid analysis, tryptic and or Glu/C peptide mapping, mass spectrometry, size exclusion chromatography HPLC, among others. These methods have allowed the verification of proteins sequences, determination of their conjugation ratio, as well as the occurrence of post-translational modifications, implementation of a new QC method for product analysis and its characterization. Conjugation ratio has been determined using two different mathematical approaches arising to similar results where an average of 2 EGF molecules is found per p64k. On the other hand, the analysis of free and modified lysines (linking points between these proteins) allowed us to predict the “hot spots” for protein conjugation. Nevertheless, no actual crosslinked peptides were detected by Mass Spectrometrical analyses. Moving forward with these studies should provide even more information on the structural heterogeneity of this product.

64. CIMAvax-EGF: A CUBAN VACCINE FOR THE LUNG CANCER TREATMENT. NEW REGULATORY APPROACH

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The biopharmaceutical products approval for marketing is a very complex activity, characterized by large periods of time among other aspects, 15 to 20 years approximately; which are necessary to recompiled evidences by means of studies and trials to support the research and development stages. Finally, all this must demonstrate the efficacy, safety and quality of the product for the design patient’s treatment. This process faced the challenge of a changing and increasing standards where the registration will take place. We described in details this problem here, as well as a quality guideline for the approval of cancer therapeutics vaccines, which are not included until now in the regulated approaches for other biologic new drugs.
CIMAvax-EGF is a novel therapeutic vaccine for the advanced lung cancer treatment and constitutes, a Center of Molecular Immunology case study that obtain after 16 years of development and investigation process, the Registration Approval in Cuba and Peru in 2008. Since it is the first and only in the world until now, faced another four innovation areas: Scientific (To validate that the concept of active immunotherapy based on an autologous protein, the Epidermal Growth Factor, can have an impact in the treatment of the lung cancer); Technological (To face the challenge of how to obtain, develop and validate a productive process with reproducible, scalable, sanitarium attributes); Clinic (To insert this drug in the clinical practice. More than 800 patients in 7 clinical trials have received this vaccine and it has been demonstrated that it is safe, immunogenic and increases the survival) and Business (To obtain financier resources from a novel negotiation models with a high level of risk of these investments).

65. 1E10 ANTI-IDIOYPE VACCINE IN NON-SMALL CELL LUNG CANCER

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1E10 is an anti-idiotype murine monoclonal antibody specific to P3 MAb, which reacts with NeuGc-containing gangliosides, sulfatides and with antigens expressed in some tumors, including those from the lung. N-glicolilated gangliosides are over-expressed in several tumors. 1E10 MAb has proved to be able to mimic N-glicolilated gangliosides in those models where these gangliosides are not self antigens. 34 stage IIIb and 37 stage IV NSCLC patients were treated with aluminum hydroxide-precipitated 1E10 in a compassionate-use basis study. The median survival time of the 56 patients who entered the study with partial response or disease stabilization and with a PS 1 after the first line of chemo/radiotherapy, was 11.50 months from starting vaccination. In contrast, the median survival time calculated for patients who started vaccination with progressive disease and/or a PS2 was 6.50 months. Furthermore, in this study, we report on the immune responses elicited in the 20 first patients that entered the study. In the hyperimmune sera from 16 of the 20 patients, a strong specific Ab response of both IgM and IgG isotypes against NeuGcGM3 ganglioside was observed. Significant immunoreactivity of IgG and IgM isotypes to NeuGcGM3 was still detected after the complete blocking of the reactivity against 1E10 mAb. These Id-Ag+ Abs could reflect the activation of an autologous idiotypic cascade into the patients. Patient immune sera were able to induce complement-independent cell death of NeuGcGM3-expressing X63 murine myeloma target cells. Patients that developed IgG and/or IgM Abs against NeuGcGM3 showed longer median times.

66. DNA IMMUNIZATION FOR THE INDUCTION OF ANTI-IDIOYPE ANTIBODIES

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The ability of anti-idiotype antibodies of mimicking protein and non-protein antigens is an attractive property for the design of vaccines. Particularly for non-protein antigens, this strategy offers several advantages, including the use of an easier-to-purify and potentially more immunogenic antigen. There are however some idiotypes that need of strong adjuvanticity for inducing a humoral response, while fewer examples are unusually immunogenic in the syngeneic model. In the present work, we compare two ways of idioype-encoding DNA delivery to elicit anti-idiotypic antibodies against an autoimmunogenic antibody (P3), specific for a tumor-associated carbohydrate antigen, and its non-autoimmunogenic anti-idiotypic antibody (1E10). We used small immune proteins (SIP) consisting on the idiotype in the scFv format, covalently linked to g1-CH3, the self-dimerizing domain of murine IgG1. By gene gun immunization of syngeneic BALB/c mice, an antibody response against both idiotypes was generated, although only in the case of P3 a single shot of DNA was sufficient to induce a strong and long-lasting anti-idiotype response. VH-specific antibodies generated by immunization with hybrid VH/VL SIPs recognized also the wild idiotypes. The requirement of T cells for the anti-idiotype response was indicated by the lack of idiotype-specific antibodies following immunization of both allogeneic C57BL/6 and athymic BALB/c mice. In addition, by delivery to BALB/c mice of the same DNA construct with a recombinant adeno-associated virus, the unique immunogenicity of the P3 idiotype was evidenced. Our results demonstrate that tolerance against self idiotypes can be broken by bacterial DNA immunization.
67. ADVERSE EVENTS ASSOCIATED WITH THE VACCINE PREPARATION NGCGM3/VSSP/MONTANIDE ISA 51 IN PATIENTS WITH METASTATIC BREAST CANCER

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Introduction: The gangliosides are among the better studied antigenic systems and they have its increased expression in the tumoral cell membrane. Several clinical trials with therapeutic vaccines containing N-Glycolidated gangliosides have been carried out in Cuba by the Center of Molecular Immunology. One of these studies is the clinical trial: Active immunotherapy specific for the vaccine preparation NGcGM3/VSSP/Montanide ISA 51 in the treatment of patients with metastatic breast cancer (stage II). With the objective of evaluating the main adverse events related with this product, the clinical records of all the patients included in this clinical trial carried out in the Oncology Service of the University Hospital “Celestino Hernández Robau” of Villa Clara were checked. Materials & Methods: The number of adverse events reported by patients were gathered and classified according to the Common Terminology Criteria for Adverse Events (CTCAE) to the Cancer National Institute of the U.S.A. Results: A total of 389 adverse events were reported. According to their intensity degree the adverse events were classified as: slight intensity (77.37%), moderate (20.05%), severe (1.54%) and very severe (1.03%). Regarding the causality relation the non-related adverse events were predominant (73.0%). The most frequent events associated to the vaccine were related with the administration site and general symptoms similar to an influenzal state. Conclusions: The adverse event of higher intensity related with the product of investigation was the abscesses in the injection site. The group of the adverse events was neither a limitation doses nor a cause for treatment interruption.

68. CRYSTAL STRUCTURE OF AN ANTI-GANGLIOSIDE ANTIBODY AND MODELS OF ITS INTERACTION WITH THE NEUGC-GM3 ANTIGEN AND AN ANTI-IDIOTYPIC ANTIBODY. STRUCTURAL BASIS OF THE VACCINE EFFECT OF THE ANTI-IDIOTYPIC ANTIBODY

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N-glycolylated (NeuGc) gangliosides are tumor-specific antigens and as such represent attractive targets for cancer immunotherapy. The chimeric antibody chP3 selectively recognizes NeuGc gangliosides, showing no cross-reactivity to the highly similar N-acetylated (NeuAc) gangliosides that are common cellular antigens in humans. Here, we report the crystal structure of the chP3 Fab and its computer-docking model with the trisaccharide NeuGc 3Gal 4Glc, which represents the carbohydrate part of the tumor-antigen NeuGc-GM3. The interaction involves only the heavy chain of the chP3 antibody. The modeled complex is consistent with all available experimental data and shows good surface complementarity. The negatively charged sialic acid residue NeuGc is buried in a pocket flanked by two arginine residues, Arg H31 and Arg H100A. We have further investigated the interaction of chP3 with its anti-idiotype antibody, 1E10, currently in clinical trials as a cancer vaccine. While many of the chP3 residues predicted to interact with the NeuGc ganglioside also feature prominently in the modeled complex of chP3 and 1E10, we do not observe structural mimicry. Rather, we suspect that the vaccine effect of the anti-idiotype is due to that 1E10 may serve as an imprint of the structural characteristics of the chP3 idiotype and, consequently, give rise to antibodies with P3-like properties upon immunization.

69. EVALUATION OF SAFETY PROFILE OF THE 1E10 ANTIDIOTIPIC VACCINE IN PATIENT WITH ADVANCED LUNG CANCER

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In this work a meta-analysis of the data of 4 Clinical Trials with the 1E10 vaccine in patients with advanced non small cell lung cancer (NSCLC) was conducted. The objective of this meta-analysis was to increase the power of the study and to develop the
Clinical Trials of preventive vaccines and Health Policies

1. THE MENINGITIS INTERNATIONAL COORDINATING GROUP: LESSONS LEARNED AFTER 10 YEARS OF COLLABORATIVE EMERGENCY RESPONSE

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The International Coordinating Group on Vaccine Provision for Epidemic Meningitis Control (ICG), a collaboration of four international partners, acting with vaccine manufacturers and countries in the meningitis belt of sub-Saharan Africa, was established in 1997 to improve the distribution and rational use of currently available meningococcal polysaccharide vaccines and related material during meningitis epidemics. Successes include: technical coordination between countries, partners and agencies, recognized epidemic thresholds, treatment efficacy for presumptive treatment protocols, stimulation of the development of a new trivalent meningitis polysaccharide vaccine and of a new qualified supplier, and establishment of criteria based on laboratory confirmation for the use of AC or ACW135 vaccines. Through ICG intervention countries have received around 40 million doses of vaccines for mass immunization campaigns within a mean of 23.8 days in 2008 after notification that the country had crossed the epidemic threshold. Delays were related mainly to time for country submission of vaccine requests due to lack of confirmation to quickly identify an outbreak. The ICG partners have streamlined the procurement mechanism and are working with countries on preparedness planning to address these gaps. The ICG has not achieved a sustainable financial base in the last 10 years; however vaccine and supplies have been shipped on short notice whenever necessary. Given the likelihood of future outbreaks of disease due to meningitis type A before the availability of an effective conjugate vaccine now under development, the Global Alliance for Vaccine Immunization (GAVI) approved in 2008 funds for 55 million dollars to finance and ICG stockpile of 45 million doses until 2013. Thus, the ICG will continue supplying polysaccharides vaccines AC and ACW to respond to epidemics.

2. WHO’S INITIATIVES TO STRENGTHEN REGULATION OF CLINICAL DEVELOPMENT OF NEW VACCINES

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Increasingly, vaccines are being developed to protect against diseases that are endemic in developing countries. To ensure suitability of clinical data generated, clinical trials must be undertaken in the target countries. Their National Regulatory Authorities (or NRAs) are responsible for authorizing and monitoring such trials. Until recently, however, many developing countries had limited experience in this area.

In 2002 WHO began working to address this gap through the creation of networks in developing countries to build competence in clinical trial oversight and evaluation of clinical data for registration of vaccines. The first network created in 2004 - the Developing Countries Vaccine Regulators Network (DCVRN)- includes representatives from Brazil, Cuba, China, India, Indonesia, the Republic of Korea, the Russian Federation, South Africa, and Thailand. Its mission is promoting and supporting the strengthening of the regulatory capacity of NRAs for the evaluation of clinical trial applications and clinical trial data for licensing purposes, through expertise and exchange of relevant information.
Some regions face challenges specific to countries in their own geographic area. In Africa, a “regional approach” was used to provide ongoing regulatory support to regulators in since 2005, with the facilitation of capacity-building activities (e.g. development of regulatory procedures, joint reviews and joint inspections of clinical trials) involving groups of countries. The establishment of the African Vaccine Regulatory Forum (AVAREF) in 2006, provided a structured approach to gather representatives from NRAs and ethics committees from 19 countries identified as targets for clinical trials and introduction of novel vaccines. AVAREF provides a platform for strengthening capacity and identifying needs for support and training, through annual plenary meetings and satellite activities.

In Asia, a mechanism to provide countries in south-east Asia with regulatory support is also in place — through the Vaccine Chapter of the Association of Southeast Asia Nations (ASEAN). The Chapter will, in collaboration with WHO, help member countries address vaccine evaluation issues and harmonization of regulatory processes.

3. DEVELOPING COUNTRY VACCINE REGULATORS’ NETWORK

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The DCVRN comprises representatives from the National regulatory authorities (NRA) of developing countries that have achieved a degree of medicine regulatory competence as assessed by the WHO. The member countries are Brazil, China, Cuba, India, Indonesia, South Korea, Russia, South Africa and Thailand.

These representatives have met twice a year, starting in September 2004, to discuss a defined agenda and propose actions for the future. In practice representatives from other regulatory authorities that have a specific and urgent interest in the agenda topics have been invited as observers. Experts from other more-developed regulatory authorities and the WHO have acted as presenters, advisors and facilitators at meetings.

The focus of activities has been to support and promote the strengthening of the regulatory capacity for evaluation of clinical trial proposals including pre-clinical data, product development processes and clinical trial data for registration of new vaccines. The DCVRN has been active in providing a list of items for consideration by the WHO Expert Committee on Biological Standardization that relate to the concerns of developing countries and introduction of new vaccines. In addition a number of guidelines have been prepared, or are in preparation, that will assist DCVRN members and the regulators of other developing countries with the regulation, control and inspection of vaccine clinical trials and trial sites. Regulatory support centres are being established in South Africa and Indonesia that are functioning at the moment as training centres. Other similar-groups of regulators have been formed in the WHO Africa and Asian Regions that receive support from the DCVRN members.

There are several future activities under consideration, including joint reviews of clinical trial applications, joint inspections of clinical trial sites, development of an IND-like process for new vaccines (already in the pilot stage), and a co-inspection program leading to possible mutual recognition of GCP Certification of vaccine clinical trial results.

The 10th meeting of the DCVRN is in progress in Cuba during this week.

4. A MALARIA VACCINE DEVELOPMENT: FROM LABORATORY STUDIES TO CLINICAL TRIALS

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Because of the emergence and rapid spread of drug-resistant parasites and insecticide-resistant mosquitoes, there is an urgent need for the development of new tools to control malaria. Vaccination is one tool that may control and even eradicate the disease from the world. Due to the complex life cycle and high antigenic diversity of the malaria parasite, multivalent and multistage malaria vaccine containing antigens derived from different stages of the parasite life cycle may necessary be constructed. We have constructed a *P. falciparum* AMA-1 (III)/MSP1-19 chimeric protein (designated PICP-2.9) via a hinge encoding Gly-Pro-Gly. This protein formulated with ISA720 adjuvant was tested in non-human primates as well as in human for safety and immunogenicity. Two clinical trials of the malaria vaccine candidate were completed in non-malaria endemic regions. In addition to PICP-2.9, we constructed another *P. falciparum* ligand, domain IIf2 of EBA-175 (PfEBA-175II F2) which has been identified as the receptor-binding domain of the molecule and a pre-erythrocytic antigen designated as PICSP-2 that is composed of region I, NANP repeats and its C-terminal region of CS protein. Immunogenicity of the combination vaccine as well as stability and potency of the vaccine formulation will be discussed.
5. THE PRODUCT DEVELOPMENT TEAM – A NOVEL APPROACH TO FACILITATING THE CLINICAL DEVELOPMENT OF NEW TB VACCINES

Barry Walker, Micha Roumiantzeff and the PDT

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During the last decade there has been significant progress in the development of new vaccines against tuberculosis, with a number of these potential candidates having entered clinical trial. Funded by the European Union Framework 6 programme, TBVAC (A cluster for tuberculosis vaccine developments) the project aims to develop improved vaccines against tuberculosis, particularly for the young adult population. A number of vaccine candidates and strategies have been optimised for evaluation in Phase I clinical trials. As part of this programme to progress vaccine candidates from the bench to the clinic, the concept of the Product Development Team (PDT) was developed and implemented.

The PDT acts as an advisory body of experts and is an informal group of experts that have a range of experience relevant to vaccine development, from project management expertise, through pharmaceutical experience, regulatory affairs and clinical trials planning. The structure of the PDT revolves around a core membership that provides consistency and oversight with additional experts being co-opted into the PDT for specific product related issues. Each PDT is product specific – a team is agreed for each Product and the Lead for each PDT is the sponsor/developer/lead scientist and a critical aspect of the methodology is that the Sponsor/Developer retains “ownership” and makes the final decisions concerning product development, the PDT acts to advise, not instruct. Meetings are response mode – called by developer, usually at developers base when the developer has a particularly pressing issue that needs input from the PDT. One member of the team remains in frequent informal contact.

Outputs from meeting with the PDT consist of a review of product development plan; targeted advice on the plan (all aspects); to identify and link to Contract Manufacturing Organisation where appropriate; to identify issues/critical steps in the development plan. A report from meeting is prepared by the PDT and sent to developers. The PDT also reports back to the TBVAC consortium through the WorkPackage Leader Paul Henri Lambert. During the process we have identified Critical Stages for input from the PDT – (1) Establish and define Go-No Go criteria for the development plan; (2) Assisting in establishing a Final Product Profile; (3) Provide guidance in determining Product Specifications and QC requirements; (4) Help identifying potential manufacturing/scale up issues. The PDT also provides an enabling linkage through to suitable CMO and focussing attention on cGMP issues, QC and Product Assays. The PDT also provides input on Regulatory issues and Clinical Trial – Ph I planning.

We will present the results from the 5 years operation of the PDT, the vaccines that have progressed and the issues and solutions that have presented themselves.

6. ASSESSMENT OF VACCINE EFFICACY BY NON-INFERIORITY CLINICAL TRIALS BASED ON IMMUNE RESPONSE

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Introduction: Conventional phase III studies are large-scale clinical trials designed to prove vaccine efficacy, which is evaluated by the reduction in the chance of developing clinical disease in vaccinated individuals regarding to that in unvaccinated ones. However, the evaluation of the immune response can also be used to assess vaccine efficacy. Materials and methods: Two non-inferiority clinical trials are shown as examples of this latter approach. They were designed to evaluate a new diphtheria/tetanus vaccine –adult formulation– and a new Salmonella Typhi Vi polysaccharide vaccine. Licensed active control vaccines were used in both trials. Validated ELISAs were used for the quantitative determination of antibodies and the seroprotection levels (serological efficacy) were estimated before and after vaccination. Results: The serological efficacy of the new candidate vaccines was not inferior to their respective controls. When vaccines containing known antigens and/or the incidence of disease are very low, it may not be feasible to perform formal studies of protective efficacy. In such instances, the efficacy can be proved by the evaluation of immune responses, especially when immunological correlates of protection are established. Non-inferiority design must be used when active control vaccines are available. Conclusions: The efficacy of a vaccine can be demonstrated not only in randomized, double-blind, placebo-controlled trials based on a clinical disease endpoint, but also can be confined to the evaluation of immune responses and comparison with recognized correlates of protection. The approval by regulatory authorities may be based on immunogenicity data.
7. CLINICAL EVALUATION OF COMBINATION VACCINES

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The development of new combination vaccines is a growing field in the market of vaccines, due to all the potential advantages related with the reduction in the number of injections and the increase into vaccination compliance, among others. However, there is also safety concerns related with the possibility of increased reactogenicity or the sub-optimal immunogenicity induced for the combination of multiple antigens, delivered in the same injection. For these reasons the evaluation of safety, immunogenicity and efficacy of a new combination before licensing represents an important area of clinical research. The aim of this lecture is to cover the main aspects related with the clinical development plan, different approaches to design clinical studies for the evaluation of this type of vaccines as well as the regulatory framework behind all these topics.

8. RANDOMIZED CONTROLLED CLINICAL TRIAL OF FRACTIONAL DOSES OF INACTIVATED POLIOVIRUS VACCINE ADMINISTERED INTRADERMALLY BY NEEDLE-FREE DEVICE

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Introduction: In 1988, the World Health Assembly resolved to eradicate poliomyelitis by the year 2000. In parallel with implementing the eradication strategies, the preparations for the post-eradication era for poliomyelitis eradication have began almost 10 years ago. The decision, to stop the routine use of oral poliovirus vaccine (OPV), was proposed in 1997. In 2007, the ACPE added the need for an «affordable IPV» appropriate for use in developing countries. As part of evaluating strategies for this necessity we conducted a clinical trial of fractional doses of IPV in Cuba. Methods: We compared the immunogenicity and reactogenicity of fractional dose IPV (0.1 ml or 1/5 of a full dose) given intradermally by needle-free jet injector device (study group) with full doses given intra-muscularly (control group) developing none blinded Phase I-II clinical trial. Subjects were randomized at birth to receive IPV at 6, 10, and 14 weeks. Results: 471 subjects were randomized, and 367 subjects fulfilled the study requirements. No significant baseline differences were detected. Thirty days after completing the 3-dose schedule of IPV, 53.2%, 85.1%, and 69.2% seroconverted to poliovirus types 1, 2, and 3 in arm A (fractional-dose IPV) compared with 89.4%, 95.5% and 98.9% in arm B (full-dose IPV; all comparisons, p<0.001). The median titers to each poliovirus serotype were significantly lower in arm A compared to arm B (p<0.001). Only minor local and no moderate or serious adverse events were reported. Conclusions: This evaluation demonstrates that fractional doses of IPV given intradermally at 6, 10, and 14 weeks were significantly less immunogenic than full doses of IPV given intramuscularly. Given the well-characterized interference of maternally-derived antibody with IPV immunogenicity, fractional dose IPV should be evaluated in a schedule that administers dose at 2 months and respects an interval of 2 months between doses. In addition, other strategies to decrease the costs of IPV should be evaluated, including addition of adjuvants, use alternative inactivation methods, and/or optimize production processes.

9. THE VACCINE GLOBAL MARKET AND ITS POLITICAL INFLUENCE IN UNDERDEVELOPED COUNTRIES

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The world today is facing thousands of unsolved problems. Some of them related to the difficult health situation of underdeveloped countries, which annually suffer from epidemics causing many deaths mainly due to AIDS, Malaria, TB, tropical diseases such as Dengue, Yellow Fever, Typhoid Fever among others. Economic disparity together with climatological events, military confrontations and governmental incapacity has contributed to the increase of deaths due to epidemics annually. Medicaments and effective vaccine shortage and the policy of big pharmaceutical companies of obtaining huge profits from their products have caused that many countries has not the minimal sanitary conditions to live. This research aims at characterizing the main stakeholders in the biotechnology field, especially those related to vaccine, and the way companies, governments, NGOs and other institutions provide solutions for global epidemics in different countries. Special focus will be done on vaccine in order to demonstrate that when there is a combination of political will with strong scientific work and other strengths it is possible to fight against the current sanitary situation of the world.
10. SAFETY AND IMMUNOGENICITY OF A COMBINED HEPATITIS B VIRUS-HAEMOPHILUS INFLUENZAE TYPE B VACCINE COMPRISING A SYNTHETIC ANTIGEN IN HEALTHY ADULTS

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Introduction: The combined HB-Hib vaccine candidate Hebervac HB-Hib® (CIGB, La Habana), comprising recombinant HBsAg and tetanus toxoid conjugate synthetic PRP antigens has shown to be highly immunogenic in animal models. Materials and Methods: A phase I open, controlled, randomized clinical trial was carried out to assess the safety and immunogenicity profile of this bivalent vaccine in 25 healthy adults who were positive for antibody to HBsAg (anti-HBs). The trial was performed according to Good Clinical Practices and Guidelines. Volunteers were randomly allocated to receive the combined vaccine or simultaneous administration of HB vaccine Heberbiovac-HB® and Hib vaccine QuimiHib® (CIGB, La Habana). All individuals were intramuscularly immunized with a unique dose of 10 µg HBsAg plus 10 µg conjugated synthetic PRP. Adverse events were actively recorded after vaccine administration. Total anti-HBs and IgG anti-PRP antibody titers were evaluated using commercial Elisa kits at baseline and 30 days post-vaccination. Results: The combined vaccine candidate was safe and well tolerated. The most common adverse reactions were local pain, febricula, fever and local erythema. These reactions were all self-limited and mild in intensity. No severe or unexpected events were recorded during the trial. The combined vaccine elicited an anti-HBs and anti-PRP booster response in 100% of subjects at day 30 of the immunization schedule. Anti-HBs and anti-PRP antibody levels had at least a two-fold increase compared to baseline sera. Even more, anti-HBs antibody titer showed a four-fold increase in 100% of volunteers in the study group. The results indicate that the combined HB-Hib vaccine produces increased antibody levels in healthy adults who have previously been exposed to these two antigens. Conclusions: This is the first demonstration of safety and immunogenicity for a combined vaccine comprising recombinant HBV and synthetic Hib antigens. The present results support phase I-II clinical trial in the target population, two months old healthy infants.

11. CLINICAL DEVELOPMENT OF THE CUBAN LIVE CHOLERA VACCINE CANDIDATE, PREPARED WITH THE VIBRIO CHOLERAE 638 ATTENUATED STRAIN 01 EL TOR OGAWA


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Introduction: World Health Organization (WHO) published alarming data on the re-emergence of cholera in many African and Asian countries, changing the earlier thinking that doubted the value of cholera vaccines, and stating that Oral Cholera Vaccines may become effective partners in rolling back the ever-more-aggressive Vibrio cholerae bacterium. In this context, promising results from a Cuban vaccine candidate Phase I/II trial are shown. The Peruvian epidemic became the basis for the attenuated, genetically modified 638 strain, the active ingredient for the new Cuban vaccine candidate. The assessment of safety and reactogenicity was particularly important, since live attenuated vaccines like ours tend to cause more adverse reactions. To assess immunogenicity and the potential protective efficacy of the attenuated strain was as important as to assess safety, reactogenicity and immunogenicity of the lyophilized vaccine candidate in subsequent stages. Materials and Methods: Since there is not an animal model that reproduces the pathology of cholera, 14 double-blind, placebo-controlled clinical trials in healthy adult volunteers in Cuba with fresh culture of 638 strain, including a challenge study with a virulent strain were carried out at Tropical Medicine Institute Pedro Kourí, Havana, with fresh culture of 638 strain. Furthermore, other two studies were similarly designed and carried out with the formulated vaccine candidate in Phase I/II trials in Cuba and Mozambique. Results: During all clinical trials only a few amount of adverse event were reported, while vibriocidal antibodies seroconversion reach more than 97 % in healthy adults volunteers in Cuba as well as in apparently healthy volunteers in Mozambique, where cholera is present together with very different nutritional, epidemiological, and environmental conditions. Conclusions: The cholera vaccine candidate 638 evaluated in non-endemic and endemic areas of cholera was safe, well tolerated and immunogenic in adults volunteers.
12. SURVEILLANCE OF THE SAFETY AND EFFECTIVENESS OF THE CUBAN SYNTHETIC HAEMOPHILUS INFLUENZAE TYPE B VACCINE, QUIMI-HIB

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**Introduction:** Quimi-Hib, the first commercial vaccine against *Haemophilus influenzae* type b (Hib) containing a synthetic antigen, was introduced into the Cuban National Immunization Program since January 2004. It replaced the Vaxem-Hib vaccine (Chiron), used since 1999. Here we present the results of the safety and effectiveness surveillance of Quimi-Hib during its massive use in Cuba for more than five years. **Materials and Methods:** The Bacterial Meningoencephalitis (BME) report system of the Ministry of Public Health (MINSAP) implemented since 1998 was used for the surveillance of BME, an indicator of the impact of Quimi-Hib vaccination in the Cuban population. Likewise the Surveillance System on Adverse Events to Vaccination introduced since 1999 by MINSAP to monitoring the adverse events of all vaccines in use in the National Immunization Program was used to the surveillance of the safety of Quimi-Hib vaccine. The CIGB receives annually from the MINSAP updated information derived from these surveillance systems. **Results:** 1 516 950 doses of Quimi-Hib have been applied in the country in the period from January 2004 to April 2008 with vaccination coverages over the 97%. In the last years, the infection rates reached very low values, indicating the capacity of this vaccine to prevent an acute invasive disease caused by this pathogen. Respect to the safety, Quimi-Hib has maintained a low reactogenicity profile. None event such as anaphylactic reaction, convulsions, deaths or adverse events with permanent sequelae have been reported after Quimi-Hib immunization. **Conclusions:** Quimi-Hib has been effective for preventing the Hib-related BME, which is the more serious form of infection by Hib and it has been well tolerated by the immunized children.

13. WHOOPING COUGH: A RE-EMERGING DISEASE

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Despite the high vaccination coverage in most of the Western world, the incidence of whooping cough has increased in all age groups during the last decades. The introduction of acellular vaccines nor the introduction of booster vaccinations in toddlers has not changed this trend. Current vaccines are not well equipped to overcome the rising incidence of whooping cough for various reasons. Therefore, there an improved whooping cough vaccine is needed.

The required characteristics of such an improved vaccine are that it (1) allows all age groups to be vaccinated with *B. pertussis* circulating strain antigens, (2) protects against whooping cough induced by *B. parapertussis*, (3) enables infants to be vaccinated earlier, (4) causes minimal adverse events after repeated vaccination, and (5) protects longer than currently registered vaccines. Consequently, vaccine compositions that can fulfill these requirements were examined, using time to market, costs and risks as constraints. The most likely candidates to succeed are oral or intranasal vaccines consisting of inactivated *B. pertussis* cells, since an oral vaccine has already shown proof of protection in a phase III study without adverse events, and an intranasal vaccine has shown proof of concept in a phase I study. Live attenuated vaccines also promising, but may take longer to commercialize. At this point it is not clear if *B. parapertussis* cells should also be included in an improved whooping cough vaccine, and what the cost-benefit ratio would be.

13. WHOOPING COUGH: A RE-EMERGING DISEASE

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Despite the high vaccination coverage in most of the Western world, the incidence of whooping cough has increased in all age groups during the last decades. The introduction of acellular vaccines nor the introduction of booster vaccinations in toddlers has not changed this trend. Current vaccines are not well equipped to overcome the rising incidence of whooping cough for various reasons. Therefore, there an improved whooping cough vaccine is needed.

The required characteristics of such an improved vaccine are that it (1) allows all age groups to be vaccinated with *B. pertussis* circulating strain antigens, (2) protects against whooping cough induced by *B. parapertussis*, (3) enables infants to be vaccinated earlier, (4) causes minimal adverse events after repeated vaccination, and (5) protects longer than currently registered vaccines. Consequently, vaccine compositions that can fulfill these requirements were examined, using time to market, costs and risks as constraints. The most likely candidates to succeed are oral or intranasal vaccines consisting of inactivated *B. pertussis*
cells, since an oral vaccine has already shown proof of protection in a phase III study without adverse events, and an intra-nasal vaccine has shown proof of concept in a phase I study. Live attenuated vaccines also promising, but may take longer to commercialize. At this point it is not clear if *B. parapertussis* cells should also be included in an improved whooping cough vaccine, and what the cost-benefit ratio would be.

**Poster Session**

### 14. DRUG RISK EVALUATION DURING CLINICAL DEVELOPMENT

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**Introduction**: Apart from some adverse reactions, drugs produce some other problems, such as inefficacy, dependency, or intoxication; these aspects should be constantly followed because they imply morbidity, hospitalization, including death. For that reason it is necessary to take into account the evaluation, estimation, monitoring, and handling associated risks to process of I + D and medical practice use. Our objective was to elaborate an algorithm to risk evaluation that could be used to classify investigational products in the research and development process and marketing authorization stage. **Methodology**: Extend Bibliographic revision, checking of risk classification according to WHO, risk analysis of PANDRH and national criteria established in Bioequivalence (BE) Regulation, working meeting were done with evaluation risk committee, looking for an instrument for evaluate a drugs risk of Essential Drugs Listed in Cuba and elements to establish drugs level risk. **Results**: Algorithm for establishment of risk categories was elaborated taking into account: Safety Information Drugs, Estimated Therapeutic Range (Limit Value 2), Post marketing and prevalence use of drugs (± 5 years), Pharmacology Classification by ATC classification (3 groups), Population target (special population and warning) and Severity of adverse reactions. Risk classification will depend on the magnitude and/or complexity and/or characteristics of the elements above (high risk 11 – 18 points, medium 6 – 10 points and low ≥ 5 point). Finally, the list with priority products for BE studies in Cuba was done. **Conclusions**: Risk evaluation drugs’ algorithm was evaluated by an exercise where its applicability was proved; that included the application of algorithm to 56 products of Essential Drugs listed in Cuba. The correspondence between the results of algorithm evaluation and reported risks in international literature was shown. Nowadays, we have some normative that includes an algorithm for risk categories establishment of drugs. Applicability and easy handling for specialists were proved by the evaluation exercise.

### 15 PROPOSAL OF AN ALGORITHM TO ASSESS CAUSALITY OF ADVERSE EVENTS IN VACCINE CLINICAL TRIALS


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**Introduction**: During clinical trials it is common to appoint a commission which is in charge of assessing the causality of adverse events which are reported and transcribed to the collection data logbooks. To be able to answer the question: “Does the vaccine under study cause an adverse event?” different pathways could be taken by decision makers, so it is obvious that the final conclusion could vary too much and a poorly assessment can generate mistaken conclusions. That confirms the needs to dispose of a procedure to define which categories must be used to analyze and to classify the relation of causality. One way to answer the mentioned question is creating an algorithm. **Materials and Methods**: We reviewed the literature and did not find any algorithm which could be considered a “master key” to set or reject causality, so this paper aims at designing and algorithm beginning with the international methodology used in post-commercialization. A first proposal was designed which was individually and collectively analyzed by the multi-disciplinary group of the Medical Management from Finlay Institute. **Results**: The projected algorithm is based on consecutive steps. If temporal association with vaccination of any adverse event is detected, and any alternative explanation is not confirmed, adverse events detected in previous clinical studies or those reported in scientific papers are analyzed to estimate the causality. Finally, the algorithm was approved by consensus. **Conclusions**: The new algorithm could be useful to apply in clinical trials inside and outside of the institution. **References**: Peña M, Valera R, Mirabal M, Rodríguez M, Armesto del Río M, Menéndez J, Baró M, Cuevas I, Estruch R, García H, Ochoa R, Casanueva V. Propuesta de un algoritmo para evaluar la causalidad de eventos adversos en ensayos clínicos de vacunas. VacciMonitor 2008, 17(3).
16. CONTRIBUTION OF THE CLINICAL STUDIES WITH CUBAN RECOMBINANT HEPATITIS B VACCINE CARRIED OUT BY “PEDRO KOURI” INSTITUTE TO HEPATITIS B PREVENTION

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Introduction: The contribution of clinical studies with Cuban recombinant hepatitis B vaccine carried out by the Tropical Medicine Institute “Pedro Kouri” for the implementation of strategies for hepatitis B vaccination and the achievements in the prevention and control of the disease in Cuba is documented in this paper. Materials and Methods: Clinical trials were conducted in subjects of all ages and included 4416 subjects. Phase II and III clinical trials were randomized. The study protocols were approved by the Ethics Committee of the Institute of Tropical Medicine “Pedro Kouri” and were conducted in accordance with the provisions of the Declaration of Helsinki. Designs included five Phase II clinical trials, a Phase III study and two Phase IV studies. Source of information on hepatitis B was obtained from the Ministry of Public Health. Results: The high immunogenic power of the vaccine in all vaccinees was demonstrated. Fourteen years after primary vaccination starting at birth, the protective efficacy in handicapped children with high hepatitis B virus infection risk was 100%. Its effectiveness with the recombinant hepatitis B Cuban vaccine in high risk neonates was 93.7%. These results contributed to the implementation of the vaccination, the prevention and control of the hepatitis B in Cuba. The disease was reduced in 99.07% (general population in 2007 in comparison with 1991 when the intervention had not been carried yet). General incidence decreased to 0.2 x 100 000 inhabitants in 2007. Conclusions: Clinical studies carried out by IPK contributed to the implementation of hepatitis B vaccination and the achievements in prevention and control of the disease in Cuba. Presently the disease is not a health problem in Cuba.

17. IMMUNOGENICITY AND SAFETY EVALUATION OF CUBAN THIOMERONTAL-FREE RECOMBINANT HEPATITIS B VACCINE

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Introduction: It has been estimated that 350 million people worldwide are chronic hepatitis B virus carriers. Vaccination continues to be the method of election to fight against hepatitis B, but recent concerns were raised regarding exposure to mercury following immunization with thioelemental-containing vaccines. Reformulation of vaccines in single-dose vials may eliminate the need for a preservative. This Clinical Trial was made to evaluate the immunogenicity and reactogenicity of the recombinant hepatitis B vaccine, Heberbiovac HB formulated without Thiomersal. Materials and Methods: A randomized double blind clinical trial was conducted according to good clinical practice principles. A total of 235 healthy volunteers between 18 to 35 years old, negative for serum HBsAg and anti-HBsAg were included. Adverse events were evaluated after the application of each dose. Volunteers received three 10 mg/0.5 mL doses of recHBsAg in the deltoid muscle, according to a 0, 1, 2 month schedule. The quantification of the anti-HBsAg titers was performed 30 days after the third dose. Results: Seroprotection rates to the Thiomersal-free lot was (98.9%), and the control lot containing Thiomersal (95.5%). The geometric mean of anti-HBsAg antibody titers (GMT) were: 727.78 IU/L and 607.89 IU/L, respectively. No statistically significant differences regarding the Seroprotection and GMT were detected. The vaccine was well tolerated: all adverse events were slight and brief. Conclusions: The Cuban Thiomersal-free Heberbiovac HB vaccine is immunogenic and safe in adults. We also concluded that the 10 mg/0.5mL dose of Heberbiovac HB is highly immunogenic in adults under 35 years old.

18. POINTS TO CONSIDER FOR CLINICAL EVALUATION IN COMBINED VACCINES

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Introduction: The development and evaluation of combined vaccines is very important because of the significant advantages that it represents: simplify and harmonize the vaccination schedule, decrease the number of injections, trauma and pain, improve immunization coverage, facilitate the introduction of new vaccines, reduce exposition to additives (preservatives,
19. DENGUE HEMORRHAGIC FEVER CAUSED BY SEQUENTIAL DENGUE 1-3 INFECTIONS AT LONG INTERVAL: HAVANA EPIDEMIC, 2001-2002

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Introduction: A DENV-3 epidemic occurred in Cuba in 2001-2002 which included cases of dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). Material and Methods: We report neutralizing antibody studies on sera from 54 of 78 DHF/DSS patients of this epidemic. Results: The results provide evidence of infections occurring in the sequence DENV-1 followed by DENV-3. No sera evidenced infection in the sequence DENV-2 followed by DENV-3. Some sera showed a pattern of infection in the sequence DENV-1 followed by 2 then 3, however definitive categorization of a tertiary infection was not possible because of broadly reactive antibodies which could have been raised by infections in the sequence DENV-1 then DENV-3. Dengue Hemorrhagic Fever has been associated with secondary infection in individuals who experienced a primary dengue infection 3-5 years earlier. Conclusions: In this paper two important observations are reported: a) secondary dengue infection is demonstrated as an important risk factor for severe disease occurring 24 years after a primary dengue infection and b) The infection sequence, dengue 1 followed by dengue 3 was associated with severe disease. There was no evidence that dengue 2 followed by dengue 3 infections resulted DHF/DSS, although infections in this sequence leading to milder illnesses were observed. These two observations are new and important to understand the pathogenesis of this disease and in vaccine safety issues.

20. SAFETY AND IMMUNOGENICITY OF CUBAN SYNTHETIC HIB VACCINE (QUIMIHIB®) IN HEALTHY INFANTS

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Introduction: A phase II clinical trial was performed to evaluate the safety and immunogenicity of one industrial lot of the novel Cuban synthetic Haemophilus influenzae type b (Hib) conjugate vaccine (Quimihib®; Heberbiotec S.A, La Habana) containing synthetic Polyribosylribitol phosphate Hib capsular polysaccharide conjugated to tetanus toxoid carrier protein (sPRP-TT). The vaccine was administered to healthy infants at 2, 4, 6 months of age. Methods: One hundred thirteen infants ~2 months of age were randomly assigned to receive sPRP-TT vaccine batch 3H0101/1. All infants received 10µg sPRP-TT (0.5mL) per dose by the intramuscular route. Results: Quimihib® vaccine was well tolerated and no vaccine-related serious adverse events were reported. Following the primary series (7-month), IgG-specific anti-PRP geometric mean concentration (GMC) was 6.6µg/mL, six times over the threshold limit considered long-term protection against Hib invasive disease. Short-term and long-term seroprotection rates were 99 and 99%, respectively. Carrier state suppression and generation of herd-immunity was attained in more than 50% of vaccinated infants. Local and systemic reactions were infrequent and consistent with established Quimihib® pre-licensure experience. More than 99% of adverse reactions were mild in intensity and resolved without medical treatment. Recorded local events included pain and purifications at the injection site (0.9%). Systemic adverse events included irritability,
9.0%; slight fever, 7.2%; febricula, 6.9%; general malaise, 2.4%; somnolence, 0.9% and cutaneous rash, 0.3%. 

Conclusions: This is the first post-licensure study with QuimiHib® vaccine and demonstrates it is highly immunogenic and well tolerated in infants when manufactured at industrial scale levels.

21. ADVERSE EVENTS ASSESSMENT IN YELLOW FEVER VACCINEES

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Introduction: Yellow fever virus (YF), prototype virus of flavivirus genus, family Flaviviridae, is transmitted to human mainly by Aedes Aegypti mosquito and might cause a potentially fatal systemic illness. Healthcare personnel and travellers to endemic regions are some of the risk groups of this viral illness. A very effective product: 17D-based vaccine is available since 1945 to protect from YF. However, an increase of serious adverse events associated to YF vaccines has been recently reported. Materials and Methods: In this study, post-vaccination data from 97 professional employees of IPK immunized against YF either with the vaccine from Biomanguinhos (Oswaldo Cruz Foundation, Brazil) or with Sanofi Pasteur (France) from 1998 to 2008 were collected.

Results: A low incidence of mild adverse events (9.27 %) after vaccination such as mild pain in the site of injection, myalgias and general malaise, followed by low-grade fevers during 1 day were reported. No serious adverse events like YF vaccine-associated neurotropic and viscerotropic disease were found. Conclusions: In our study serious adverse events were not reported in the vaccinees, however minor symptoms were found.

22. STUDY OF PREVALENCE OF MEASLES ANTIBODIES IN THE CHILDREN POPULATION BY INDIRECT ELISA


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Introduction: To know the efficacy of vaccination, it is necessary to determine the immune status of population. Measles vaccine is included in the Cuban National Immunization program. The last case of the disease was reported in 1993. This paper described a study of prevalence of measles IgG antibodies in children by an ELISA developed in our laboratory and compared to Hemagglutination Test (HI) used as reference technique described by Norrby (1992). Material and methods: A total of 200 samples of children from 0 to 14 years old were randomly collected at the Pediatric Hospital “Juan Manuel Marquez” to determine measles antibody level. To be carried out the ELISA was made a concentrated and purified measles antigen in the laboratory. Results: 180 out of 200 serum samples showed protective measles IgG antibody, while 18 were negative by both techniques, 2 serum samples resulted negative for HI and positive for ELISA. The 18 negative samples coincided with infants in ages among 7 to 11 months, due to the lost maternal antibodies. In general ELISA detected IgG class antibodies, whereas HI test was only able to detect hemagglutination inhibitor antibodies. The high seroconversion obtained was prospective, because Cuba has a high vaccination coverage which corroborates the appropriate strategy used in our national vaccination program that it is started at one year old with viral triple vaccine. Conclusions: The ELISA developed in our laboratory will provide a high protection against measles in this population.
Introduction: Since 1992 the World Health Assembly launched the initiative of 100% of the vaccines employed in the National Immunization Programs with assured quality. In consequence, since 1996 till now the Word Health Organization (WHO) is coordinating several activities including the prequalification of vaccines, a process which consists of the assessment of vaccines, their manufacturers and also the National Regulatory Authority (NRA), which is expected to be fully functional, independent and meeting all the regulatory functions. This investigation was addressed to evaluate the impact on the regulatory system for vaccines in Cuba and its NRA, the Centre for State Control of Drug Quality (CECMED) of WHO´s assessments. Methodology: Data was collected using the general methods of archival study and interview of key informants, data was analysed, summarized and tables were prepared. Results: It was found that CECMED obtained satisfactory results in regulating vaccines in all the WHO´s assessments received and learned lessons from the interchange of experiences with the WHO assessors team, reviewed its processes with a better approach of the basic functions, identified weaknesses and strengths, and strategies for solving insufficiencies. CECMED also collaborates with WHO and the Pan American Health Organization (PAHO) developing tools for the assessment, teaching courses for other NRAs in the Latin American region, and participating in the assessment visits as part of the teams of international experts convened by WHO. Conclusions: The first general conclusion was that impact on Cuban vaccines regulatory system and CECMED of WHO assessments has been its consolidation and improvement as NRA, which means better quality support for all products on the Cuban market. It was remarked that CECMED is playing an active role collaborating with WHO/PAHO in the development and improvement of the NRA assessment process, including capacity building for another NRAs.

2. EVOLUTION IN CUBA OF THE POSTMARKETING SURVEILLANCE SYSTEM FOR VACCINES
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Introduction: The Cuban National Immunization Program (CNIP) has obtained very important results and its impact on health of the population is a success in terms of eradication of preventable diseases. In this context, importation and national production of vaccines are well developed pharmaceutical activities in Cuba. The National Regulatory Authority, the Centre for State Control of Drug Quality (CECMED), paid special attention to the regulation and control of the system for the surveillance of the behaviour of the vaccines on the market, as a particularity of postmarketing drug control, one of its basic regulatory functions. This investigation was performed for characterizing the organization, structure and regulatory support of postmarketing surveillance system for vaccines in Cuba, and its evolution. Materials and Methods: Data was collected using general methods of archival study and interview of key informants, data was analysed, summarized and diagrams and flow charts were prepared. Results: The system operating in Cuba has evolved and now is decentralized and coordinated by CECMED. It is based on voluntary reporting and consists of national surveillance systems of manufacturers, importers, distributors, the Centre for the Development of Pharmacoepidemiology, CNIP, and National Centre for Toxicology. Alerts and statistics are generated and regulatory actions are taken by CECMED. Regulations of good practices, guidelines and rules for the postmarketing surveillance has been issued, updated and implemented in order to precise responsibilities and improving the organization and information/retrieve mechanisms of the system, according to current trends, focused in the key elements of the World Health Organization (WHO) assessment tools, and according to recommendations from the assessment visits. Conclusions As a conclusion it was found that the system has been strengthening during the last years, coordination among the different institutions is becoming more effective and regulation is more complete, playing an important role in this process the assessments of WHO to CECMED.
3. LOT RELEASE BY THE NRA OF CUBA: PROCESS IMPROVEMENT AND GENERALIZATION

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Introduction: Lot release of vaccines and other biological, performed by National Regulatory Authorities (NRA) is part of the overall regulation of biological products to assure their quality, safety and efficacy. This is one of the six NRAs critical functions (WHO reference) and has an important impact on the availability and use of those products in public health. This process is independent on the manufacturer’s quality control testing and release. Some manufacturing process can be considered different as biological products have an intrinsic variability and some molecules are not well defined. The basis of this additional control include verification of fulfillment of specifications, manufacturing process conditions and test results based on approved Marketing Authorizations. The work’s objective was development and generalization assessment of this process in CECMED, as Lot Release is a dynamic process which requires continuous adjustments ensuring an up-to-date scientific approach as a basis for evaluating and releasing vaccines. Methodology: Three stages of assessment were defined: diagnosis, development/ implementation of corrective action and final results. Results: The outcomes are reflected in: legal framework update which includes new version of Lot Release Regulation, normative related to specifications for procurement, new SOP and work instructions and applicant access to guideline with more detailed information on how to present adequate lot release documentation to be assessed, timeframes and official documents templates. As tool for evaluation of every Summary Protocol, Lot Release Certificate emissions and consistency monitoring, a functional Data Base System (Excel application) has been improved and is in place. In 2000 WHO CECMED assessment Lot Release obtained 67% of function implementation; indicators have been changing and in last WHO assessment (2008) the result was 100%. Conclusions: In the last 3 years more than 700 lots of about 45 products, national/imported, were released by CECMED in a properly and timely manner, according to the product quality standards and avoiding delay on worldwide supply.

4. EVALUATION OF THE EXPERIMENTAL REDUCTION AND REMOVING OF THIOMERSAL OVER THE MICROBIOLOGICAL AND BIOLOGICAL PROPERTIES OF VACCINES

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Introduction Thiomersal is an Organo – mercury preservative included in vaccines for human use. In Cuba, the most of vaccines that conforms the National Immunisation Program contain thiomersal, including single – dose vaccine formulations like recombinant Hepatitis B and Meningococcal BC vaccines. Although there is no evidence of harm due to the level of exposure from vaccines, different manufacturers and regulatory bodies have promoted the reduction, substitution or elimination of thiomersal. In fact, thiomersal-free or reduced formulations are already available in the market. Nevertheless, issues concerning quality, safety and efficacy of vaccines could limit these options. The aim of this Paper is demonstrate that the elimination and or reduction of thiomersal should not impact the microbiological quality and biological activity of vaccines. Materials and Methods For that, we prepared some experimental thiomersal-free and reduced vaccine samples by dialysis and evaluated the protein content (by Lowry method), in vitro and or in vivo potency and sterility against thiomersal-containing vaccines. The effectiveness of preservative was also evaluated. Results There was no significant difference between thiomersal-containing and non-containing samples regarding important quality parameters like protein content, immunogenicity and sterility. Some in vitro tests yielded different results. Likewise, the effectiveness of preservative showed a variable pattern of protection regarding the removal or decreasing of the amount of thiomersal. Conclusions This approach provides useful and predictable information in order to produce large – scale batches with removed or reduced thiomersal without affecting the quality and efficacy of vaccines.
5. THE PHARMACEUTICAL INSPECTION TO VACCINES WITH RISK APPROACH

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Introduction: The thematic risk management, addressed at international level in documents such as the ISO 14971:2000 and the ICH Q9, is a systematic process for the evaluation, control, communication and revision of the risks in the processes and products through its life cycle. Within the group of parenteral medications, vaccines are highlighted by its highest level of demands; this is due to its nature and widespread use in healthy populations, mostly children. These risks can be attributed to the design, production process, storage, transportation and use, as well as factors involved in these processes, as personnel, equipment, facilities and documentation. Materials and Methods: This work presents the analysis of cuban vaccines manufacturing processes, from the point of view of the national regulatory demands and using the FMCEA tool (Analysis of the Way, Effect, Fails and Criticality). For that, the Priority Risk Numbers (RPN) of the Good Practices requirements that have to be considered in these processes are determined, according to the severity of their impact, as well as the probability of their occurrence and detection. Results: A hierarchy of the requirements was established for every aspect of the regulations applying to this kind of products. Conclusions: Using this risk approach, the State Pharmaceutical Inspections to vaccine manufacturers could be performed considering the risk associated. It would make easy the decision-making process with a scientific basis and a rational use of resources.

Symposium: New trends in manufacturing and quality assurance for vaccines in Cuba

Poster Presentations

1. INCREASING PRODUCTION CAPACITY FOR THE MANUFACTURING OF QUIMI-HIB® VACCINE ACTIVE PHARMACEUTICAL INGREDIENT

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Introduction: The number of companies producing vaccines nowadays is dropping, mainly because they are moving to produce more lucrative products. The market for Hib vaccines is growing and there is an unsatisfied demand. Quimi-Hib is the Cuban vaccine against *Haemophilus influenzae* type b which is manufactured in the CIGB facilities. In order to increase the production capacity of the Quimi-Hib® Active Pharmaceutical Ingredient (API), a new production strategy was performed. Materials and Methods: A new production strategy was defined using SuperPro-designer Software. Three large-scale - batches were manufactured using the defined strategy and previously installed technology. Purity and identity of each batch were tested by nuclear magnetic resonance spectroscopy (NMR). A statistical analysis was made by Minitab15 Software in order to evaluate process consistency before and after to introduce the new strategy. Results: The analytical results and the NMR spectrum of the batches showed compliance with the quality specification. The statistical analysis showed stability in the manufacturing process and no significant differences between batches produced with the two scales. There was an improvement on PRP yields media values and the cost per doses was reduced in to 41.4 percentage. Conclusions: The new strategy is economically suitable and allowed to improve productivity of the manufacturing of Quimi-Hib® vaccine active pharmaceutical ingredient in the same operations times and with previously installed equipment.
2. PROCESS VALIDATION OF RECOMBINANT HEPATITIS B SURFACE ANTIGEN (HBSAG) AS ACTIVE PHARMACEUTICAL INGREDIENT (API)


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Introduction: The production of HBsAg as API of Hepatitis B vaccine was established on Plant 1 at CIGB in 1991. Since 2000, this production was evaluated and certificated by World Human Organization. In 2008, validation strategy of this product was redefined including: qualification of equipment and facilities, validation of utilities systems, equipment and facilities cleaning, in process product and buffer hold times, the chromatographic resin and filter elements lifetime, impurity and contaminant removal, validation of final sterilizing filtration and consistency of inspection points and API quality controls. Materials and Methods: The new Process Validation Protocol defines the maintenance of validation stage by Annual Review of Product and continues process analyses (to every produced lot). The Performance Qualifications of technological systems must be needed when equipments are changed or facilities maintaining was made. The specific characterization and contaminant removal must be conducted when change has been introduced in the process, to demonstrate that the validation stage hasn’t been affected. Results: In 2008, a general maintenance was made in Plant 1 facilities. A lot of equipment was moved out of the production locations. At the end of the maintenance, before starting process validation activities, appropriate qualification of equipment and facilities was completed. This included Installation Qualification and Operational Qualification activities. In the performance Qualification, assembling of all components in each technological system was checked. A process with negative Pichia pastoris (yeast without genetic transformation) was performed until Propagation to Primary Purification Steps. The specific characterization including: removal of host cell proteins and product-related impurities; consistent product quality, purity and process yield were completed successfully. A complementary retrospective validation was made to evaluate the chromatographic resin lifetime. Conclusions: Qualification of equipment and facilities was completed successfully and it was demonstrated that process fulfills the validation requirements.

3. COMPUTERIZED SYSTEMS VALIDATION AT FINLAY INSTITUTE

Sánchez E


Introduction: Finlay Institute uses SCADA systems to manage sterilization and fermentation process for tetanus vaccine production and to preserve the classification of clean areas in the production area of DPT plant. The use of computerized systems in biopharmaceutical production requires an exhaustive process of validation because it is required by the organizations regulating this industry. As a result of WHO inspection on March, 2007 was established the following non-conformity for SCADA systems: “Computer controller equipment – no validation of SCADA used to control fermenter and HVAC”. The main objective of this work is to show how it was solved. Methodology: In order to obtain the validated state for the systems was elaborated a Master Plan to validate SCADA systems and a Protocol Validation for each system. In these documents are established the needed tests and acceptance criteria to assure that pressure, temperature and humidity parameters are effectively controlled during the operation in critical process and data are saved as electronic records to be stored and processed. Results: The different tests performed during IQ, OQ and PQ assured fundamentally the unity, integration and functionality of computer system modules. Acceptance criteria established assured the integrity of electronic records due to they can’t be modified or deleted, and access to those data only could be allowed to authorized personal. It was verified that data values read by sensor are equals to those that are shown and saved by the computer system as electronic records. When a parameter is out of established range, an alarm signal is generated and it is recorded by the system too. System security was also assured with an user account for each person that operates the system. Conclusions: The deviation was solved by taking actions for validating the computerized systems, as demonstrated during the WHO follow-up inspection.
4. INTRODUCTION OF A NEW REACTION SYSTEM FOR HYDROGENATION OF SYNTHETIC OLIGOSACCHARIDE

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Introduction: Polysaccharide polyribosylribitol phosphate (PRP) antigen for Quimi-Hib® vaccine is produced synthetically. The oligosaccharide synthesis groups around 15 chemical reactions, where selected protective groups are introduced and removed carefully. Materials and Methods: Deprotection and azide reduction of synthetic Oligomer with an average of 11 repeating units was made in two different reaction systems using H₂, Pd-C, and EtOH-H₂O-ETOAc-AcOH at 1.5 atm. In this paper we compare three process variables: hydrogenation reaction advance, mass and yield of processed PRP obtained as a result of using both large-scale reaction systems. Results: No statistically significant differences were found in product yield and at 48 hour reaction advance at the 95.0% confidence level between both reactions systems. However, installation capacity increased from 10 to 24-g scale per batch of highly pure functionalized PRP. Process remained stable and under control and was capable to produce consistently a hydrogenated oligosaccharide which meet all its specifications. Product losses, due to fatigue Teflon-flask breaking, were minimized after new reaction system introduction. Conclusions: New reaction system was operated as a complete close system at higher level of security and reliability.

5. IMPROVEMENT OF CULTURE CONDITIONS FOR PRODUCING POLYSACCHARIDES VACCINES FROM STREPTOCOCUS PNEUMONIAE

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Introduction: Many studies for the optimization of cultures during the production of polysaccharides from Streptococcus pneumoniae are not reported. That’s why, for what we intended to evaluate the influence of the composition of the medium and culture conditions in the prefermentation stage. Materials and Methods: The growth was obtained by direct pass starting from seed bank stored to -70 °C to a flask with 200ml of half triptic soy diafiltrated of non animal origin. The culture conditions evaluated were: elimination of the solid medium, shaker at 50 rpm, static cultivation at 37 °C and static cultivation at 37 °C with 5% CO₂. It was also evaluated the influence of the concentration of glucose and the pH of the culture medium. Results: The results demonstrated that the CO₂ acidifies faster the culture so the growth is slow, however there were not significant differences between the cultures in shaker and without CO₂. Concentrations of glucose higher than 5 g/L inhibit the growth. The growth kinetics in all replicates assayed was the typical one for this microorganism, so it is able to recover its physiologic conditions quickly even when it has been under freezing. Growth kinetic was similar for all the serotypes evaluated. Conclusions: This result demonstrated that the time for obtaining the inoculum during the fermentation can be reduced considerably for obtaining a better production consistency.
Vaccinology has been very effective to preventing infectious diseases. However, in several cases, the conventional approach to identify protective antigens, based on biochemical, immunological and microbiological methods, has failed to deliver successful vaccine candidates against major bacterial and parasitic pathogens. Nowadays there is a contrast between the rapid development seen in the understanding of basic immunology and the very slow progress to bringing new vaccines into use. This relatively slow progress is the lack of inherent immunogenicity of many pure antigens, and the lack of appropriate licensed adjuvants able to efficiently enhance immune responses. The recent development of powerful biotechnological tools applied to genome-based approaches has revolutionized vaccine development, biological research and clinical diagnostics. The availability of a genome provides an inclusive virtual catalogue of all the potential antigens from which it is possible to select the molecules that are likely to be more effective. These advances in the fields of genomics, proteomics and molecular immunology offer tremendous opportunities for the development of novel vaccines in infectious diseases like cholera, tuberculosis, Neisseria meningitides, Herpes Virus and Malaria among others. The purpose of this work is to evaluate main strategies for new vaccine candidates including selected antigens by bioinformatic and biotechnologic tools, and new adjuvants, which constitutes important topics of research in our institution.