TECHNOLOGICAL ADVANCES IN VACCINE DEVELOPMENT

Organizer: Laurence Lasky

January 30 - February 6, 1988

Page

Keynote Address 2

Plenary Session

January 31:
Attenuated Virus Vaccines 2
Inactivated Virus Vaccines and Naturally Derived Subunit Vaccines 3

February 1:
Production of Pathogen Surface Antigens in Bacteria and Yeast 4
Production of Pathogen Surface Antigens in Mammalian and Insect Cells 6

February 2:
Vaccines Based on Synthetic Peptides (joint session) 7

February 3:
Humoral and Cytotoxic Responses to Infectious Agents 8
Vaccinia-based Vaccines 9

February 4:
Other Viral Systems for the Delivery of Antigens 10
Adjuvants 11

Poster Sessions

January 31:
Attenuated, Inactivated and Naturally Derived Subunit Vaccines 14

February 1:
Production of Pathogen Surface Antigens by Recombinant DNA 19

February 2:
Vaccines Based on Synthetic Peptides 26

February 4:
Vaccinia and Other Viral Delivery Systems 33

February 5:
Anti-idiotype Vaccines; Immunological Constraints; Immunological Enhancement with Adjuvants and Biologicals 39

Technological Advances in Vaccine Development

Keynote Address

F 001 PHENOMENOLOGY AND SCIENCE OF VACCINOLOGY, Jonas Salk, The Salk Institute for Biological Studies, P.O. Box 85800, San Diego, CA 92138-9216.

As a backdrop to the discussions of the technology of vaccines, I will present a picture of the phenomenology and science of vaccinology, about which I have been reflecting for some time. A review of experiences and approaches that were explored before the advent of high technology—an era of low technology, or no technology, as compared with what is now possible—might be helpful to those who are now engaged in advancing the technology of developing and improving vaccines. For this purpose, I shall draw largely on my own experiences to reveal the process by which we arrived at useful results and their introduction into practice. Such an historical view may not contribute directly to the solution of the technological problems with which this symposium is concerned, but it may provide a way of thinking for those who wish to relate what they do to a practical end result. I shall also discuss the difference between the use of immunogens (or vaccines) for therapy as well as for prophylaxis where this may be applicable as for the control of AIDS and other similar infectious diseases.

Attenuated Virus Vaccines

F 002 CLASSICAL AND NEOCLASSICAL LIVE VIRUS VACCINES, Stanley A. Plotkin, Children's Hospital of Philadelphia, the University of Pennsylvania and the Wistar Institute, Philadelphia, PA 19104.

The current fashion is to develop vaccines by the genetic engineering of non-living antigens thought to be immunogenic and protective. Although there is much promise in this approach, live vaccines, both historically and at present, provide the surest means of stimulating all relevant immune responses. Live vaccines against varicella-zoster and cytomegalovirus exemplify the utility of attenuated strains to prevent disease of complex pathogenesis. These viruses are given parenterally and stimulate antibody and cellular immune responses. Rotavirus vaccines now under investigation are attenuated viruses that replicate in the intestine and stimulate humoral and probably local immunity. Both naturally derived heterospecific strains and laboratory-derived reassortant strains are being tested.

Inactivated Virus Vaccines and Naturally Derived Subunit Vaccines

F 003 IMMUNOBIOLOGY OF THE EXTERNAL HIV ENVELOPE GLYCOPROTEIN, Dani P. Bolognesi, Department of Surgery, Duke University Medical Center, Durham, NC 27710.
Studies will be described which identify epitopes on HIV gp120 which are important targets for immune attack. These will include regions of the molecule important for virus neutralization. Other segments of the molecule can serve as targets for antibodies that mediate destruction of infected cells through various mechanisms including ADCC. The efficiency of various vaccine regimens to induce immune responses to such epitopes will be discussed.

F 004 *Abstract Withdrawn*

F 005 CANDIDATE SUBUNIT FLAVIVIRAL VACCINES: IMMUNOGENIC CHARACTERIZATION OF YELLOW FEVER AND DENGUE VIRUS STRUCTURAL AND NON-STRUCTURAL PROTEINS, Jacob J. Schlesinger, Michael W. Brandriss and Edward E. Walsh. University of Rochester School of Medicine and the Rochester General Hospital, 1425 Portland Avenue, Rochester, NY 14621.
Immunogenic and structural characterization of those viral proteins involved in the protective immune response is requisite to the design of candidate synthetic peptide or recombinant vaccines. Cumulative evidence derived from mouse protection experiments involving passive transfer of monoclonal antibodies has so far suggested as components of a subunit flaviviral vaccine two virus-specified proteins: the virion envelope glycoprotein, E (51-60 kd), which subserves cell attachment and neutralization and a non-virion cell-associated glycoprotein, of uncertain function, NS1 (44-49 kd). We have purified these proteins from lysates of yellow fever (YF) and dengue 2 (DEN) virus-infected cells by immunoaffinity chromatography and HPLC. Affinity-purified YF E could not be identified as the intact protein but bore antigenic determinants of E as defined by selective reactivity with anti-E monoclonal antibodies. The reactivity correlated with a 33 kd band, the predominant component on PAGE. Immunization of mice with this material provided protection against lethal intracerebral challenge with the virus and hyperimmunized rabbits produced neutralizing antibodies to both YF and DEN, indicating a potential for cross-protection. Active immunization of mice and monkeys with YF NS1 stimulated production of complement-fixing antibody in high titer and conferred protection against lethal challenge with the virus. Plasma membrane expression of NS1, but not E, demonstrated by immunofluorescence, radiobinding, and monoclonal antibody-dependent complement-mediated cytolytic assays, suggests that immune recognition of this protein on the surface of infected cells may provide an alternative to direct viral neutralization as a mechanism of host defense against flaviviral infection. Recognition of tryptic peptides of NS1 by monoclonal antibodies and monospecific antisera suggests that synthesis of potentially protective peptides may be feasible.

Production of Pathogen Surface Antigens in Bacteria and Yeast

F 006 **TOWARD DEVELOPMENT OF A RECOMBINANT PERTUSSIS VACCINE.** W. Neal Burnette,[1] Vernon L. Mar,[1] Witold Cieplak,[2] Charles F. Morris,[1] Kaarel Kaljot,[2] Hiroko Sato,[3] and Jerry M. Keith.[2] [1]Amgen Inc., Thousand Oaks, CA 91320, [2]NIAID Rocky Mountain Laboratory, Hamilton, MT 59840, and [3]NIH, Tokyo, Japan.

Despite the wide availability of an efficacious pertussis vaccine developed half a century ago, whooping cough remains a major contributor to worldwide infant morbidity and mortality. Whole-cell pertussis vaccine components have been implicated in both mild and severe reactions to immunization. Recent litigation surrounding issues of pertussis vaccine liability have had a chilling effect on all new vaccine development programs, threatened the nation's vaccine supply, and led to a decrease in vaccine acceptance with a subsequent resurgence of whooping cough in certain parts of the world.

We have taken a recombinant DNA approach to eliminate the reactogenicity problems encountered in traditional pertussis vaccines. Pertussis toxin (PTX) is a major virulence factor of Bordetella pertussis and has been demonstrated to be a necessary, and perhaps sufficient, protective immunogen. We have previously cloned the PTX operon and determined its cistron order and sequence (Locht et al., Nucl. Acids Res. 14:3251, 1986; Locht and Keith, Science 232:1258, 1986). The genes encoding the five individual subunits of PTX were subcloned and directly expressed to high levels in E. coli as non-fusion proteins. The recombinant S1 subunit (26,026 d) contains a major protective epitope and possesses enzymatic activities associated with the toxicity of PTX and reactogenicity of pertussis vaccines. The S1-related ADP-ribosyltransferase has been shown to catalyze the ADP-ribosylation of the N_i inhibitory protein of the adenylate cyclase complex. Deletion mutations of the S1 subunit gene resulted in protein species with extensive terminal truncations which allowed us to assign the protective epitope and enzymatic sites to locations near the amino terminus. More precise mapping was accomplished by producing a progressive series of amino-terminally truncated recombinant proteins by exonucleolytic digestion of the S1 gene followed by expression in E. coli. Analysis of these products, both for enzymatic activity and for reactivity with a monoclonal antibody capable of providing passive immune protection in challenged mice, defined a discrete region of the S1 protein, delineated by tyrosine 8 and proline 14, which possesses at least a portion of the enzyme active site and the protective antigenic epitope. These studies will permit us to construct recombinant site-specific S1 mutants that retain an important protective epitope, yet eliminate enzyme-related reactogenicity.

F 007 YEAST AS A HOST CELL FOR THE EXPRESSION OF VACCINE ANTIGENS, Ronald W. Ellis, Department of Virus & Cell Biology, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486.

Bakers' yeast Saccharomyces cerevisiae is a versatile host for the expression of foreign genes. It has been especially useful for the expression of hepatitis B surface antigen as a vaccine, the first recombinant-derived vaccine for humans ever licensed. Applications to present- and future-generation hepatitis B vaccines as well as to vaccines in general will be discussed.

F 008 MAPPING OF THE PRINCIPAL HIV NEUTRALIZING EPITOPE, Scott D. Putney[1], Joan Petro[1], Debra L. Lynn[1], Raymond Grimaila[1], Shuzo Matsushita[2], Majorie Robert-Guroff[3], Robert C. Gallo[3], Dani P. Bolognesi[4], Thomas J. Matthews[4] and James R. Rusche[1]; [1]Repligen Corporation, Cambridge, MA 02139, [2]The Second Department of Internal Medicine, Kumamoto University Medical School, Kumamoto 860, Japan, [3]Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, MD 20892, [4]Department of Surgery, Duke University Medical School, Durham, NC 27710.

Antibodies to the entire HIV envelope, gp160, or the carboxyl-terminal portion of the external envelope, PB1, inhibit fusion of virally infected cells and neutralize free virus infection. These activities are HIV isolate specific. Antibodies to either gp160 or PB1 from the HTLV-III_B (III_B) or HTLV-III_{RF} (RF) isolate neutralize and block fusion of cells infected with the homologous isolate but not two heterologous isolates.

To map the location of the neutralizing and fusion inhibiting epitope, subfragments of PB1 were synthesized. A 24 amino acid long peptide (RP135) blocks a large majority of the fusion inhibiting antibodies and elicits antibodies that neutralize cell free virus and inhibit fusion of infected cells. RP135 also blocks antibodies from a rhesus monkey infected with recombinant vaccinia virus expressing gp160 and from a chimpanzee infected with HTLV-III_B. In addition, a monoclonal antibody (0.5B) was produced to gp120 from III_B and 0.5B neutralizes infection by III_B but not RF or MN. 0.5B binds PB1 from only the III_B isolate and also binds to RP135. The sequence of the RP135 region is highly variable.

These results show that the RP135 region contains the principal HIV type specific neutralizing and fusion inhibiting epitope. This does not rule out, however, the possibility that other envelope segments, perhaps those that are more conserved and play a role in CD4 binding, may elicit neutralizing antibodies. An effective HIV vaccine may need to contain both conserved and variable envelope regions.

Production of Pathogen Surface Antigens in Mammalian and Insect Cells

F 009 THE EFFECTS OF POST-TRANSLATIONAL MODIFICATIONS ON THE ANTIGENIC PRESENTATION OF EXPRESSED PROTEINS, Emilio A. Emini, M. Silberklang, L. D. Schultz and R. W. Ellis, Department of Virus and Cell Biology, Merck, Sharp and Dohme Research Laboratories, West Point, PA 19486.
The effects of the host cell on the antigenic presentation of expressed protein immunogens was investigated. The model protein employed was the Epstein-Barr virus (EBV) major membrane antigen, gp350/220. This protein is highly glycosylated; approximately fifty percent of its molecular mass is contributed by the carbohydrate added onto the protein subsequent to translation.
Yeast cells (Saccharomyces cerevisiae) and several mammalian cell lines (GH3 rat pituitary cells, mouse L cells and monkey kidney vero cells) were engineered to express the gp350/220 antigen. Purified preparations of each expressed protein, as well as gp350/220 purified from EBV-producing B95-8 lymphoid cells, were compared for their antigenic presentation. Each antigen was evaluated for its ability to bind to a panel of anti-gp350/220 monoclonal antibodies and for relative binding to a series of anti-EBV positive human sera.
The yeast cell-expressed protein exhibited a significant degree of hyperglycosylation, its molecular weight being in excess of 400 Kd. This excessive carbohydrate modification rendered the antigen unreactive with the large majority of monoclonal antibody and human serum samples. The purified protein was found to bind strongly to normal anti-yeast antibodies, presumably due to the unique nature of the yeast cell-dependent glycosylation.
In contrast, the mammalian cell-derived proteins all exhibited a degree of carbohydrate modification equivalent to that of the native lymphoid cell-expressed protein. Nonetheless, each antigen proved unique in its pattern of binding to the monoclonal antibodies and human sera. Each antigen was also unique in the pattern of biologically active antibodies induced following inoculation into small animals. Hence, antigenic presentation of expressed proteins are significantly influenced by the specific post-translational modifications imposed by the host cell.

F 010 EXPRESSION AND MODIFICATION OF HEPATITIS B SURFACE ANTIGEN, Rolf E. Streeck*, Nicole Chenciner, Jean-Francois Houssais[+], Bruno Blondel, Radu Crainic, Sylvie van der Werf and Francis Delpeyroux, Institut Pasteur, Paris and [+]Institut Curie, Orsay, France. *Present address: Institute of Medical Microbiology, University of Mainz, Germany.
The hepatitis B virus (HBV) envelope protein carrying the surface antigen (HBsAg) is assembled in mammalian cells into 22-nm particles corresponding to empty viral envelopes. We have developed cellular systems for expression of HBsAg at high level, including gene amplification (1) and fusion of cell lines with primary hepatocytes. Monkey hepatocytes fused to monkey Vero HGPRT$^-$ cells, previously transfected by plasmids carrying the S and pre-S coding regions of HBV, induced a large increase of HBsAg synthesis. Fusion with primary monkey kidney cells had no stimulatory effect at all. Hybrid clones established as cell lines had a constant karyotype and exhibited stable high-level expression of HBsAg. - In a study to evaluate the capacity of HBsAg to present foreign peptides in a biologically active form, we have created mutant envelope proteins by insertion of various sequences into the S gene of HBV (2). One of the sequences inserted corresponded to an 11-amino acid poliovirus type 1 (Mahoney) neutralization epitope. Expression of the modified gene in mammalian cells has yielded hybrid particles which induce in mice neutralizing antibodies against poliovirus but only a weak immune response to HBsAg (3). By cotransfection of cells with plasmids carrying either modified or unmodified S genes we have obtained mixed particles presenting both HBsPolioAg and HBsAg. When inoculated to rabbits, antibodies to both poliovirus and HBsAg were induced. The titers of neutralizing antibodies to poliovirus induced by HBsPolioAg were much higher than previously observed in mice. The design of multivalent particles presenting several heterologous epitopes may therefore be possible.

1. M.L. Michel, E. Sobczak, Y. Malpièce, P. Tiollais and R.E. Streeck (1985) Bio/Technology 3, 561-566.
2. F. Delpeyroux, N. Chenciner, A. Lim, M. Lambert, Y. Malpièce and R.E. Streeck (1987) J. Mol. Biol. 195, 343-350.
3. F. Delpeyroux, N. Chenciner, A. Lim, Y. Malpièce, B. Blondel, R. Crainic, S. van der Werf and R.E. Streeck (1986) Science 233, 472-475.

Technological Advances in Vaccine Development

Vaccines Based on Synthetic Peptides

F 011 THE NEXT GENERATION OF FOOT-AND-MOUTH DISEASE VACCINES, F. Brown, Wellcome Biotech, Langley, Court, Beckenham, Kent BR3 3BS, U.K.

A peptide corresponding to the major immunogenic site of the protein VP1 of foot-and-mouth disease virus (FMDV) will elicit a protective neutralizing antibody response in guinea pigs, cattle and pigs. The response is much greater when the peptide is presented as a linear dimer or tetramer fused to β-galactosidase and pigs receiving as little as 40μg of the tetramer are protected against challenge infection. A response approaching that obtained with virus particles is obtained when the peptide is presented as part of the core protein of hepatitis B virus. Moreover, responsiveness to the peptide can be enhanced by the presence of a covalently linked T cell epitope. These results indicate that a molecular vaccine based on peptides is feasible for foot-and-mouth disease.

F 012 STUDIES ON THE DEVELOPMENT OF A SYNTHETIC PEPTIDE VACCINE TO FELINE LEUKEMIA VIRUS, John H. Elder, Department of Molecular Biology, Research Foundation of Scripps Clinic, La Jolla, CA 92037.

We have prepared synthetic peptides corresponding to the predicted amino acid sequences of the envelope gene products of several variants of feline leukemia virus. Antisera were prepared to these peptides conjugated to carrier proteins and tested for ability to elicit *in vitro* neutralizing responses. A number of sites were identified which may serve to neutralize virus infectivity, including epitopes in both the major envelope glycoprotein, gp70, as well as the small membrane-spanning envelope protein, p15E. We next tested the antipeptide antisera for ability to cross-neutralize a panel of FeLV variants, including members of all three major subtypes of FeLV. We found that in certain instances, the ability of the antisera to neutralize the variant viruses correlated directly with sequence conservation or diversity at a given epitope; i.e., conserved epitopes served as broadly cross-neutralizing sites whereas epitopes which varied in amino acid sequence facilitated only type-specific neutralization. However, we also found that certain conserved sites, located in the C-terminal portion of gp70 and within p15E, did not serve as broadly cross-neutralizing epitopes. The implication from this observation is that the exposure of certain epitopes varies from one FeLV variant to the next (generally along major subtype lines), either by differences in folding of the molecule or via differential glycosylation. Another possibility is that localized changes in the conformation of the epitope results in lowered affinity of antibody binding in certain variants, resulting in failure to neutralize the virus. We have found an example of the latter in a detailed analysis of one particular conserved neutralizing epitope. Using a monoclonal antibody prepared to intact virus, but reactive with one of our peptides, we were able to precisely map a five amino acid epitope required for antibody binding. A variant which is not neutralized at the site contains this five amino acid epitope, but also has a single amino acid change (leucine for proline) three amino acids N-terminal to the epitope. Synthetic peptides were prepared corresponding to the region of both the susceptible and resistant FeLV isolates and used to compete for antibody binding to whole virus. The results indicate that this single amino acid change causes a ten-fold diminution in the ability of the peptide to compete for antibody binding. We postulate that the leucine for proline substitution alters the beta turn potential of the antibody binding epitope, resulting in lower antibody binding affinity and failure to neutralize the variant virus.

F 013 SYNTHETIC HEPATITIS B IMMUNOGENS, A. Robert Neurath[1], Stephen B.H. Kent[2], Nathan Strick[1], Karen Parker[2], Linda Martin[2], [1]The New York Blood Center, 310 E. 67th Street, New York, 10021; [2]California Institute of Technology, Pasadena, CA 91125.

Mapping of epitopes on the hepatitis B virus (HBV) envelope (env) protein(s) involved in neutralization of infectivity and in protection against infection is difficult, since virus infectivity titrations can be carried out only in chimpanzees. Nevertheless, it was possible to identify such epitopes in distinct regions of the HBV env protein using synthetic peptides and monoclonal antibodies. The HBV env consists of three distinct, but related protein species: S-protein, M-protein (S + preS2) and L-protein (S + preS2 + preS1). Studies with synthetic peptides derived from the S, preS2 and preS1 regions of the HBV env protein have shown that each of these regions contains an epitope(s) involved in the process of virus-neutralization. In considering the development of an immunogen with potential for vaccination, one has to consider virus-neutralization epitopes on each of these three regions of the HBV env. Therefore, delineation of dominant epitopes on the S, preS2 and preS1 region is required. The dominant B-cell epitopes on the preS1 and preS2 region appear to be contiguous and are mimicked with relative ease by synthetic peptides. Antigenic determinants of the S-region appear to be non-contiguous and are dependent on the maintenance of disulfide bonds. Results of delineation of contiguous determinants essential for biological functions of the preS1 + preS2 sequence of the HBV env protein and attempts to mimic with synthetic peptides S-protein epitopes will be described. The design of fully synthetic immunogens containing essential portions of each the S, preS1 and preS2 sequence will be discussed.

The preS2 sequence was reported to react with gluteraldehyde-polymerized human serum albumin. It was postulated, without any direct evidence, that immunization with preS2 sequences may result in auto-immune responses to human serum albumin and to human hepatocyte cell membranes. Experimental evidence disproving this postulate and supporting the safety of vaccines containing preS sequences will be presented.

Humoral and Cytotoxic Responses to Infectious Agents

F 014 ABERRANT RESPONSES TO INFECTIOUS AGENTS: IMMUNE ENHANCEMENT OF VIRAL INFECTIVITY, James S. Porterfield, Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, England.

The response of a vertebrate host to an infectious agent contains both specific and non-specific elements. Unless a virus produces a rapidly fatal infection, recovery is marked by the appearance of antiviral antibodies and of T cell immunity with specificity against the virus in question which together provide substantial protection against reinfection with the same agent. Viral vaccines are intended to produce a comparable degree of protection without the host having to undergo the hazards of exposure to unmodified viruses. It is difficult to assess the efficacy of anti-viral vaccines except by challenge experiments; assays of antiviral antibodies can give misleading results even when neutralization tests are used, and assays of T cell responses are difficult to perform and to interpret.

Some viral vaccines induce antibodies that are not protective in vitro, and such antibodies may indeed enhance the replication of viruses. Antibody dependent enhancement (ADE) of viral infection can occur in vitro with viruses in many different families, and there are now several examples of ADE in vivo. The best understood mechanism underlying ADE involves the more efficient viral infection of cells bearing Fc receptors in the presence of poorly neutralizing antiviral antibodies, but ADE can also occur in some cells which lack Fc receptors, and this mechanism does not adequately explain in vivo enhancement. Vaccines that fail to induce potent neutralizing antibodies, or that induce unbalanced B and T cell responses may potentiate viral infection with consequences that may be detremental to the host.

F 015 **T CELL-MEDIATED IMMUNITY AND MONOCLONAL ANTIBODY HETEROCONJUGATES TO HUMAN IMMUNODEFICIENCY VIRUS (HIV)**, Joyce M. Zarling[*,**], Patricia Moran[*], Laura S. Grosmaire[*], Shiu-Lok Hu[*], Kathleen Shriver[+], Jorg W. Eichberg[**], Elaine Kinney-Thomas[*], and Jeffrey A. Ledbetter[*]. [*]Oncogen, Seattle, WA 98121; [+]Genetic Systems, Seattle, WA 98121; [**]Southwest Foundation for Biomedical Research, San Antonio, TX 78284.

HIV may spread as cell-free virus or by fusion of infected cells with uninfected cells. Neutralizing antibody may help prevent spread of HIV whereas killing of HIV-infected cells may be mediated by HIV specific T cells or by or monoclonal antibodies that could specifically target T cells or large granular lymphocytes (LGL) to lyse HIV infected cells. Uninfected individuals might therefore be protected against HIV infection by immunization with vaccines that induce neutralizing antibody and HIV-specific T cell-mediated immunity (CMI). Individuals, such as babies born of HIV-infected mothers, who are already infected with HIV might be protected from developing widespread infection with HIV and developing AIDS by passively transferring antibodies that could neutralize infectivity of HIV and/or render peripheral blood lymphocytes (PBL) lytic for HIV-infected cells. We have carried out experiments aimed at determining whether potential HIV vaccines induce CMI in primates and also whether monoclonal antibodies to HIV can target PBL to kill HIV-infected cells.

In studies of CMI to HIV, PBL of healthy HIV seropositive humans and chimpanzees were stimulated in vitro with HIV-1 and with HIV-1 glycoproteins gp41 and gp120. Whereas PBL from HIV-infected chimpanzees showed strong proliferative responses to intact HIV, gp41 and gp120, PBL from asymptomatic HIV-infected humans showed little, if any, proliferative response to HIV or HIV glycoproteins. Since both HIV-infected humans and chimpanzees develop antibodies to HIV, the difference is the incidence of AIDS in these species may be related to the levels of T CMI to HIV. Immunization of macaques and chimpanzees with recombinant vaccinia viruses which express either HIV <u>env</u> or <u>gag</u> antigens induced T cell proliferative responses to HIV. Additionally, <u>env</u> specific cytotoxic T cell clones were isolated from immunized chimpanzees.

Antibody heteroconjugates were constructed that contain a monoclonal antibody to gp110 (110.4), which neutralizes infectivity of HIV, cross-linked to a monoclonal antibody to CD3 (G19-4) or to the CD16 Fc receptor on LGL (Fc2). Such antibody heteroconjugates bridge HIV-infected target cells to T cells or LGL, activate their lytic mechanisms and enable PBL from HIV seronegative as well as seropositive humans to lyse HIV infected cells. With the 110.4 x G19-4 and 110.4 x Fc2 heteroconjugates, lysis is mediated by $CD8^+$ T cells and $CD16^+$ LGL, respectively. Pre-treatment of PBL with monomeric anti-CD3 on solid phase or with interleukin-2 or interferon-β markedly augments the ability of PBL to lyse HIV infected cells in the presence of the heteroconjugates. Such monoclonal antibody heteroconjugates, particularly ones containing a neutralizing antibody to HIV, may be of prophylactic or therapeutic value in individuals infected with AIDS virus.

Vaccinia-Based Vaccines

F 016 **USE OF RECOMBINANT VACCINIA VIRUS AS AN APPROACH TO VACCINES AGAINST AIDS AND MELANOMA.** Shiu-Lok Hu,[1] Joyce M. Zarling,[1] Patricia N. Fultz,[2] Jorg W. Eichberg,[3] Elaine Kinney-Thomas,[1] Pennathur Sridhar,[1] Bruce Travis,[1] and Charles D. Estin,[1] [1]Oncogen, Seattle, WA; [2]CDC and Yerkes Regional Primate Center, Atlanta, GA; [3]Southwest Foundation for Biomedical Research, San Antonio, TX

Recombinant vaccinia virus has been used extensively as an approach to vaccines against various infectious diseases. We are interested in using this approach for generating potential vaccines against AIDS. Toward this end, we have constructed several recombinant viruses that express the surface antigens of human immunodeficiency virus (HIV). One such recombinant, v-env5, which contains the entire coding sequence of the HIV <u>env</u> gene, has been studied in detail. Upon infection of tissue culture cells, v-env5 synthesized immunoreactive glycoproteins that corresponded to the precursor (gp160), and the mature envelope glycoproteins (gp120 and gp41) of HIV. Macaques and chimpanzees immunized with v-env5 generated HIV <u>env</u>-specific antibodies and T-helper cells. In addition, <u>env</u>-specific cytotoxic T-lymphocytes were detected in chimpanzees immunized with this recombinant. Results from preliminary studies indicated that although immunization of chimpanzees with v-env5 did not protect them from challenge infection of HIV, it might have affected the development of disease, since no v-env5-immunized animal developed persistent lymphadenopathy as one of the control animals did. The significance of these results as well as their relevance to vaccine trials will be discussed. We have also constructed recombinants that contain the <u>gag-pol</u> region of HIV. These recombinants synthesized immunoreactive polypeptides of 55K, 40K, 24K and 18K, corresponding respectively to the precursor (p55), the processing immediates (p40) and the mature core proteins (p24 and p18) of HIV. The immunogenicity of these recombinants are currently being studied. Preliminary results indicated that mice and macaques immunized with these recombinants were able to generate antibodies and T-helper cells specific for HIV core antigens. We are also interested in exploring the potential of recombinant vaccinia virus as an approach for cancer immunotherapy. Infection with a live recombinant virus could result in the presentation of tumor-associated antigens in a form favorable for the generation of cell-mediated immunity, which is an essential consideration for an anti-tumor vaccine. To demonstrate this potential, we have constructed a recombinant virus, v-p97, which expresses a human melanoma-associated antigen, p97. Both humoral and cell-mediated immunity (T-helper cells and delayed-type hypersensitivity) against p97 were elicited by v-p97 immunization of animals. This was observed not only in mice, but also in macaques, which express an antigen highly cross-reactive with human p97. Furthermore, tumor regression was observed in v-p97-immunized mice following transplantation of syngeneic tumor cells expressing human p97 antigens. The potential use of this recombinant virus as a form of immunotherapy for metastatic melanoma patients will be studied.

F 017 VACCINIA VIRUS EXPRESSION VECTORS: RECENT ADVANCES, Bernard Moss, Thomas Fuerst, Charles Flexner, Ambros Hugin, Patricia Earl

Vaccinia virus has been developed as a vector for expression of proteins in vitro and in vivo. Advantages of the system include a large capacity for foreign genes, cytoplasmic site of transcription, wide rage of susceptible tissue culture cells, ability to infect wild and domesticated animals as well as man, and extensive experience with vaccinia virus as a smallpox vaccine. Two types of vectors have been devised: the first utilizes vaccinia transcriptional regulatory sequences for expression; the second involves the incorporation of the bacteriophage T7 DNA-dependent RNA polymerase into the vaccinia genome and the use of T7 regulatory sequences for foreign gene expression. Methods to facilitate the selection and screening of recombinant viruses are now available. Efforts to improve the safety of vaccinia virus as a live recombinant vaccine include the inactivation of individual vaccinia genes and the introduction of lymphokine genes. Currently, vaccinia virus vectors are being used to determine the targets of immunity to human immunodeficiency virus and to develop both subunit and live AIDS vaccines.

Other Viral Systems for the Delivery of Antigens

F 018 VARICELLA-ZOSTER VIRUS: A VIRAL VECTOR FOR THE EXPRESSION OF FOREIGN GENES, Ronald W. Ellis, Department of Virus & Cell Biology, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486.

Varicella-zoster virus (VZV) is the etiologic agent of chickenpox. A live attenuated viral vaccine has been shown in clinical trials to have activity in preventing disease in young children. It is hoped that eventually this vaccine will be available for wide-spread use in healthy children. The vaccine strain of VZV has been developed into a live viral vector for the expression of foreign genes, especially those encoding immunogens of other viruses. Vector development and a relevant in vivo model system will be discussed.

F 019 CLONING AND EXPRESSION OF GLYCOPROTEIN GENES IN HUMAN ADENOVIRUS VECTORS, Frank L. Graham, Ludvik A. Prevec, Mary Schneider, Goutam Ghosh-Choudhury, Mark McDermott, and David C. Johnson, Departments of Biology and Pathology, McMaster University, Hamilton, Ontario, CANADA. Adenovirus vectors have proven useful for achieving high level expression of a variety of foreign genes and have many features which make them attractive for use as recombinant viral vaccines. The human adenovirus genome contains at least two regions into which DNA can be inserted to generate helper independent vectors: early region 3 (E3) which is nonessential for replication of virus in cultured cells, and E1, which is nonessential for replication in 293 cells which provide E1 functions in trans. Without deletion of viral sequences, the virion capsid can accommodate genomes with up to 2 kb of extra DNA and if compensating deletions of viral DNA are made, as much as 7 kb of foreign DNA can be inserted. We have developed novel approaches for rescue of insertions into both E1 and E3 and have used these methods as well as conventional techniques to construct vectors carrying and expressing the genes for dihydrofolate reductase, Herpes Simplex Virus thymidine kinase (HSV TK), Vesicular Stomatitis Virus glycoprotein G (VSVG), and HSV glycoprotein B (HSVgB). Both the VSVG and HSV gB recombinants produced high levels of the corresponding glycoprotein in infected human cells and were able to express in cells of other species as well. The VSV vector has been used to elicit neutralizing antibody in a variety of species including swine, murine, bovine and canine. The HSVgB expression vector has been shown not only to induce production of circulating antibody in infected mice, but to be able to protect mice against a subsequent challenge with HSV.

Adjuvants

F 020 INFLUENCE OF PHYSICAL PRESENTATION FORM OF GONOCOCCAL OUTER MEMBRANE PROTEIN IB ON THE HUMORAL IMMUNE RESPONSE. Tom Teerlink[1], Gideon F.A. Kersten[1], Wim Jiskoot[2], Marcel Paques[1], Daan J.A.Crommelin[2] and E.Coen Beuvery[1]. [1]Departments of Bacterial Vaccines and Inactivated Viral Vaccines, National Institute for Public Health and Environmental Hygiene (RIVM), P.O.Box 1, 3720 BA Bilthoven, The Netherlands; [2]Department of Pharmaceutics, University of Utrecht, Croesestraat 79, 3522 AD Utrecht, The Netherlands.
The major outer membrane protein of *Neisseria gonorrhoeae* (PI) is an attractive vaccine component. In order to contribute to protection against infection antibodies induced by the vaccine must be directed against regions of PI that are surface exposed. In the first place we have determined, which regions of PI are surface exposed. Therefore PI was degraded with cyanogen bromide (CNBr) and the order of the 3 resulting peptides in the intact PI was determined. By a combination of proteolytic and chemical degradations of PI it was established that most of the central fragment (CB2) and part of the C-terminal fragment (CB3) are surface exposed, whereas the N-terminal fragment (CB1) is completely buried in the membrane (1). Secondly, four delivery systems were used to present PI to the immune system. i. PI complexed with detergents and $AlPO_4$, ii. PI incorporated into liposomes, iii. PI incorporated in immunostimulating complexes (iscoms), and iv. native outer membranes (2). The resulting antisera were analyzed for their content of antibodies against intact PI and the three CNBr peptides. Both iscoms and detergent complexes induced high anti-PI antibody levels, with a clear booster effect. Liposomes induced much lower antibody levels and caused only a small booster effect. Liposome induced antibody levels were increased by simultaneous incorporation of an amphiphilic adjuvant (e.g. dimethyl-dioctadecyl-ammoniumbromide (DDA)). We also determined the IgG response against the individual CNBr peptides. Immunization with native outer membranes induced mainly antibodies against the surface exposed CB2 and to a lesser extent against the partly exposed CB3, whereas almost no antibodies against the membrane embedded CB1 were formed. Almost the same pattern was observed after immunization with PI iscoms. On the other hand immunization with detergent complexes or liposomes resulted in relatively high titres against the membrane embedded CB1 fragment. The results show that it is possible to direct the immune response against specific parts of a protein by varying the physical presentation form of that protein.

REFERENCES

1. Tom Teerlink, Hanneke Versantvoort and E.Coen Beuvery. 1987. Antigenic and immunogenic properties of cyanogen bromide peptides from gonococcal outer membrane protein IB. J. Exp. Med. 166: 63-76.
2. Tom Teerlink, E.Coen Beuvery, Dolf Evenberg and Toon L.van Wezel. 1987. Synergistic effect of detergents and aluminium phosphate on the humoral immune response to bacterial and viral membrane proteins. Vaccine, in press.

F 021 SYNTHETIC ANTIGEN-POLYELECTROLYTE COMPLEXES INDUCING ENHANCED IMMUNOGENICITY AND PROTECTIVE IMMUNITY Rakhim M. Khaitov. Institute of Immunology, 24-2 Kashirskoye Shosse, 115478 Moscow, USSR.

A principle of creation of artificial T-independent antigens was proposed. It consists in joining haptens or relatively weak protein antigens to polymer fragments (synthetic carbochained and heterochained polyelectrolytes of controlled structure) capable of multisite cooperative interaction with immune system outer cell membranes. The polyelectrolytes used were relatively weak polyclonal B-lymphocyte activators, possessing a pronounced mitogenic and adjuvant effect. The activating effect of synthetic polyions on ion-transporting pumps was shown. This corresponds with the data on permeability increase of lymphocyte cell membrane under their influence. Series of antigens (albumin, gammaglobulin, (T,G)-A-L, influenza virus haemagglutinin, Salmonella typhimurium polysaccharide) complexed to the polyelectrolytes caused primary IgM response 50-100 times higher than the original antigen and induced a heightened immunologic memory to the antigenic part of the complex. Secondary immunization with the antigen without the polyelectrolyte after priming with the antigen-polyelectrolyte complex caused an immune response 2-3 orders higher as comparedto the control (double immunization with the pure antigen). IgM and IgG antibody secreting cells were formed. Immunization of animals with the viral and bacterial antigens conjugated to immunostimulating polyelectrolytes manifested in a high vaccinating effect. The mechanism of action of such antigen-polymer complexes was the induction of B-cell response irrespective of T-cell and Ir-genic control of immunogenesis. Presently these methods allowed to create experimental artificial vaccines against influenza, salmonellosis and some other infectious diseases.

F 022 INTERLEUKIN-2 AS AN ADJUVANT TO VACCINATION, Jack H. Nunberg, Michael V. Doyle, Arthur D. Newell, Gary A. Anderson[1], Charles J. York[2]. Cetus Corporation, Emeryville, CA 94608; [1]University of Nebraska, Lincoln, NE 68583; [2]BioTrends International Inc., Winters, CA 95694.

Interleukin-2 (IL-2) occupies a central position in the cascade of cellular events involved in the immune response. IL-2 supports the growth and proliferation of antigen-activated T cells and the generation of effector T cells, including helper, suppressor, and cytotoxic T cells. Proliferating T cells also produce a variety of other lymphokines that affect other arms of the immune system, supporting B cell development and macrophage activation. Taken together, the data suggest that IL-2 may function as a potent adjuvant to vaccination, to increase the specific and durable response to vaccine immunogens.

Here we present data demonstrating the use of IL-2 as an adjuvant to *Haemophilus pleuropneumoniae* (Hpp) bacterin in swine. In these studies, pigs are treated with IL-2 in conjunction with vaccination. These experiments demonstrate that daily systemic administration of IL-2 at the time of vaccination is able to markedly enhance protection against subsequent Hpp challenge. These pigs show minimal clinical signs of infection, gain weight at a normal rate, and are free of typical Hpp lung lesions. Animals receiving only a single injection of IL-2 at the time of vaccination show substantially less protection.

We were interested to extend these findings in a more manipulable experimental system. We chose to investigate the ability of IL-2 to act as an adjuvant to inactivated Rabies virus vaccine in mice in the NIH Test of Rabies Vaccine Potency. In an initial study, continued systemic administration of IL-2 in conjunction with immunization was shown to markedly increase the potency of inactivated Rabies virus vaccine. Under conditions where challenge is fatal in 100% of mice receiving vaccine alone, there are no deaths in the group receiving vaccine plus IL-2. This effect is vaccine dose-dependent and is not attributable to the IL-2 treatment per se. Extrapolating from survival curves, we estimate that IL-2 is able to increase the potency of this inactivated Rabies vaccine at least 25-fold.

The adjuvant effect of IL-2 is not universal; e.g., IL-2 does not affect the potency of another bacterin in a septicemia challenge model. Further work is underway to dissect the mechanism of IL-2 action and the mechanism of disease protection. Nonetheless, IL-2 appears to offer potential as a well-defined and potent adjuvant to enhance the efficacy of vaccination in an outbred population.

F 023 MOLECULARLY ENGINEERED MICROBIAL IMMUNOSTIMULATORS, Jon A. Rudbach, John L. Cantrell and J. Terry Ulrich, Ribi ImmunoChem Research, Inc., Hamilton, MT 59840. Microbes and extracts therefrom are known to possess immunostimulatory (adjuvant) properties. However, their routine use to enhance the immunogenicity of vaccines for veterinary and human applications have been limited by toxic and allergenic properties associated with crude microbial products. Renewed interest in adjuvants was generated by the availability of new generations of "designer" antigens produced by synthesis *in vitro* or from genetically engineered microbes. Scientists at our Company have been studying structural-functional relationships of microbial immunostimulators for over 30 years. During the course of these investigations molecular engineering techniques, coupled with precise biochemical and physicochemical fractionation procedures have yielded products in which the desirable properties of immunostimulation have been retained, while the toxic and allergenic potentials have been greatly reduced or eliminated. When adjuvants prepared with various combinations of three of these materials, cell wall skeleton, trehalose dimycolate and monophosphoryl lipid A were tested with a prototype antigen, 80 to 600 fold enhancement of immune responses were obtained in mice. Similar levels of adjuvant activities were obtained with a synthetic polypeptide antigen specific for the hepatitis B virus, a bacterial subunit antigen, and two viral proteins produced from bacteria genetically altered by recombinant DNA technology. Furthermore, RIBI adjuvants enhanced the immune response to polysaccharide antigens, one of which was pneumococcal capsular polysaccharide. Both IgM and IgG class antibody responses were increased to the pneumococcal polysaccharide; such antibodies with opsonic activity are protective against pneumococcal pneumonia. Also, protection against a lethal challenge with influenza virus was obtained with a killed viral vaccine incorporating RIBI adjuvants. The amount of influenza antigen required in a protective dose of vaccine was reduced over 30-fold and the induction period was only 4 days when these adjuvants were employed. Thus, in a variety of systems the utility of these immunostimulatory microbial products is demonstrated.

Attenuated, Inactivated and Naturally Derived Subunit Vaccines

F 100 HIV ENVELOPE GLYCOPROTEIN: EVALUATION AS A PROTOTYPE AIDS VACCINE, Larry O. Arthur[1], Stephen W. Pyle[1], Julian W. Bess, Jr.[1], Peter L. Nara[2], John C. Kelliher[3], Bror Morein[4], Raymond V. Gilden[1], and Peter J. Fischinger[2]. [1]Program Resources, Inc., National Cancer Institute-Frederick Cancer Research Facility (NCI-FCRF), Frederick, MD 21701; [2]Office of the Director, Virus Control Unit, NCI-FRCF, Frederick, MD; [3]Primate Research Institute, Alamogordo, NM 88330-1027; [4]The Royal Veterinary College Biomedical Center, S-751 23 Uppsala, Sweden.

The outer envelope glycoprotein (gp120) of HIV has been purified from HIV-infected cells and used to immunize experimental animals including chimpanzees. All chimpanzees were immunized with 4 to 5 inoculation of 50 ug of gp120 formulated in alum as the adjuvant. These animals responded to the immunizations by developing antibodies which neutralized HTLV-IIIB. Maximum neutralization titers detected were 1:16. HTLV-IIIB virus stocks titered in chimpanzees were used to challenge the gp120 vaccinated chimpanzees. HIV was recovered following challenge of the vaccinated chimpanzees indicating no protection. To enhance the immunogenicity of the gp120, alternative adjuvants and antigen presentation vehicles are being evaluated. Immunostimulatory complexes (iscoms) have been constructed from the purified gp120. A rhesus monkey injected three times with only 5 ug of gp120 iscoms has developed neutralization titers of 1:128 and ^{125}I-gp120 precipitation titers of greater than 1:6250. These studies will be extended by inoculation of additional animals. Research sponsored, at least in part, by the National Cancer Institute under contract NO1-CO-74102 with Program Resources, Inc.

F 101 DIFFERENT EPITOPES ON EBV-gp350/220 CHARACTERIZED BY A PANEL OF MONOCLONAL ANTI-BODIES, G. Bertoni, R.E. Humphreys and T. Sairenji, University of Massachusetts Medical School, Worcester, MA 01655-2397.

Development of the most efficacious Epstein-Barr virus (EBV) vaccine to prevent infectious mononucleosis, nasopharyngeal carcinoma and Burkitt's lymphoma could be accelerated by understanding the physiological roles of several EBV-coded, membrane (MA) in the immune defense to EBV. gp350/220 MA is one of the principal targets of the humoral immune defense to EBV. Antibody-mediated EBV neutralization and inhibition of EBV release from EBV-productive, lymphoblastoid cell lines, P3HR-1 and B95-8, was probed with two anti-MA mAbs. 72A1 and 2L10, which immunoprecipitated the same gp350/220 MA found with 1B6 mAb with which inhibition of EBV release from P3HR-1 cells was first described. These three mAbs were not equivalent in either MA reactivities or functional effects, reflecting the variable expression of different epitopes of gp350/220. 1B6 recognized MA on P3HR-1 cells, which expressed predominately the gp220 form of MA. 1B6 did not recognize (or barely recognized) a determinant on B95-8 cells. 2L10 and 72A1 reacted as well with B95-8 cells as they did with P3HR-1. 1B6 and 2L10 neutralized neither P3HR-1 nor B95-8 EBV, but 72A1 neutralized both viruses. 1B6 and 72A1 inhibited P3HR-1 virus release, as measured by the assay for infectious virus and by DNA hybridization analysis of released virus, but 2L10 had no such activity. 72A1 (but not 1B6) inhibited release of EBV from B95-8 cells. These experiments pointed to the presence of three different epitopes on gp350/220, identified with respective mAbs and having varying involvement in virus neutralization and virus release inhibition.

F 102 CELLULAR DNA IN POLIO VACCINE PREPARATIONS, CHRISTIAN HOURS AND JOSÉE FLYNN, INSTITUT ARMAND-FRAPPIER, LAVAL-DES-RAPIDES, QUÉBEC, H7N 4Z3.

Production of polio vaccines in established cell lines raises the concern about potential oncogenicity carried by cellular nucleic acids. Besides considerable efforts achieved by producers to improve purification methods in order to eliminate as much as possible residual cellular material, quality control laboratories have to be able to detect picograms of cellular DNA; this is the limit of detection by the present techniques and, therefore, that represents the actual world wide accepted regulation. Techniques able to reach these quantities are based on molecular hybridization. We have systematically explored different parameter ranges in order to optimize and to detect routinely few picograms of cellular DNA in polio vaccine preparations made from Vero cells. Furthermore, our technique allowed us to detect cellular DNA adsorbed on and/or encapsidized in highly purified poliovirus particles.

F 103 NATURALLY ATTENUATED NEONATAL ROTAVIRUS STRAIN: A NEW VACCINE CANDIDATE?, S. Jayashree, M.K. Bhan, P. Raj, R. Kumar, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi-110029, India. A rotavirus (RV) strain asymptomatically infected 58.7% of 274 neonates born at the maternity services of an urban hospital, during the period Dec. 1985 to Nov. 1986. The RNA electropherotype of the neonatal RV strain was different from the electropherotype of the RV strains that caused severe acute gastroenteritis in older children admitted to the same hospital. Humoral and local immune responses following neonatal RV infection were evaluated among 36 infants infected with RV and 16 non-infected controls, by an ELISA using simian RV SA-11 as the antigen. None of the RV infected infants showed a rise in RV specific IgG levels (over base-line cord sera levels) at 3 weeks or 3 months following infection, and only 2 infants showed a weak RV specific IgM response in serum. In contrast, 19 of the 36 RV infected infants showed a positive RV specific IgA response in their saliva (>2SD of mean value of RV non-infected controls). Our results indicate that asymptomatic RV infection in neonates is caused by a virus strain that is probably attenuated. Our finding that this strain evokes salivary antibody response reflecting gut immunity, suggests that it holds promise as an attractive vaccine candidate.

F 104 IDENTIFICATION OF EPITOPES ON RICKETTSIAL ANTIGENS THAT STIMULATE MURINE AND HUMAN T-CELL LINES, CLONES AND T-CELL HYBRIDOMAS, Thomas R. Jerrells, Carole J. Hickman and Gregory A. McDonald, Dept. of Pathology, UTMB, Galveston, TX. 77550, Rickettsial Dis. WRAIR, Washington, D.C., 20307 and RML, NIH, Hamilton, MT., 59840.
Antigen-reactive T-cells are critical for immunity to rickettsiae and it is important to develop the technology to identify antigens that contain the epitopes that stimulate the T-cell for candidate vaccines. We have developed cloned T-cells and T-cell hybridomas from murine and human sources that are responsive to antigens of *R. tsutsugamushi*, *R. conorii*, and *R. rickettsii*. Based on requirements for MHC matched antigen presentation, surface markers, and production of lymphokines these T-cell hybridomas are considered representatives of the T-helper cell population. Some cloned T-cell hybridomas have differential response to various lots of rickettsiae used as antigen and Western blot analysis of these preparations have suggested differences in various antigenic bands especially the 56 kd band. We also have isolated a T-cell line using peripheral blood lymphocytes from an individual that was infected with a spotted fever group rickettsia that reacted to both *R. rickettsii* and *R. conorii* antigens. These are T-cells ($CD3^+$) of the helper type ($CD4^+$). In preliminary studies this T-cell line is responsive to a recombinant *R. rickettsii* 155 Kd antigen. These studies suggest that these cloned T-cells will be useful in future studies to screen antigenic preparations for their ability to stimulate T-cells and perhaps to epitope map antigens for T-cell epitopes.

F 105 MEASUREMENT OF CELL AGGREGATION AND SURFACE CHARGE OF MYCOBACTERIUM BOVIS-BCG VACCINE, TICE™ SUBSTRAIN, Melvin E. Klegerman, Michael J. Groves and Amy Zhang, University of Illinois at Chicago, Chicago, IL 60612. Examination of the vaccine with a light-blockage counter (HIAC/Royco) demonstrated a log-normal (Poisson) distribution between cumulative number and size between 2 and 50 μm equivalent circular diameter. This data can be manipulated to provide a total number of organisms per ampule. Using a Coulter Multisizer fitted with a 50 μm orifice tube, which can detect single bacteria, allows the total number of BCG particles to be counted within the vaccine. The count obtained by this method was 3-4 times higher than the CFU count, but the instrumental method does have the prospect of providing a very rapid quality control procedure that may be directly related to the CFU. Measured as zeta potential, the TICE™ vaccine cells were found to have a positive surface charge below pH 4.4. At neutral pH the addition of anionic surfactant, sodium tauroglycholate, increased the negative charge, but only doubled the number of particles in the system. Above 10^{-5} M cetylpyridinium chloride, the charge was reversed, becoming positive, but again there was only a small effect on the size and number of aggregates as determined by the Coulter Multisizer. This suggests that the aggregation mechanism between the primary *M. bovis* cells is predominantly by a hydrophobic interaction which may only be affected by high concentrations of wetting agent.

F 106 A RANDOMLY ACTIVATED BACTERIAL KILLING SYSTEM AS A BASIS FOR LIVE VACCINES. Per Klemm and Soren Molin, Technical University of Denmark, DK-2800 Lyngby, Denmark.
The design of a biological debilitating system to be employed in live vaccines is reported. The key elements are A) a gene, hok, encoding a small polypeptide of 52 amino acids that is lethal when expressed in a variety of bacterial species, and B) them fimA promoter, responsible for the phase variable expression of type 1 fimbriae in *Escherichia coli*. The fimA promoter is located on a stochastically invertible DNA segment. The orientation of this segment is controlled by two site-specific recombinases encoded by the fimB and fimE genes, located proximal to the invertible segment. When the orientation of the "switch" results in expression of the hok gene, killing of the host ensues. The percentage of cells killed per generation can be regulated by the number of "killing casettes" harbored by the host. The system allowes non-pathogenic wild-type strains to be employed as host for the expression of various antigens, and therby used as live vaccines.

F 107 **PLASMODIUM FALCIPARUM ANTIGENS IN RECOMBINANT HSV-1**, Christopher J. Langford[1], Tony Triglia[1], Stirling J. Edwards[2], Alan W. Hampson[2] and Michael Sheppard[1]. [1]The Walter and Eliza Hall Institute of Medical Research and [2]The Commonwealth Serum Laboratories, Parkville, Victoria, 3050, Australia

In general, the levels of expression of *P.falciparum* antigens in recombinant vaccinia viruses is low, usually requiring detection by immunoblotting techniques. This may partly account for the poor immunogenicity of many of these recombinants, particularly in mice. The poor expression may be influenced by the AT-rich vaccinia transcription termination signal sequences (1) found in all of these AT-rich *P.falciparum* gene sequences. One approach to test the effect of these transcription termination sequences would be to delete them by site directed mutagenesis. As an alternate solution, we have re-expressed a number of these antigen genes in recombinant herpes simplex virus type-1 (HSV-1) under the control of strong herpes virus promoter elements. The level of expression of the *P. falciparum* antigens in cells infected with these recombinants has been compared with that observed in recombinant vaccinia virus infected cells. The immunogenicity of the *P. falciparum* antigens expressed by each of the recombinant viruses is also being tested in rabbits and mice.
(1) Yuen L. and Moss B.(1987) Proc. Natl. Acad. Sci. USA. 84, 6417-6421.

F 108 PROTECTIVE SPOROZOITE IMMUNITY INDUCED BY ORAL IMMUNIZATION WITH ATTENUATED SALMONELLA EXPRESSING *P. BERGHEI* CS PROTEIN, J.C. Sadoff*, R.N. Brey+, L.S. Baron*, W.R. Majarian+, P.S. Pillai+, W.R. Ballou*, and W.T. Hockmeyer+; *Walter Reed Army Institute of Research, Washington, D.C. 20307 and +Praxis Biologics, Inc. Rochester, NY. 14623.
Recent evidence suggests that immunization with irradiated sporozoites induces potent cell mediated immunity directed against pre-erythrocytic stage parasites sharing circumsporozoite (CS) protein epitopes. Subunit recombinant or peptide carrier CS vaccines do not induce cell mediated protection but instead, less protective antibody mediated immunity. Though irradiated sporozoite immunization is efficacious, little is known about the nature of the cellular responses which are induced, and the inability to obtain large quantities of pure sporozoites precludes vaccine development based on this approach. In an effort to mimic immunity induced by irradiated sporozoites, plasmids were constructed in which the lambda P_L promoter directed transcription of the *P. berghei* CS protein or a portion of this molecule fused to the binding subunit of *E. coli* heat labile toxin (LT-B). These constructions were transferred to attenuated *Salmonella typhimurium* and *S. dublin* and shown to direct expression of epitopes recognized by Mab specific for *P. berghei* CS repeat region epitopes. *Mice immunized i.p. or s.c. with attenuated Salmonella strains induced significant CS antibody measured by* ELISA or IFA but failed to protect against a 1.5 or 2.0 x 10^3 sporozoite dose challenge. In contrast, oral immunization with these strains did not induce detectable antibody but protected 70-90% of animals challenged with the same sporozoite dose. Control animals immunized with attenuated Salmonella without the CS gene were not protected. These data suggest that oral vaccination with attenuated Salmonella expressing CS epitopes elicits protective cell mediated immunity targeted toward pre-erythrocytic stages in the liver similar to that induced by irradiated sporozoites.

F 109 IMMUNISATION OF MICE AGAINST TOXOPLASMA GONDII USING MEMBRANE ANTIGENS RECONSTITUTED INTO LIPOSOMES, C. McColgan and J. Alexander, University of Strathclyde, Glasgow, G4 0NR

Toxoplasma gondii is a protozoan parasite which can infect almost any warm blooded animal. Although most human infections are assymptomatic toxoplasmosis can be fatal in immunosuppressed individuals. Furthermore, the infection can be transmitted vertically which may lead to blindness and brain damage in the foetus. To date there is no successful immunoprophylatic treatment against Toxoplasma infection. Our approach was to develop a vaccine which consisted of membrane antigens of the parasite reconstituted into the phospholipid bilayer of liposomes. This permitted the antigens to be presented to the host's immune system in their native form.

A membrane preparation of Toxoplasma tachyzoites, which consists of four major protein bands as determined by SDS-PAGE, was obtained by detergent extraction with octyl glucoside. The sample was reconstituted into liposomes by detergent dialysis and injected into mice by either s.c. or i.p. routes. This vaccine was able to stimulate high titres of specific antibody. When challenged with 2×10^4 RH strain tachyzoites the survival time of the immunised mice was significantly prolonged when compared with controls.

F 110 HIV BLOCKS THE NEUROTROPHIC ACTIVITY OF NEUROLEUKIN ON NEUROBLASTOMA CELL LINES Mizrachi Y, Wallace B, Gurney ME, Chen I, Ho DD. UCLA School of Medicine and The University of Chicago.

Neuroleukin is an important mediator in both the nervous and the immune system. It promotes the in vitro survival of embryonic spinal neurons, skeletal motor neurons and sensory neurons. It also has a stimulatory effect on B-cell immunoglobulin synthesis. Neuroleukin shares partial sequence homology with HIV envelope protein (gp120). We previously found that HIV gp120 interfered with the neurotrophic action of neuroleukin on sensory neurons from the chick embryo dorsal root ganglion. In this study, we have developed a new bioassay for neuroleukin using neuroblastoma cell lines. The proliferation rate of the responsive neuroblastoma cells was reduced by 50% with the addition of neuroleukin, a reflection of cell differentiation. Crude lysates of HIV, or purified gp120, blocked this effect of neuroleukin. Furthermore, two synthetic peptides homologous to the neuroleukin-like domain of HIV env also inhibited neuroleukin activity on neuroblastoma cells. Similarly, neuroleukin inhibition was observed with the addition of antibodies to neuroleukin or to the synthetic peptides. These findings emphasize the potential of these neuroblastoma cells in studying the possible mechanisms by which HIV indirectly interferes with brain function. The results also raise the possibility that HIV candidate vaccines based on gp120 or env peptides may be neurotoxic because of neuroleukin antagonism.

F 111 MOLECULAR SIZE VARIATIONS IN AN IMMUNOPROTECTIVE PROTEIN COMPLEX BETWEEN ISOLATES OF ANAPLASMA MARGINALE. S. Oberle, G. Palmer, A. Barbet, T. McGuire. University of Florida, Gainesville, FL, 32610. A surface protein complex from the Florida isolate of Anaplasma marginale has been shown to induce protection in immunized cattle, and has been proposed as the basis of a subunit vaccine. This complex in the Florida isolate is composed of two noncovalently associated polypeptides with molecular weights of 105 and 100 kDa. The analogous protein complex from four geographically different isolates of Anaplasma marginale was immunoprecipitated and compared with the protein complex of the Florida isolate. The proteins of the complex varied in apparent molecular weight between isolates. Using antibodies recognizing epitopes on the polypeptides of the Florida complex, the antigenic identity of the polypeptides in the analogous complexes was determined. The polypeptides recognized by the neutralizing monoclonal antibody $22B_1$, which recognizes the 105 kDa polypeptide in the Florida isolate, ranged from 70 to 100 kDa in the other isolates. Those polypeptides recognized by rabbit antiserum R911, which recognizes a 100 kDa polypeptide in the Florida isolate, ranged from 97 to 100 kDa. The surface exposed peptides of the polypeptides were compared by a limited enzymatic digestion in order to assess structural homology between isolates. Despite the variations in apparent molecular weight, there were surface exposed peptides conserved between the $22B_1$ reactive polypeptides of each isolate and between the R911 reactive polypeptides of each isolate. Determination of the possible function of the conserved peptides in inducing immunity may be critical to the application of the 105 kDa polypeptide as the basis of a subunit vaccine for bovine anaplasmosis.

F 112 THE ROLE OF HUMAN PARAINFLUENZA 3 VIRUS ENVELOPE GLYCOPROTEINS IN INDUCTION OF A PROTECTIVE IMMUNE RESPONSE.
Ranjit Ray[1], Brenda Glaze[1], Tracie Burnett[1], and Richard W. Compans[2], [1]Molecular Engineering Associates, Birmingham, AL; [2]Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL

The hemagglutinin-neuraminidase (HN) and the fusion (F) glycoproteins of paramyxoviruses are known to be involved in initiation and progress of the infection processes. These two glycoproteins have been found to induce a complete protective immune response when tested as a subunit vaccine in hamsters. To investigate the role of individual glycoproteins in the induction of a protective immune response, the two glycoproteins were purified from virus-infected cell lysates by affinity chromatography using specific monoclonal antibodies and used to vaccinate hamsters through the intranasal route. The efficacy of vaccination was tested by challenge infection of the immunized as well as unimmunized control hamsters. Results of virus recovery from lungs suggested that vaccination with purified HN or F alone could only provide partial protection. Similar observations were also noted after passive transfer of monospecific rabbit antibody to purified HN or F glycoproteins in baby hamsters. However, passive transfer of mixtures of antibodies to HN and F conferred a higher level of protection from challenge infection. Also, the animals showed complete protection from challenge infection when a mixture of the two affinity purified glycoproteins was administered intranasally. Thus, the two virus envelope glycoproteins appear to have a synergistic effect in conferring protection from infection.

F 113 ATTENUATION OF RECOMBINANT VACCINIA VIRUSES.
John D Williamson, Ruth W. Reith (St. Mary's Hospital Medical School, London W2 1PG, England), *John R. Arrand and *Michael Mackett (*Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester M20 9BX, England).

A murine model has been developed to compare the virulence of WR and Wyeth vaccinia virus strains with recombinant viruses expressing the Epstein-Barr (EB) virus membrane antigen gp340. The recombinant viruses were constructed by insertion of the foreign gene into the thymidine kinase (TK) gene of the parental virus genomes. All 3-week old CBA and 4- to 5-week old DBA/2 mice infected intranasally with 10^6 pk.f.u. of WR virus died within 7 days. There were no mortalities after similar infection of other mice with the recombinant WR/EB virus and no lethal effects were obtained with the TK-deficient variant of WR. No deaths occurred after mice were infected with either the Wyeth strain of vaccinia or the recombinant Wyeth/EB virus. Studies of the pathogenesis of these murine infections showed significant titres of infective virus recoverable from the lungs, blood and brain of all mice infected with WR, WR/EB and WRTK- viruses until 7 days post-infection. No infective virus was recoverable at later times from mice infected with WR/EB or WRTK- viruses. Although virus could be recovered from the lungs of mice infected with Wyeth or Wyeth/EB viruses until 7 or 5 days post-infection, respectively, little or no virus could be recovered from brain or blood at any time. These results show marked attenuation of the WR/EB recombinant and no exacerbation of the avirulent Wyeth/EB recombinant.

F 114 EXPRESSION OF REPETITIVE ELEMENTS OF RESA,
G.C. Woodrow, B. Norman, J.Kyngdon, R. Gibbs, G. Schevzov, B. Murray and D.O. Irving, Biotechnology Australia Pty. Ltd., Sydney, NSW 2069, Australia

One of the antigens that has been identified as a potential component of a molecular vaccine against the asexual stages of the malaria parasite, Plasmodium falciparum, is the 155 kDa, ring-infected erythrocyte surface antigen (RESA). When several fragments of the RESA molecule were used as immunogens in monkeys, it was found that polypeptides containing repeating 11 amino acid subunits from the N-terminal (5') portion, as well as polypeptides containing 8 amino acid repeated sequences from the carboxyl (3') end of the molecule, induced protection against subsequent challenge with P.falciparum. Accordingly, it appeared reasonable to deduce that protective immunity may be induced by vaccinating with peptides containing either the 5' or 3' repeats of RESA. A number of genetic constructs were prepared by ligating synthetic oligonucleotides encoding the repeating units in head-to-tail tandem arrays such that varying numbers of the repeats were obtained. The constructs were then expressed in bacteria and their antigenicity tested by Western blotting using monoclonal antibodies specific for either the 5' or 3' repeats. Although constructs containing more than ten of the basic repeat unit were genetically unstable, those with fewer repeats were stable and produced significant quantities of antigenic polypeptide. Experiments in small animals aimed at characterizing the immune responses to the expressed material are under way. The approaches used here are of general applicability for producing molecules containing repeated epitopes that could be used as components of a vaccine.

Production of Pathogen Surface Antigens by Recombinant DNA

F 200 EXPRESSION OF ANTIGENS FROM M.TUBERCULOSIS IN E.COLI, Åse Andersen,
Statens Seruminstitut, Copenhagen, Denmark.
Tuberculosis caused by M.tuberculosis is still a serious world health problem, especially in developing countries. The efficacy of vaccination with M.bovis BCG was seriously questioned because of the outcome of a major field trial in India 1968-1971 (1). To examine if some/which components of M.tuberculosis are able to induce protective immunity, there is a need for isolation of single mycobacterial antigens. A genomic DNA library from M.tuberculosis in lambda gtll constructed by R.A. Young (2) were screened with a panel of monoclonal antibodies (m.abs.). Twelve recombinant phages were isolated encoding eight different mycobacterial proteins. Some of the phages expressed proteins possessing epitopes specific for M.tuberculosis and M.bovis BCG, others produced proteins with epitopes widely distributed among mycobacterial species. Physical maps of the genes encoding those eight mycobacterial antigens have been generated.
A gene designated pab encodes a protein of molecular weight 38000. It possesses two distinct epitopes specific for M.tuberculosis and M.bovis BCG. By generation of truncated versions of the molecule these epitopes have been mapped and the nucleic acid sequence of the gene have been determined.
1) WHO Technical Report Series, No. 651, 1980
2) R.A.Young et al. (1985) Proc.Natl.Acad.Sci. USA 82: p. 2583-2587

F 201 FUNCTIONAL PROPERTIES OF VACCINIA VIRUS-PRODUCED HIV GP120.
Paul Barstad(1), Clifford Snyder(2), Klaus Strebel(2), Patricia Earl(3), and William Todd(4). NIAID Vaccine Development Program, BCDP, Program Resources Inc., Frederick Cancer Research Facility, Frederick, MD 21701(1); Laboratory of Molecular Microbiology(2) and Laboratory of Viral Diseases(3), NIAID, NIH, Bethesda, MD 20892; Vet. Micro. and Vet Science, LAES, Louisiana State Univ., Baton Rouge, LA 70803(4).
The envelope glycoprotein(gp120) of Human Immunodeficiency Virus Type 1 has been produced in mammalian cell culture using the vaccinia virus vector. A gene encoding the entire gp120 molecule has been inserted into vaccinia and expressed under the control of T7 polymerase. Standard biochemical techniques for the isolation of the intact molecule are presented. The use of this material in screening patients' sera as an indicator of HIV infection using a single step dot blot assay has been tested. Investigations to determine the binding activity of the vaccinia-produced material to CD4 molecules are presented. Antisera made against the purified material have been evaluated for the ability to block binding to CD4.

F 202 PRIMARY STRUCTURE OF KERA (LYSINE/GLUTAMATE RICH ANTIGEN), A PLASMODIUM FALCIPARUM ANTIGEN LOCATED AT THE MEROZOITE SURFACE AND IN THE PARASITOPHOROUS VACUOLE.
Jeffrey D. Chulay, Jeffrey A. Lyon, Ruth H. Wolfe, Ted Hall and James L. Weber, Walter Reed Army Institute of Research, Washington, DC 20307-5100.
We have previously characterized a 101 kD P. falciparum schizont antigen located at the merozite surface and within the parasitophorous vacuolar space (J Immunol 1987; 139: 2768). We obtained Camp strain genomic DNA clones encoding the entire protein and FCR3 strain cDNA clones encoding 89% of the protein. A β-galactosidase fusion protein expressed by one clone was recognized on immunoblots by monoclonal and affinity-purified polyclonal antibodies against the native antigen. Mouse antiserum against a synthetic peptide based on the deduced amino acid sequence of this clone detected a protein with electrophoretic mobility identical to the native schizont antigen on immunoblots. The gene encodes a protein of 743 aa which, except for a 22 aa hydrophobic leader sequence, is hydrophilic and highly charged. Near the N-terminus a hexapeptide with consensus sequence TNDEED is tandemly repeated 8 times. Near the C-terminus a stretch of 58 aa contains alternating KE and KEE sequences. Because K and E each constitute >14% of the total aa residues, we refer to this protein as KERA (lysine/glutamate rich antigen). The Camp and FCR3 nucleotide sequences differ in only 3 positions, each of which results in an amino acid substitution. This apparently conserved antigen is accessible at the merozoite surface and complexes with antibodies when schizonts rupture in the presence of growth-inhibitory immune serum (*op. cit.*). Thus KERA is an attractive blood-stage malaria vaccine candidate.

F 203 ANALYZING RECOMBINANT SCHISTOSOME ANTIGENS. John S. Cordingley, James R. Chambers, Karen White, and Kim Wells, Univ. of Wyoming, Dept. of Molecular Biology, Laramie, WY 82071. We have identified cDNA clones of a number of antigens from the human trematode parasite, *Schistosoma mansoni* and have characterized these clones by mRNA hybrid selection, sequencing and expression cloning [1,2]. Using both recombinant antigens and synthetic peptides we have analyzed the immunogenicity and antigenicity of these molecules by combinantions of immunoprecipitation, western blotting and ELISA assays. Using overlapping synthetic peptides we are trying to define epitopes on one of these molecules, a 40,000 dalton antigen which is abundantly synthesized in parasite eggs [1]. This polypeptide is very immunogenic in human patients and it is possible that a recombinant fragment or synthetic peptide analogue of part of the antigen will be useful as an immunodiagnostic test. However, the levels of antibody against individual epitopes in infection sera is low and it is technically not simple to detect specific binding of antibodies to synthetic peptides using infection sera. As a complement to this approach we have been making recombinant deletion fragments of the 40,000 dalton antigen to identify fragments which bear multiple antigenic sites on somewhat larger polypeptide fragments. The potential usage of some of these recombinant polypeptides for immunoprophyllaxis will be discussed. 1) Nene,V., Dunne,D.W., Johnson,K.S., Taylor,D.W., and Cordingley, J.S. (1986) Mol. Biochem. Parasitol. 21, 179-188. 2) Cordingley,J.S., Haddow,W.J., Nene,V. and Taylor,D.W. (1986) Mol. Biochem. Parasitol. 18, 73-88.

F 204 AN APPROACH TOWARDS DEVELOPMENT OF SYNTHETIC PROTOTYPE MODEL VACCINES FOR ALPHAVIRUSES, Haim Grosfeld, Baruch Velan, Udi Olshevsky, Bat-El Lachmi, Moshe Pinto and Avigdor Shafferman, Israel Institute for Biological Research, 70450 Ness-Ziona. Sequence analysis of the E_2 proteins of the alphaviruses Semliki Forest virus (SFV) and Sindbis, which belong to different subclasses within the alphavirus group, demonstrates that although the overall analogy of the amino acids (a.a.) is only 40%, there exist numerous completely conserved stretches of 3-8 a.a., dispersed between nonconserved regions. A stretch of nonconserved a.a., bounded by sequences of conserved a.a. in the alphaviruses, is defined here as a "cassette". Analysis of hydropathic plots of various E_2 peptides and of the serological characteristics of alphaviruses suggest that at least some of the protective epitopes could reside within these cassettes. In search for the fragments in the SFV cDNA which code for cassettes carrying protective epitopes, we cloned Sau3A restriction fragments into expression plasmids. The cassette-related peptides are expressed from these plasmids in the form of a product fused to the N-terminus of β-galactosidase. Five such protein hybrids - BC, DE, FG, HIK, and L(MN)-β-galactosidase - have been efficiently expressed in *E. coli* transformants and purified to homogeneity. Antibodies raised in mice or rabbits against intact Semliki Forest virions recognized all the SFV-E_2-β-galactosidase cassettes, while they did not react with β-galactosidase. However, we found a large variation in the specificity of different sera. Sera of certain mice could detect only one cassette, while others could interact with four or all the cassettes. We conclude that all the five different SFV-$E2_2$-β-galactosidase fusion products possess antigenic properties of SFV. Immunization experiments revealed that mice injected with one of the cassettes - L(MN) - became protected against high challenge (250 LD_{50}) of SFV.

F 205 PRODUCTION OF PLASMODIUM FALCIPARUM BLOOD STAGE ANTIGENS IN RECOMBINANT HOSTS AS POTENTIAL CANDIDATE VACCINE MOLECULES, Mitchell Gross*, Timothy W. Theisen*, Gail Wasserman+, Carol Silverman+, and James F. Young*, Depts. of Molecular Genetics* and Protein Biochemistry+, Smith Kline & French Laboratories, King of Prussia, PA 19406. Advent of hybridoma and recombinant DNA technologies has resulted in a great deal of interest toward developing an anti-malarial vaccine. To date, we and other laboratories have made significant progress in the development of several candidate anti-sporozoite vaccines which have been evaluated in human clinical trials. We have recently initiated studies to express various *P. falciparum* blood stage antigens in recombinant hosts as potential vaccine candidate molecules. These proteins have been shown to react with sera from immune animals and antibodies made to the recombinant proteins react with the native molecule in parasite infected erythrocytes. Results will be presented on the expression, purification and the potential utility of these molecules as candidate vaccines.

F 206 **Presentation of Foreign Epitopes at the Surface of Live Recombinant Bacteria. Immunization Studies.**

Charbit, , A. Molla, S.Van der Werf, E. Sobczak, M.L. Michel, M. Girard ,P. Tiollais, and M. Hofnung*
CNRS UA271, INSERM U163, Institut Pasteur, 25 rue du Dr Roux, 75015, Paris, France.

In order to elaborate vaccines from defined epitopes, it is essential to present the epitopes in an appropriate manner to the immune system of the host, so that immunization can lead to long term protection. We developed a general method to insert genetically a foreign epitope into the continuity of a carrier protein without affecting appreciably the activities of the protein or the antigenic properties of the epitope. By performing the insertion into an external loop of LamB, an outer membrane protein of *E coli* as used as a carrier, we were able to expose a foreign epitope at the surface of a *gram*⁻ bacteria (Charbit *et al.*, 1986,1987).

We have investigated the immunogenicity of three different peptides inserted into this site. One peptide corresponds to the C3 neutralization epitope of the VP1 protein from type 1 poliovirus (residues 93 to 103) ; and the two other peptides correspond to two distinct portions of the preS2 region of the envelope proteins from Hepatitis B virus (region A : residues 132 to 145 ; region B : residues 153 to 171). Bacterial strains expressing the hybrid proteins were used directly as immunogens, in rabbits and mice. The viral sequences were found to be immunogenic in the two animal species and the antibodies raised recognized, in all cases, the virus.

Thus, epitope presentation at the surface of live recombinant bacteria can be an efficient system for the induction of anti-viral antibodies. Our results indicate that the LamB site can accomodate a broad spectrum of foreign sequences having very different characteristics (hydrophobicity *etc*...). This system opens interesting perspectives for the development of bacterial live vaccines. Our current studies involve 1) expression of the hybrid genes in other enterobacteria more suitable for vaccination than *E.coli*, as avirulent mutants of *S. typhimurium* or *typhi*. 2) Comparison of different routes of administration. 3) Development of other carrier proteins than LamB.

Charbit, A., J.C. Boulain, A. Ryter, and M. Hofnung. 1986. Probing the topology of a bacterial membrane protein by genetic insertion of a foreign epitope ; expression at the cell surface. EMBO J. 5: 3029.

Charbit, A., E. Sobczak, M.L. Michel, A. Molla, P. Tiollais, and M. Hofnung. 1987 b. Presentation of two epitopes of the preS2 region of Hepatitis B on live recombinant bacteria. J. Immunol. 139:1658.

F 207 EXPRESSION OF THE FUSION PROTEIN FROM RESPIRATORY SYNCYTIAL VIRUS IN ESCHERICHIA COLI, Antonia Martin-Gallardo, Karen Fien, Algis Anilionis, and Peter Paradiso, Praxis Biologics, Inc., Rochester, NY 14623.

A bacterial plasmid that directed expression of the respiratory syncytial virus fusion protein (F) gene under the control of the P_R promoter was constructed. Upon heat induction, E. coli cells harboring this plasmid produced peptides of apparent size 45, 23 and 18 KDa which were immunologically reactive with anti-F antisera. The 45 KDa peptide, which showed the characteristics of the unglycosylated form of the F_1 subunit, reacted also with a neutralizing, fusion-inhibiting anti-F monoclonal antibody. Expression of the F gene, though in low levels, resulted in severe growth inhibition of the bacterial host. Truncated derivatives of the F gene were inserted into pUC plasmids, and expression induced with IPTG. We found that inhibition of E. coli growth was not overcome by deletion of the hydrophobic carboxy-terminal domain of the F protein. This inhibitory effect was overcome by deletion of the sequences encoding the hydrophobic amino terminal domain (signal peptide) at the amino terminus of the F_2 subunit. The mechanism of toxicity and the possible role of the hydrophobic fusion-related domain (the amino-terminus of the F_1 subunit) will be discussed. Expression of the non-toxic, truncated F genes showed that the carboxy-terminal domain, though it seemed to have no apparent effect on bacterial growth, had a negative effect on protein accumulation. We conclude that the RSV F protein has to be modified so that its expression is not toxic to E. coli and high levels of expression can be reached in bacteria.

F 208 MOLECULAR CLONING AND EXPRESSION OF HAEMOPHILUS INFLUENZAE TYPE b OUTER MEMBRANE PROTEIN P1, Robert Munson, Jr., Washington University School of Medicine, St. Louis, Mo. 63110.

The structural gene for outer membrane protein P1 of Haemophilus influenzae type b was isolated from a genomic library produced in λEMBL3 by immunologic screening. A 4.2kb EcoRI-BamHI fragment was subcloned into pBR322. The full size gene product was produced in this construct and the recombinant protein exhibits the same heat-modifiability as the protein produced in Haemophilus. Recombinant P1 was detectable on the surface of E. coli. Together, these data suggest that the recombinant protein is processed correctly. Sequence data indicates that the signal peptide is 22 amino acids in length and shares features common to many procaryotic signal peptides, that is, it contains 2 lysine residues near the n-terminus, a hydrophobic stretch and Ala at the -1 and -3 positions. In order to produce larger quantities of recombinant protein, the P1 gene has been placed under control of the lac promotor in the lipoprotein expression vector pIN-III-A3 constructed by Inouye and coworkers.

F 209 PSEUDORABIES VIRUS GP50: AN EFFECTIVE SUBUNIT VACCINE AND INTERFERENCE WITH VIRUS REPLICATION IN CELL LINES, E.A. Petrovskis, A.L. Meyer, D.R. Thomsen, P.J. Berlinski, R.C. Wardley and L.E. Post. The Upjohn Company, Kalamazoo, MI.
Pseudorabies virus (PRV) is a herpesvirus of swine. All of the PRV glycoproteins sequenced to date have homologues in herpes simplex virus (HSV), the prototype herpesvirus. PRV produces a glycoprotein, gp50, which has been shown to be the target of neutralizing antibodies and to have homology to glycoprotein D of HSV. Due to the extensive work done on HSV gD in animal models, gp50 was evaluated as a subunit vaccine candidate for PRV. Gp50 protected mice from PRV-induced mortality either when delivered via infection with a recombinant vaccinia virus or when administered as a subunit vaccine produced in Chinese hamster ovary (CHO) cells or in baculovirus infected cells. The gp50 synthesized in CHO cells protected swine, the natural host, from lethal infection with PRV. In the course of generating various mammalian cell lines expressing gp50, it was noted that such cell lines have a reduced capability to propagate PRV. Reduction in virus yield was observed in Vero, HeLa, and pig kidney (MVPK) cell lines producing gp50. The Vero and HeLa cells producing gp50 showed an even greater reduction in yield of HSV than PRV. This phenomenon may be an example in a herpesvirus of the interference observed in retroviruses, or cross protection in plant virus systems.

F 210 EXPRESSION AND SECRETION OF PERTUSSIS TOXIN SUBUNITS IN BACILLUS HOSTS, Per E.J. Saris, Airi Palva, Marjo Simonen and Ilkka Palva, University of Helsinki, Finland.
Pertussis toxin (PT) is an exoprotein complex produced by Bordatella pertussis, the causative agent of whooping cough. PT is composed of five different subunits, designated S1-S5. For immunization, a whole-cell vaccine has been routinely used. This vaccine, however, can give rise to some undesired side effects like erythema, fever, reversible and nonreversible neurological damages and, occasionally, even death. To obtain a safer vaccine chemically detoxified PT purified from the culture supernatant of Bordetella pertussis has been tested and shown to be, in animal models, comparable to the cellular vaccine in the effectiveness with less side effects. In order to obtain pure PT, recombinant expression systems will be the method of choice for vaccine production. In E. coli, the expression of all five subunits as fusion proteins or intracellular aggregates has been shown. In B. subtilis, expression and also efficient secretion of S1 has been demonstrated. We have cloned the entire PT-operon in E. coli and created, with site directed mutagenesis, a restriction site at the cleavage site of the signal peptide for all the subunits. To characterize expression in various Bacillus hosts, we have used different expression/ secretion vectors to separately clone the coding region of each subunit. Expression and secretion data will be discussed.

F 211 Conformational peptide carrier: grafting an epitope from Influenza Virus Hemagglutinin into Staphylococcal Nuclease. *Xavier Soberon, Judith White and David A. Agard. Dept. of Biochem. and Biophys. University of California San Francisco. San Francisco, Ca. 94143.*
In order to study the posibility of enhancing the immunogenicity of a small, preselected antigenic determinant from one protein we set out to display it on the surface of another protein in order to restrain its conformational freedom. We chose as epitopes parts of antigenic site A from Influenza Virus Hemagglutinin (HA). These segments fail to induce antibodies specific for the native conformation when given as peptides, but the region is highly antigenic and immunogenic in the intact protein. For the carrier protein we used Staphylococcal nuclease (SN). Using Molecular Graphics and the crystal structures of both proteins, we selected loops of SN and looped segments of HA site A and explored the most favorable points for substitution, trying to find pairs of residues in SN that were superimposable with pairs of residues of the HA loop. Applying site directed mutagenesis and other recombinant DNA techniques to a cloned SN gene we replaced two regions coding for nuclase loops with DNA coding for segments of HA site A. In order to explore more conformational alternatives, another construction, with randomized contact points was also designed. Characterization of the products is in progress, aimed at evaluating both their antigenicity and their immunogenicity, using mono and polyclonal antibodies directed to the HA epitope and immunizing animals with the recombinant proteins.

F 212 SEQUENCE, TRANSCRIPTIONAL ANALYSIS AND TRANSIENT EXPRESSION OF THE HUMAN CYTOMEGALOVIRUS (TOWNE) GLYCOPROTEIN B GENE, R. Spaete[1] R. Thayer[1] W. Probert[1] L. Rasmussen[2], T. Merigan[2], and C. Pachl[1]. [1]Chiron Corporation, Emeryville, CA 94608; [2]Stanford University School of Medicine, Stanford, CA 94305. The gene encoding glycoprotein B of human cytomegalovirus (CMV) strain Towne was cloned, sequenced and expressed. Secondary structure analysis of the 907 amino acid protein predicted a 24 amino acid N-terminal signal sequence and a transmembrane region comprised of two domains, 38 amino acids and 21 amino acids. Computer analysis also predicted the major antigenic sites on the gB molecule. The CMV (Towne) gB gene had a 94% nucleotide similarity and a 95% amino acid similarity to the CMV (AD169) gB gene (M.P. Cranage *et al*., 1986. EMBO J. 5:3057-3063). Transcriptional analysis of the CMV (Towne) gB coding strand revealed that three transcripts, 12.0 kb, 8.5 kb and 3.9 kb, were transcribed from this region at both early and late times after infection. These transcripts appeared to be similar to the transcriptional pattern seen in HSV infected cells at the homolgous loci, i.e., gB, ICP18.5, and ICP8 (L.E. Holland *et al*., 1984. J. Virol. 49:947-959). Full length and truncated versions of the gB gene were expressed in COS-7 cells using expression vectors where transcription was driven by the SV40 early promoter or the CMV major immediate early promoter. Expression was detected by immunofluorscence and ELISA using the virus neutralizing murine monoclonal antibody 15D8 (L.E. Rasmussen *et al*., 1985. J. Virol. 55:274-280), which was previously shown to recognize a 55 kD CMV virion glycoprotein and a related 130 kD intracellular precursor. These results suggest that CMV gB is a target for neutralization and a likely component of a subunit vaccine.

F 213 NON-POLYMORPHIC SEQUENCES OF THE MAJOR SURFACE ANTIGEN, P190, FROM *P.FALCIPARUM* MEROZOITES. Bela Takacs, Ulrich Certa, Andrea Crisanti, Reiner Gentz, Hubert Jacot, Hugues Matile, J. Richard L. Pink, and Francesco Sinigaglia. Central Research Units, F. Hoffmann-La Roche & Co. Ltd., Basel, Switzerland.
The p190 antigen of *Plasmodium falciparum*, a 190 kilodalton (kDa) glycoprotein, is synthesized during schizogony and then processed to 83, 42, and 19 kDa products, which are major components of the merozoite surface coat. The p190 antigen is an attractive candidate for the development of a blood stage malaria vaccine because it can induce protective immunity in monkeys against *P. falciparum* challenge. However, the genetic polymorphism of p190 has rendered development of a p190-related vaccine problematic.
We cloned and expressed, in *E.coli*, a number of DNA fragments, comprising almost the entire p190 gene of the K_1 isolate. Pooled human endemic-area sera, and rabbit antibodies raised against p190, isolated from K_1 parasites, reacted only with a limited number of recombinant proteins. We selected two antigenic polypeptides that contain conserved amino acid stretches of the otherwise highly polymorphic protein. Human monoclonal antibodies were obtained from malaria-exposed donors. Three of these antibodies react with a polypeptide from a conserved region near the p190 N-terminus and also react, by immunofluorescence and Western blotting, with all 14 *P. falciparum* isolates tested. Human T-cell clones, reacting with this polypeptide, also recognized the 14 different isolates. According to these results a region near the p190 N-Terminus is indeed conserved among different parasite isolates and has the requisites to be immunogenic in humans.

F 214 EXPRESSION STUDIES FOR CORONAVIRUS SUBUNIT VACCINE DEVELOPMENT. H.Vennema, R.de Groot, M.Dalderup and W.Spaan. State University of Utrecht, the Netherlands.
The causative agents of feline infectious peritonitis (FIP) and avian infectious bronchitis (IB) are both members of the family Coronaviridae. Coronaviruses are enveloped positive-stranded RNA viruses which are studied in our laboratory for their mode of replication, virion assembly, pathogenesis and antigenic variation.
The virion contains the positive-stranded RNA genome and three classes of structural polypeptides, namely the nucleocapsid protein and two membrane proteins E1 and E2.
The E2 protein forms the spikes which give coronaviruses their characteristic appearance as seen by electron microscopy. The spike protein contains the antigenic determinants for virus-neutralizing antibodies and mediates receptor binding and cell fusion.
For this reason the spike protein is considered to be a good candidate for the development of live carrier and subunit vaccines.
The gene coding for the E2 polypeptide has been identified, cloned and sequenced for several coronaviruses. For production of subunits a number of eukaryotic expression systems are tested. The transient expression system based upon a vaccinia virus recombinant producing T7 RNA polymerase is used to test and optimize or alter gene constructs. Vaccinia virus recombinants are used to test the feasability of the use of live carrier vaccines and for immunogenicity studies. Furthermore stable cell lines constructed with bovine papilloma virus vectors are considered for subunit production.
Initial results of expression studies will be presented.

F 215 THE GENE IN EQUID HERPESVIRUS 1 ANALOGOUS TO THE HERPES SIMPLEX VIRUS GENE ENCODING A MAJOR ENVELOPE ANTIGEN GLYCOPROTEIN gB, J. Millar Whalley, Norma A. Scott, Graham R. Robertson, Grant C. Hudson and Christopher W. Bell, School of Biological Sciences, Macquarie University, Sydney, Australia, 2109.
A gene in equid herpesvirus-1 (equine abortion virus) (EHV-1) colinear with and homologous to the gB glycoprotein gene of herpes simplex virus (HSV) has been identified by DNA hybridization and nucleotide sequencing. The EHV-1 gB (gp14) sequence (map location 0.40-0.43) specifies a polypeptide of 933 amino acids and hydropathy plots show hydrophobic regions near the N- and C-termini consistent with a cleavage signal sequence and transmembrane domains respectively. The extent of conservation of this gene is indicated by amino acid homologies with its counterpart in HSV-1 and -2, varicella-zoster virus, human cytomegalovirus and Epstein-Barr virus, and by overall similarity of predicted secondary structure. The glycoprotein gB of HSV is essential for virus infectivity (required for virus penetration) and is able to induce protective immunity in experimental animals. The EHV-1 gB counterpart described here is expected to have similar properties and is therefore a good candidate for a vaccine antigen. One useful approach to assess its potential is to use a recombinant vaccinia virus containing the EHV-1 gB sequence.

F 216 IMMUNIZATION WITH A VACCINIA-HSV-1 gB RECOMBINANT VIRUS: IMMUNE RESPONSE AND PREVENTION OF GANGLIONIC LATENCY IN MICE, Dru E. Willey, Edouard M. Cantin, Elizabeth Taylor-McLaughlin, Bernard Moss* and Harry Openshaw, City of Hope National Medical Center, Duarte, CA 91010. * National Institutes of Health, Bethesda, MD 20205.
We have constructed V11, a vaccinia recombinant containing the glycoprotein B (gB) gene of herpes simplex virus type 1 (HSV 1) (in collaboration with B. Moss, NIAID). The mature gB produced by V11 is glycosylated, expressed at the surface of infected cells, and indistinguishable from authentic HSV 1 gB in terms of electrophoretic mobility. We report here the effect of V11 vaccination in BALB/c mice. Serum anti-HSV ELISA antibody titers were undetectable in contrast to high titers in littermates vaccinated with V52, a vaccinia recombinant virus which expresses HSV 1 gD. Preliminary data suggest that V11 induces an anti-HSV cytotoxic T lymphocyte response. Animals vaccinated with V11 were subsequently challenged by the corneal inoculation of the McKrae strain of HSV 1 and comparisons were made to control mice vaccinated with V36, a vaccinia recombinant virus which expresses influenza virus hemagglutinin antigen. In animals immunized with V11, mortality from the HSV 1 challenge was reduced from 78% to 2%, HSV 1 shedding in the tear film (3-7 days after challenge) reduced from 90% to 18%, detection of infectious virus in the trigeminal ganglia (3-5 days after challenge) reduced from 90% to 15%, and detection of latent virus in the trigeminal ganglia (30 days after challenge) reduced from 100% to 55%. We obtained a similar degree of protection with V52, the vaccinia recombinant virus which expresses HSV 1 gD. In conclusion, both the gB and gD recombinant vaccinia viruses show promise as anti-HSV vaccines and as useful reagents to analyze separate components of the anti-HSV immune response.

F 217 CLONING OF FILARIAL SURFACE ANTIGEN, Maria Yazdanbakhsh, David A. Denham*, Felix Partono**, Rick M. Maizels and Murray E. Selkirk, Imperial College, London University, London SW7, U.K.. *L.S.H.T.M., **University of Indonesia.
Lymphatic filariasis is a major tropical disease of man caused by the parasitic nematodes Brugia and Wuchereria, currently estimated to infect 100 million people. Surface labelling studies of adult worms has shown a major antigen of 29000 molecular weight to be present on all species of human lymphatic filariae. This major surface antigen is a glycoprotein released in hydrophilic form following mechanical disruption in aqueous media. Enzymatic cleavage and absorption experiments have confirmed that the 29K is exposed on live worms and accessible to antibody binding. This protein is recognized early in infection and seems to be present on larval stages of the parasite 48 hours after the injection of the infective larvae into the host. Therefore this is the best candidate for immunoprophylaxis.
A 29K preparation was obtained by direct excision of the 29K fraction from SDS-polyacrylamide gels, electroelution and concentration. Antisera raised to this preparation recognized the 29K surface glycoprotein. The serum immunoprecipitated a 40K product from in vitro translation of adult Brugia malayi mRNA. This is probably a precursor of the 29K. The serum was used to screen cDNA library constructed in bacteriophage lambda gt11 using as template RNA isolated from adult Brugia pahangi. Eighteen clones were isolated and by hybridization analysis these fall into three separate families. Affinity purification of antibody with β-galactosidase fusion protein was used to verify clones that encode the surface glycoprotein. Structural data will be presented on the products of two of these cDNAs.

F 218 BLOOD STAGE P. FALCIPARUM ANTIGENS INVOLVED IN PROTECTIVE IMMUNE RESPONSE IN EXPERIMENTAL INFECTION IN SQUIRREL MONKEYS, M. Jendoubi, J.M. Postal, Y. Gysin, Ph. Dubois and L. Pereira da Silva. Unite de Prasitologie Experimentale, Institut Pasteur, 25 rue du Dr Roux 75015 Paris.

In previous studies we have identified a series of parasite proteins which are strongly recognized by antibodies present in protective sera of experimentally infected squirrel monkeys (1). Two parasite protein fractions purified from culture forms and containing these polypeptides are able to induce protective antibodies in monkeys when injected with Freund adjuvant (2). Four of these polypeptides have now been well characteized *E. coli* recombinant clones corresponding to these antigens have been obtained using expression vectors and monospecific sera have been produced using the recombinant antigens. In the present communication we will present a comparative study of the immunogenecity of proteins purified from the parasite or synthesized by *E. coli* recombinant clones.

1 Ph. Dubois, J.P. Dedet, Th. Fandeur, Ch. Roussilhon, M. Jendoubi, S. Pauillac, O. MercereauPuijalon and L. Pereira da Silva. Proc. Natl. Acad. Sci USA, Vol 81: 229. 1984.
2 Ph. Dubois, M. Jendoubi, S. Pauillac, H. Jouin and L. Pereira da Silva. Parasitologia 27: 31. 1985.

Vaccines Based of Synthetic Peptides

F 300 ADJUVANT-INDEPENDENT IgG RESPONSES TO PROTEIN AND SYNTHETIC PEPTIDE ANTIGENS INDUCED IN MICE BY IMMUNIZING WITH ANTIGEN COUPLED TO ANTI-CLASS II MHC MONOCLONAL ANTIBODIES, Brian H. Barber and George Carayanniotis, Dept. of Immunology, Medical Sciences Building, University of Toronto, Toronto, Ontario, Canada M5S 1A8.
We have investigated an adjuvant-free antigen delivery system based on the hypothesis that antigen coupled to monoclonal antibodies (MAbs) specific for Class II major histocompatibility complex (MHC) determinants should be "targeted" onto antigen presenting cells, thus facilitating recognition by helper T cells. As reported earlier (Carayanniotis and Barber, Nature 327,59 (1987)), an avidin-specific IgG response is induced in mice, without adjuvant, when avidin is injected coupled to biotinylated anti-class II MHC MAbs. We have extended this system to generate IgG responses to biotinylated proteins such as bovine serum albumin, or influenza hemagglutinin by using the avidin as a "bridge" linking the antigen to the MAb. Similarly, using the avidin as a peptide carrier, we have induced specific IgG responses to two Herpes glycoprotein D peptides (2-21 and 256-267). In all cases, a targeting effect was demonstrated because only mice bearing the appropriate class II antigens responded. This approach merits further exploration as a new option in the construction of adjuvant-free vaccine agents. (Supported by the National Research Council of Canada and Connaught Laboratories Ltd.)

F 301 **PASSIVE IMMUNIZATION AT A MUCOSAL SITE: SECRETORY IgA DIFFERS FROM SERA Ig IN PROTECTION AGAINST GROUP A STREPTOCOCCAL INFECTION, Debra Bessen and Vincent A. Fischetti, The Rockefeller University, New York, NY 10021.**
The influence of secretory IgA (sIgA) on colonization of mucosal surfaces by group A streptococci was explored. The surface of the streptococcus is covered with M protein, a fibrillar molecule with antiphagocytic properties. Over 80 distinct serological types of M protein exist, and only IgG specific for the M serotype in question will mediate phagocytosis of the organism in whole blood. We sought to determine the protective role, at the mucosa, of anti-M protein-specific Ig derived from opsonic serum and sIgA isolated from the saliva of opsonic donors. Secretory IgA was prepared from pooled human saliva by binding to the lectin jacalin. Whole opsonic serum and jacalin-purified sIgA were passed over an affinity column of the type 6 M protein (M6). The immunoreactivity to M6 protein of affinity-purified anti-M6-specific sera Ig exceeded that of sIgA by about 100-fold, as determined by ELISA. The anti-M6-specific antibody preparations were mixed with live type 6 streptococci and administered intranasally (i.n.) to mice. We measured the mortality rate of the animals due to the bacteria colonizing and subsequently invading the mucosal barrier and disseminating to distal sites. The data indicate that passively acquired sIgA (i.n.) protects mice against challenge (i.n.) with a lethal dose of streptococci, whereas passively administered (i.n.) serum antibodies are without effect. We describe a new model for evaluating antibody-mediated protection against infection by passive immunization at a mucosal site.

F 302 THE HLA RESTRICTED RECOGNITION OF IDIOTYPE BY HUMAN ANTI IDIOTYPIC T CELL CLONES DOES NOT REQUIRE PROCESSING BY ANTIGEN PRESENTING CELLS,
Ivo J.T.M.Claassen, Gerhard M.ten Hoor, Hans J.Bunschoten, Ab D.M.E.Osterhaus and Fons G.C.M.UytdeHaag, National Institute of Public Health and Environmental Hygiene, Bilthoven, The Netherlands.
Although amply documented in experimental murine models, only limited experimental data are available supporting the existence of idiotype specific T helper cells in man. Recently we described the cloning and characterization of human anti idiotypic T cell clones with specificity for an idiotope on rabies virus specific immunoglobulin. In this paper we show that the idiotype recognized is associated with λ light chain bearing immunoglobulins. Furthermore data are presented which indicate that the HLA-DQw2 restricted presentation of this idiotype to these T cell clones does not require processing of idiotype by antigen presenting cells.

F 303 ESCHERICHIA COLI HEAT-LABILE ENTEROTOXIN POSSESES ADJUVANT ACTIVITY AND PREVENTS THE INDUCTION OF ORAL TOLERANCE IN MICE TO UNRELATED PROTEIN ANTIGENS, John D. Clements, Nancy M. Hartzog, Frank L. Lyon, and Laura S. Levy, Tulane University Medical Center, New Orleans, LA 70112.
The ability of Escherichia coli heat-labile enterotoxin (LT) to influence the induction and maintenance of tolerance in animals primed orally with a soluble protein antigen, ovalbumin (OVA), or in animals primed orally with two unrelated protein antigens administered simultaneously, OVA and bovine serum albumin (BSA), was examined. In this study, simultaneous administration of LT with OVA was shown to prevent the induction of tolerance to OVA and to increase the serum anti-OVA IgG response 30 to 90 fold over OVA primed and PBS primed animals, respectively. This effect was determined to be a function of the enzymatically active A subunit of the toxin, and probably reflects the ADP-ribosylation activity of the subunit and subsequent intracellular increase in cAMP. Animals fed LT with OVA after the initial OVA prime developed a significantly lower serum IgG and mucosal IgA anti-OVA response than those fed LT with OVA in the initial immunization, indicating that prior exposure to the antigen reduces the effectiveness of LT to influence tolerance and its ability to act as an adjuvant. LT was not able to abrogate tolerance once it had been established. Serum IgG and mucosal IgA responses in animals receiving LT on only a single occasion, that being upon first exposure to antigen, were equivalent to responses after three OVA/LT primes, indicating that commitment to responsiveness occurs early and upon first exposure to antigen. An additional finding of this study was that LT can serve as an adjuvant for orally administered antigens and that it elicits the production of both serum IgG and mucosal IgA against antigens with which it is delivered. We are currently investigating the use of LT as an adjuvant for oral immunization against retroviral disease.

F 304 IMMUNE EVASION MECHANISMS: POLYMORPHISM IN THE CIRCUMSPOROZOITE PROTEIN OF PLASMODIUM FALCIPARUM, Vidal F. de la Cruz, Altaf A. Lal, Michael F. Good and Thomas F. McCutchan, Malaria Division, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892.
Efforts towards the development of anti-sporozoite malaria vaccines have focused on the repeats of the circumsporozoite protein. Because of genetic restriction of the immune response to the repeats, it has been suggested that the inclusion of parasite derived T cell determinants along with target B cell determinants (i.e. the repeats) will help overcome these obstacles by increasing vaccine construct immunogenicity, as well as provide for natural boosting in the field. However, we have recently observed that the regions containing the proposed T cell determinants are polymorphic in sequence. The pattern of variation at the DNA and protein levels are consistent with the hypothesis that the polymorphisms are being selected for by the immune system of humans. But since cross-reactivity of T cells among dissimilar sequences has been documented, the polymorphisms in this molecule are of unknown impact. We show using the mouse immune system as a model that T cells primed by one of these variants do not proliferate when challenged with another, nor do they provide help for antibody production. The immunological consequences of the variation thus further complicate vaccine development.

F 305 IMMUNE-STIMULATING COMPLEXES ('ISCOMS') AS A NOVEL HEPATITIS B VACCINE, Colin R. Howard, Bo Sundquist, Janice Allan, Sheila E. Brown and Shih-Hui Chen, London School of Hygiene and Tropical Medicine, London, UK.
Immune-stimulating complexes (iscoms) have been prepared by the mixing of solubilized HBsAg polypeptides expressed in yeast and the triterpenoid-like compound Quil-A. Immunization of BALB/c mice with a single dose of hepatitis B iscoms in saline resulted in a high titre antibody response to HBsAg equivalent to that obtained in mice immunized with the original HBsAg preparation adsorbed onto alum. Analysis of sera from mice receiving iscoms revealed high affinity antibodies directed against the major a determinants of HBsAg, and the antibody levels were detectable at 50% of peak values even after 18 months. High secondary antibody responses were observed in immunized animals previously inoculated with a sub-immunogenic dose of HBsAg indicating that hepatitis B iscoms represent a suitable immunogen for use in individuals in whom a course of immunization with currently licenced hepatitis B vaccines has failed to produce a significant anti-HBs response. Iscoms containing synthetic peptide analogues of HBsAg determinants have been prepared and immune responses to these quantified in mice.

F 306 STUDIES ON SYNTHETIC PEPTIDES FROM ENV GLYCOPROTEIN OF HIV, A.K. Judd,[1] M.A. Winters,[2] R.C. Humphres,[2] I.K. Sharma,[3] G. Bhatia,[3] S. Smith,[3] and W.W. Robinson.[3] [1]Bio-Organic Chemistry Laboratory, Life Sciences Division, SRI International, Menlo Park, CA 94025; [2]Immunobiology Section, Biomedical Research Laboratory, Life Sciences Division, SRI International, Menlo Park, CA 94025; [3]Department of Medicine, Division of Infectious Diseases, Stanford University School of Medicine, Stanford, CA 94305

The goal of this research is to investigate the potential of synthetic peptides as vaccines for AIDS. Synthetic peptides attached to appropriate carriers may lead to stimulation of antibodies capable of reacting with proteins containing the peptides in their sequence.

Using a solid phase technique, we have synthesized and purified a series of peptides from the HIV env glycoprotein sequence.

These peptides were conjugated to protein carrier for immunization studies. Initial screening of peptides was done by enzyme-linked immunosorbent assays for the binding of peptides to human immune sera. Then antisera to peptides were raised in guinea pigs. Antisera are now being tested for neutralization of viral infection.

Results of these experiments will be presented in the poster session.

F 307 SYNTHETIC MALARIA FALCIPARUM SPOROZOITE VACCINE - PILOT CLINICAL TRIAL

M. Just, R. Berger, C. Müller (Univ.Childrens Hospital); D. Stürchler, H. Etlinger, D. Gillessen, H. Matile, R. Pink, F. Sinigaglia, B. Takacs
(F. Hoffmann-La Roche & Co.); Basel, Switzerland

A safe, immunogenic, and protective malaria vaccine could contribute to the control of a disease, which involves nearly 100 million clinical cases each year. A candidate vaccine containing the synthetic dodecapeptide (NANP)3 - epitope of the circumsporozoite protein (CSP) of Plasmodium falciparum -, coupled to tetanus toxoid as a carrier was tested in 10 healthy adult volunteers for tolerability and immunogenicity. Following this a double blind study was initiated on 37 volunteers divided into 3 groups: one group receiving 2 injections of the vaccine i.m. 8 weeks apart, two other groups receiving recombinent Inteferon alpha simultaneously with the vaccine in doses of 1.5 or 0.5 x 10^6 IU.

Besides ordinary hematologic and blood chemistry tests the following immunological parameters were tested:

a) humoral immune response to: (NANP)50 by Elisa, sporozoites by IFA and Western blot, asexual blood stages by IFA; tetanus-toxoid by Elisa.
b) lymphocyte stimulation was tested with (NANP)50, (NANP)3, and with tetanus-toxoid. Hepatocyte invasion inhibition in vitro was also assayed.

The tolerability was excellent; local reactions were only mild even in tetanus-hyperimmunized volunteers. The immunological results will be presented in detail.

F 308 EFFECT OF DIETHYLCARBAMAZINE ON TITERS OF ANTIBODY TO FELINE ONCORNAVIRUS-ASSOCIATED CELL MEMBRANE ANTIGEN (FOCMA) IN FELINE LEUKEMIA VIRUS (FeLV) INFECTED CATS AND CATS GIVEN FeLV VACCINE. Lynn W. Kitchen, Division of Infectious Diseases, Tulane University Medical Center, New Orleans, LA 70112. Diethylcarbamazine (N, N-diethyl-4-methyl-1-piperazine;DEC) is the mainstay drug for prevention and treatment of the human filariases. An opsonic action of DEC may generate increased immune responses to microfilariae. In the present study, 9 domestic cats infected with FeLV that tested negative for antibody to FOCMA before treatment tested positive ($\geq$ 1:10 serum dilution, geometric mean titer [GMT]=278) for antibody to FOCMA after DEC treatment (10 mg/kg/day po x 1 mo). Among 19 cats initally testing positive for FOCMA antibody, higher titers were noted after treatment in 17 (pretreatment GMT=264; posttreatment GMT=6, 158). DEC treatment given for 2 weeks with a single IM dose of NORDEN FeLV vaccine increased the titer and duration of antibody to FOCMA in 3 cats (2 FeLV-naive; 1 FeLV-exposed) in comparison to 3 vaccinated/untreated controls. Use of DEC as an antifilarial agent could conceivably alter serologic outcomes of vaccine trails in Africa regarding HIV.

F 309 ANTI-IDIOTYPE ANTIBODY AS A PROSPECTIVE VACCINE AGAINST HEPATITIS B

E. Korec, I. Hlozanek, J. Stara, V. Nemecek
Institute of Molecular Genetics, Czechoslovak Academy of Sciences, Flemingovo 2, 166 37 Prague 6, Czechoslovakia.

The idea that regulation of the immune response can take place at the level of the idiotype recognition via an idiotype-anti-idiotype reaction was first proposed by Jerne in 1974. Manipulation of the immune response by injection of antibodies to idiotype has been documented in several systems. In this work we prepared rabbit and mouse sera containing anti-idiotype antibody against a monoclonal and against some polyclonal anti-HBsAg antibodies. The immunoglobulin fractions of these sera provoked the formation fo anti-HBsAg antibody when injected into BALB/c mice. The sera of humans repeatedly immunized with human anti-HBsAg antibodies as a means of passive protection against hepatitis B were shown to contain anti-idiotyope antibody. These anti-idiotype antibiodies elicitated an anti-HBsAg response after immunization of BALB/c mice and Syrian hamsters. Such sera may be a potential source of human anti-idiotype vaccine.

F 310 PROTECTION AGAINST VIRAL ENCEPHALITIS BY A SYNTHETIC PEPTIDE SELECTED FOR SURFACE PROBABILITY,

Pierre J. Talbot[1], Gervais Dionne[2] and Martial Lacroix[1,2], [1]Virology Research Center and [2] IAF BioChem International Inc., Institut Armand-Frappier, Université du Québec, 531 boulevard des Prairies, Laval, Québec, CANADA H7N 4Z3.

The JHM strain of murine hepatitis virus (MHV-JHM) is a neurotropic member of the Coronaviridae, a family of enveloped viruses that contain a single stranded RNA of positive polarity. The agent is involved in encephalitis and demyelination in mice and rats. This experimental model of virus-induced disease of the central nervous system may have direct relevance to some human neurological disorders such as multiple sclerosis or Parkinson's disease. Monoclonal antibodies have identified critical determinants of virus biology and neuropathogenicity on the external peplomer glycoprotein E2 of MHV-JHM.

In order to physically map such determinants on the E2 protein, potential immunogenic peptides were selected from the amino acid sequence predicted from the published DNA sequence of the cloned gene. The surface probability method of Emini et al. was used to select a decapeptide homologous to 0.8% of the E2 residues. This sequence of amino acids corresponded to a minor peak on a hydrophilicity plot, the usual selection strategy. Immunization of mice with the chemically synthetized peptide coupled to keyhole limpet hemocyanin elicited high levels of neutralizing antibody and protected against a lethal virus challenge. Protection correlated with a critical level of anti-peptide antibody, which could be reached after a single inoculation. These results suggest that an appropriate antibody response to a highly restricted, surface exposed domain of this viral protein is critical in determining the outcome of infection of the central nervous system.

F 311 **SYNTHETIC MALARIA, TRYPANOSOME OR STREPTOCOCCAL PEPTIDES HYDROPHOBICALLY COMPLEXED TO PROTEOSOMES VIA HYDROPHOBIC AMINO ACID OR LAURIC ACID FEET BECOME HIGHLY IMMUNOGENIC VACCINES,**

George Lowell, Lynette Smith, Wendell Zollinger, W. Ripley Ballou, Wayne Hockmeyer, Jeffrey Chulay, Edwin Beachey, Michelle Pace & Isaac Chalom. Walter Reed Army Institute of Research, Wash, DC 20307 and VA Med Ctr & Univ of Tennessee, Memphis, TN 38104.

When meningococcal outer membrane proteins are isolated, their hydrophobic interactions result in the formation of whole or fragmented vesicles which we have called "proteosomes." We have found that addition of a hydrophobic foot (HFt) consisting of a lauroyl group or a series of hydrophobic amino acids to one end of a peptide allows it to hydrophobically complex to proteosomes after simple dialysis. Such proteosome vaccines, made with synthetic peptides representing malaria circumsporozoite proteins, malaria merozoite gp113 & gp101, trypanosome VSG and group A streptococcal M proteins were highly immunogenic in mice without any other adjuvants. A cysteine inserted between the epitope and the HFt was essential for this immunogenicity perhaps due to epitope stabilization induced by cysteic dimerization. Differences were also found when the HFt was moved from the amino to the carboxy terminus. Although adding the HFt and a cysteine made many peptides immunogenic without either proteosomes or adjuvants, vaccines with proteosomes were invariably far more immunogenic (ELISA IgG & IFA titers exceeded 10^4-10^6) and were effective even in mouse strains that were non-responsive to the peptide. Proteosomes' hydrophobic multimolecular structure and the fact that they are B cell mitogens suggest that they may serve in vaccines not only as T cell carrier proteins but also as adjuvants perhaps via enhancement of antigen processing. Since proteosomes have already been shown to be safe for human use as components of meningococcal vaccines, and since the IgG induced can recognize native proteins, this novel system has immediate application for the development of peptide vaccines for humans.

F 312 **ENHANCEMENT OF IMMUNOGENICITY BY ANTIGEN STRUCTURE MODIFICATION.**
J. Gabriel Michael, Department of Microbiology and Molecular Genetics, University of Cincinnati Medical Center, Cincinnati, Ohio 45215
We have reported on a new approach of achieving enhanced immunogenicity through structural modification. This approach involves substitution of carboxylic groups on a protein antigen by amino groups. The substitution results in increase of positive charges and resistance to enzymatic degradation of the antigen. Several protein antigens which were modified by this technique, such as bovine serum albumin, hen egg albumin, ferritin, tetanus toxoid all demonstrated dramatic enhancement of immunogenicity, as much as 1000 fold, as determined by antibody formation and T cell proliferation isolated from immunized mice. The modified antigens were capable of inducing significant antibody response in low-responder strains of mice and were immunogenic when administered in a tolerogenic mode, intravenously or orally. The modified proteins were found to be presented very efficiently by antigen presenting cells to T helper cells.
In summary, our experimental data provide strong evidence that this new approach in antigen structure modification may prove useful in development of vaccines.

F 313 AN OLIGOSACCHARIDE VACCINE FOR HUMAN COLON CANCER, Christopher L. Reading and J. Milburn Jessup, M.D. Anderson Hospital, Houston, TX 77030.
Analysis of structural studies and studies with murine monoclonal antibodies reveal that there are a large number (at least 19) of human colon carcinoma-associated carbohydrate structures. Analysis of the specificity of humoral and cellular immune responses of patients treated with autologous colon tumor cell vaccine will define which of these structures are immunogenic in humans and provide evidence for or against autoimmune complications from boosting immunity to these structures. Transplantable syngeneic colon tumor CoLo26 in Balb/c mice and _de novo_ carcinogen-induced colon tumors in CF-1 mice provide a preclinical model to analyze immunization schedules, doses, presentation, side effects, and efficacy. It may be possible to use a synthetic carbohydrate vaccine approach to boost immunity in patients who have had a colon tumor classified as Dukes B_2 or C resected. Prolongation of disease-free survival in the absence of autoimmune complications would provide evidence of efficacy and safety.

F 314 DETECTION OF HUMAN HORMONE-LIKE MOLECULES ELABORATED _IN VITRO_ BY _NEISSERIA GONORRHOEAE_, Edward N. Robinson, Jr. and Zell A. McGee, the University of Louisville, Louisville, Kentucky, 40292 and the University of Utah, Salt Lake City, Utah, 84132.
Published descriptions of the ability of bacterial toxins to bind to human hormone receptors and the elaboration of hormone-like molecules _in vitro_ by tumor-associated bacteria prompted a search for hormone-like molecules expressed by gonococci. Monoclonal antibodies to human chorionic gonadotropin hormone (hcg) were used in dot blot and electroblot assays to detect hcg-like molecules in whole cell lysates of agar-grown _Neisseria gonorrhoeae_ and _N. subflava_. Antibody to the alpha subunit of hcg bound a 60kd protein present in gonococcal lysates but not in a similarly prepared lysate of _N. subflava_. Antibody to the beta subunit of hcg bound numerous gonococcal proteins of a wide range of molecular weights. Only one protein in the subflava lysate was recognised by beta-hcg directed monoclonal antibody. The pathogenic role of these hormone-like molecules is unknown. The implications of bacterial mimicry of human substances for vaccine production include the inability to elicit significant antibody titers due to host tolerance and the risk of producing autoimmune disease in vaccine recipients.

F 315 A COMPARISON OF ADJUVANT EFFICACY FOR A RECOMBINANT HERPES SIMPLEX VIRUS GLYCOPROTEIN VACCINE, Lisa Sanchez-Pescador, Rae Lyn Burke, Gary Ott, Philip Ng, Cate Larsen, Barbara Gervase and Gary Van Nest, Chiron Corporation, Emeryville, CA.

The emergence of recombinant DNA derived subunit vaccines has intensified the need for safe and effective adjuvants. We have used one such recombinant vaccine candidate antigen, the herpes simplex virus type 1 glycoprotein D (gD), to compare the ability of several adjuvants to stimulate immunity in guinea pigs. A truncated, secreted form of gD expressed in Chinese hamster ovary cells was purified and formulated with the adjuvants complete Freund's, aluminum hydroxide, muramyl dipeptide and a lipophilic muramyl tripeptide (MTP-PE). The muramyl peptides were delivered in a variety of vehicles including a 50% oil emulsion, a 4% oil emulsion, and liposomes (MTP-PE only). In the first phase of the study gD, combined with MTP-PE in a 4% oil emulsion was especially effective in generating anti-gD antibody titers. In the second phase, the adjuvants were compared for the elicitation of protective immunity against a genital challenge with herpes simplex virus type 2. Animals were immunized 3 times with gD and the various adjuvants prior to virus challenge. Thereafter, animals were monitored daily for herpetic lesions, urinary retention, and mortality. Results indicated that clinical symptoms of the MTP-PE and Freund's adjuvant groups were similar in magnitude and were markedly reduced compared to those of the aluminum hydroxide or unimmunized control groups.

F 316 STRUCTURAL SIMILARITY OF Ia-BINDING REGIONS FROM UNRELATED ANTIGENS, A. Sette, S. Buus, and H. Grey, Natl. Jew. Ctr. for Immunol. & Resp. Med., Denver, CO 80206.

Considerable evidence has accumulated to support the concept of specific interactions between Ia molecules and peptide antigens. It is not clear, however, how a very limited number of Ia molecules could bind the almost endless variety of potential immunogenic determinants. In a previous study of the affect of single amino acid substitutions on Ia binding the very permissive nature of antigen-Ia interaction was demonstrated. We also showed that unrelated peptides that are good IA^d binders share a common structural motif, and speculated that recognition of such motifs could represent a mechanism to achieve a very permissive type of interaction that yet retained some degree of specificity. The purpose of the present set of experiments was two fold: First, to determine whether the peptide regions, predicted on the basis of structural similarity to be involved in IA^d binding, were in fact involved: Second, in instances in which the same peptide bound more than one Ia-molecule, to determine whether the same peptide region was used to interact with the different Ia molecules. We tested the Id binding capacity of overlapping peptides derived from sperm whale myoglobin (Myo102-125), and influenza hemagglutinin (Ha121-146). In both cases the IA^d-interacting determinants contained the sequence motif postulated to be important for IA^d binding. Furthermore, since HA121-146 and Myo102-125 also contained IA^k and IE^d interacting determinants, the same peptides were tested for IA^k and IE^d binding. Both the IA^k-interacting determinant in Ha121-146 and the IE^d-interacting determinant in Myo103-125 mapped to the same regions involved in IA^d interaction, suggesting that different Ia molecules may recognize closely related structures.

F 317 THE ROLE OF LAUROYL AND TETRAPEPTIDE CYGG IN T CELL ACTIVATION BY SYNTHETIC PEPTIDES FROM *P. FALCIPARUM* CIRCUMSPOROZOITE PROTEIN. LF Smith, GH Lowell, WR Ballou and U Krzych. Depts. of Bacterial Diseases and Immunology, WRAIR and Dept. of Biology, CUA, Wash., DC.

We have observed that priming with *P. falciparum* circumsporozoite (CS) synthetic peptide (NPNA)4 [(F4)], without any adjuvants, generates secondary IgG responses in responder C57Bl/6 mice only if the peptide is conjugated to lauroyl-CYGG (LC). To determine the role of LC in T cell activation, mice were immunized peripherally with either F4, C-F4 or LC-F4 in Complete Freund's Adjuvant (CFA). Eight days later lymph node (LN) T cells were tested for their ability to respond to F4 and F4 constructs in the T cell proliferative assay. Results demonstrated that F4-specific T cells responded equally to F4 and F4 constructs. In constrast, C-F4-specifc T cells showed a significant response upon *in vitro* challenge with the priming antigen or LCF4, but not with F4. However, additon of the lauroyl group during priming did not enhance the CF4 effect. It is conceivable that the addition of CYGG to F4 creates an immunodominant epitope distinct from the epitope on F4, thereby activating T cell specificities other than F4. The potential enhancing effect of the lauroyl group might have been obscured by the presence of CFA during *in vivo* priming therefore, *in vitro* T cell activation of two (NANP)40-specific T cell clones was utilized for this assessment. Upon stimulation with F4, C-F4 or LC-F4, both clones demonstrated a striking preference for LC-F4. C-F4 induced an intermediate response and F4 was least effective. These data clearly demonstrate that both the lauroyl and the tetrapeptide CYGG augment T cell activation by small peptides. Whereas the enhancement seen with peptides conjugated to lauroyl may be related to the unusual ability of hydrophobic moieties to interact with antigen presenting cells, addition of CYGG to the peptide may stabilize the conformation of a T cell epitope and thereby promote antigen presentation. The cellular basis of the mechanism of LC enhancement is currently being studied. This information should be extremely useful in the planning and design of synthetic vaccines.

F 318 POTENTIATION OF ANTIBODY RESPONSES TO SYNTHETIC HEPATITIS B PEPTIDE; Michael W. Steward, Sheila E. Brown and Colin R. Howard; London School of Hygiene and Tropical Medicine, London WC1E 7HT, U.K.
The ability of monoclonal and polyclonal antibodies to potentiate the level and affinity of antibody responses has been assessed in mice. In preliminary experiments, model antigens (hapten:protein conjugates) were complexed with monoclonal anti:hapten and anti:carrier antibodies. The results showed that either IgG or IgM antibodies were able to enhance the antibody response to both hapten and carrier moiety of the conjugate. Furthermore, a monoclonal IgG anti:HBsAg antibody was shown to potentiate the response to HBsAg compared to the response to HBsAg given alone in terms of both affinity and antibody levels. On the basis of these results, we assessed the ability of both human polyclonal and murine monoclonal anti:HBsAg antibodies to influence the response in mice to a synthetic peptide representing residues 139-147 of HBsAg. This region corresponds to part of the 'a' determinant of HBsAg, antibodies to which confer protection to viral infection. Immunization with complexes of peptide and each antibody resulted in enhanced immunogenicity and the development of high affinity antibodies to the peptide which cross-reacted with native HBsAg. Immunization with immune complexes represents an alternative approach for enhancing the immunogenicity of synthetic peptides.

F 319 MODULATION OF THE IMMUNE RESPONSE TOWARDS ANTI-FELV IMMUNITY WITH ANTI-IDIOTYPE ANTIBODIES. Weijer, K., Osterhaus, A., Overman, P. and UytdeHaag, F; The Netherlands Cancer Institute, Amsterdam, The Netherlands; National Institute of Public Health and Environmental Hygiene, Bilthoven, The Netherlands.

Among the approaches to develop a FeLV vaccine, we considered the generation of an anti-idiotype vaccine. Virus neutralizing mouse monoclonal antibodies (MoAb1) were generated against an epitope of gp70 shared by all three subtypes. Against one of these, MoAb 3-17, a panel of 15 anti-idiotype monoclonal antibodies (MoAb2) were generated. They proved to recognize partially overlapping private idiotypes within the paratope of MoAb 3-17. Repeated immunizations with these MoAb2 coupled to KLH, resulted in low-titered anti-FeLV serum titers in BALB/c mice. In a second series of experiments rabbits were immunized with an affinity purified polyclonal cat anti-FeLV neutralizing antibody preparation. Immunization of BALB/c mice with Ig from these rabbits resulted in high anti-FeLV antibody titers within 3 weeks.

F 320 CHARACTERIZATION OF ANTIBODIES RECOGNIZING PROTEIN I OF *NEISSERIA GONORRHOEAE* PRODUCED BY INJECTION WITH PROTEIN I - LIPOSOME CONSTRUCTS. *Wetzler, L.M.; Blake, M.S.; Gotschlich, E.C.; Laboratory of Bacteriology and Immunology, The Rockefeller University, New York, N.Y. 10021 A major goal of gonococcal research is the development of a gonorrheal vaccine. A vaccine candidate is the major outer membrane protein (PI) of the gonococcus which has limited antigenic variability. Two main subtypes, PIA and PIB, and 9 main serotypes have been described. In order to avoid raising anti-Protein III (PIII) blocking antibodies, PI was chromatographically isolated with minimal PIII contamination (less than 1%) from Pgh 3-2 (PIB), a serum sensitive gonococcal strain and UU1 (PIA), a serum resistant gonococcal strain. Alum was first used as an adjuvant and antibodies raised did not agglutinate the organisms and were not opsonic, and bactericidal titers were not increased. In order to present PI in a form mimicking its *in vivo* disposition, it was inserted into liposomes. Antibodies raised did agglutinate the organism and they contained opsonic and bactericidal activity greater than the preimmune and alum generated sera. The PIB-liposome antisera also had higher ELISA titers to a synthetic peptide equivalent to the exposed portion of PIB and a higher percentage of antibodies absorbed by whole organisms than the PIB-alum antisera. We speculate that the differences in these results can be attributed to the method by which PI is presented as an immunogen. When PI is presented absorbed to alum, the specificity of the antibodies produced is mainly to buried epitopes; however, when PI is presented in liposomes, the antibodies raised mainly recognize surface exposed epitopes of the protein. The liposome seems an ideal vehicle to present membrane proteins as vaccine candidates.

Vaccinia and Other Viral Delivery Systems

F 400 DESIGN CONSIDERATIONS FOR VACCINES AGAINST RELAPSING DISEASES, Michael W. Clarke and Vern B. Carruthers, University of Western Ontario, London, ON N6A 5C1.
Pathogens which persist in the infected host producing chronic or relapsing disease present a great challenge for the vaccine designer. Persistence is usually due to the presentation of novel antigens on the surface of the organism and the consequent appearance of new serotypes. The mechanisms which various organisms utilise to generate a repertoire of antigenic variants are diverse and include point mutations and complex chromosomal rearrangements. We are studying the variant surface glycoproteins (VSG's) from African trypanosomes, protozoan parasites which are responsible for a chronic relapsing disease of humans and domestic animals in Africa south of the Sahara. These molecules constitute a very large and diverse family of polymorphic glycoproteins that represent a very useful model of antigenic variation. Four VSG's will be described which, although not identical, share immunochemical and structural features. Monoclonal antibodies have been derived some of which cross-react with all four VSG's and others which are specific for one VSG. These epitopes are mapped and their precise structure determined and located on the sequence of the VSG's. Differences between epitopes are at the level of certain critical amino acid residues which define the immunochemical specificity of a VSG. These findings are important for the design of pan-specific synthetic peptide vaccines.

F 401 CONSTRUCTIONS AND IMMUNOGENECITY OF *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE PEPTIDE CONJUGATE VACCINES. Stan J.Cryz [1], John U. Que[1], Jerry C.Sadoff[2] and Ripley Ballou[2]. Swiss Serum and Vaccine Institute, Berne, Switzerland[1] and Walter Reed Army Institute of Research, Washington, DC[2].
Conjugates were formed between the immunodominant tetrapeptide, Ala-Asn-Ala-Pro, of the *Plasmodium falciparum* circumsporozoite protein (CSP) and various carrier proteins suitable for human use. Synthetically prepared $(Ala\text{-}Asn\text{-}Pro\text{-}Ala)_3$ (R_3) or $(Ala\text{-}Asn\text{-}Ala\text{-}Pro)_6$ (R_6) were coupled to tetanus toxoid, choleragenoid, cholera toxin or *Pseudomonas aeruginosa* toxin A via reductive amination using adipic acid dihydrazide as a spacer. Conjugates were stable, nontoxic and non-pyrogenic. Immunogenecity was determined by immunizing rabbits intramuscularly with conjugates adsorbed to $Al(OH)_3$. Both R_3 and R_6 when coupled to carrier proteins were capable of eliciting high anti-CSP immunoglobulin G (IgG) ELISA titers. The magnitude of the immune response was dependent upon the carrier used. Cholera toxin and toxin A conjugates were the most immunogenic while tetanus toxoid conjugates were poorly immunogenic. Repeated immunization were only moderately effective at increasing anti-CSP antibody levels.

F 402 INDUCTION OF ANTIBODY PRODUCTION TO SYNTHETIC PEPTIDES VIA PEPTIDE PHOSPHOLIPID COMPLEXES. Goodman-Snitkoff , P. Heimer , W. Danho , M. Felix and R.J. Mannino . Dept. Micro. and Immunol. Albany Med. Col., Albany, NY 12208. Peptide Research Dept. Hoffmann-LaRoche Inc., Nutley, NJ 02110.
We have found that cross-linking peptides to phospholipid and complexing this with additional phospholipid results in a strong immune reponse to the peptide in the absence of any additional protein carriers or adjuvants. Peptides that are neutral or hydrophilic in a Hopp and Woods analysis do not stimulate antibody production when complexed in this way. However, peptides which are amphipathic can induce significant antibody titers (>25,600) after two immunizations, which increase to more than 100,000 after a third immunization and are predominantly of the IgG_1 and IgG_2 subclasses. These data are indicative of T helper cell involvement. The immunogenicity of the amphipathic peptides is dependent upon their being covalently linked to and complexed with phospholipids. If the peptide is injected in saline or in the presence of phospholipid without covalent linkage to phospholipid, there is no immune response. Furthermore, the covalent linkage of a non-immunogenic, hydrophilic peptide to the immunogenic, amphipathic peptide stimulates production of antibody to the previously nonimmunogenic peptide. The findings here lead to the following working hypothesis: 1) crosslinking of a peptide to and complexing it with phospholipid allows the peptide to be presented to the immunocompetent cell in a multivalent configuration similar to that on a cell surface; 2) multivalency alone is not sufficient to assure an immune response, but amphipathicity of the peptides is also required. This method of complexing amphipathic peptides and phospholipid molecules allows the presentation of B and T cell determinants in a multivalent complex which stimulates antibody production by providing the components necessary for activation of both cell types.

F 403 VARIABLE BINDING SITE FOR HIV NEUTRALIZING ANTIBODIES HAS A CONSERVED ARCHITECTURE WITHIN THE EXTERNAL ENVELOPE PROTEIN, Jaap Goudsmit and Rob H. Meloen; Human Retrovirus Laboratory, AMC, Amsterdam, the Netherlands and Central Veterinary Institute, 8200 AB Lelystand, the Netherlands.
The host range of HIV appears to be restricted to humans and chimpanzees, limiting the opportunities for vaccine efficacy studies to these two species. Therefore sera from HIV infected numans and chimpanzees and the PEPSCAN method were used to identify neutralizing epitopes across the entire gp120. An immunodominant domain in the carboxyl terminal region V3 that bound HIV neutralizing natibodies was identified. HTLV-IIIB and LAV inoculated chimpanzees recognized the amino-acid sequence IRIQRGPGRAFVTIG (aa307-321) of HTLV-III B, but not the corresponding region within HTLV-III RF. A HTLV-III RF inoculated chimpanzee recognized the RF sequence: ITKGPGRVIYATG at the same location, but not corresponding region of HTLV-III B. The HTLV-III B residues IQR and AF and the HTLV-III RF residues TK and VI flanking a highly conserved β-turn (GPGR) appeared to be crucial for binding these type-specific antibodies of HIV-infected humans and chimpanzees. This suggests a general architecture of this epitope for all virus variants.

F 404 RECOMBINANT MOLECULES AND THE DEVELOPMENT OF NEW VACCINES AGAINST WHOOPING COUGH.
A. Bartoloni, M.G. Pizza, R. Gross, G. Grandi* and R. Rappuoli.
Sclavo Research Center, Siena, Italy; *Eniricerche, Milan, Italy
By engineering of the genes encoding pertussis toxin (PT) we tried to construct new protective molecules for the development of a new vaccine against whooping cough. These molecules include the 5PT subunits expressed in E. coli as fusion proteins and the 5 subunits expressed in B. subtilis. All these constructions were unable to cause a protective antibody response in mice from the intracerebral challenge with B. pertussis. Studies on the immunogenicity of these molecules have led us to understand some of the reasons which make these molecules unsuitable as a vaccine. These analyses provide the rationale for the construction of new vaccines based on PT.

F 405 IMPORTANCE OF THE SECOND CONSERVED DOMAIN OF gp120 FOR HIV INFECTIVITY AND ANTIBODY NEUTRALIZATION. Ho DD, Alam M, Gurney ME. UCLA School of Medicine and The University of Chicago.
Rabbits were immunized with three overlapping synthetic peptides with sequence homology to the second conserved domain of the human immunodeficiency virus (HIV) external envelope glycoprotein (gp120). All antisera were reactive with the immunizing peptides by immunoassay and with gp120 by radioimmunoprecipitation. One antiserum (versus amino acids 254-274 of env) was also very efficient in neutralizing three diverse isolates of HIV (HTLV-IIIB, HTLV-IIIRF, ARV-2) in vitro, with titers significantly higher than those of anti-gp120 sera. However, the binding of HIV to CD4-positive cells was not affected by these antisera. Therefore, this conserved region of gp120 appears to be critical in a post-binding event during virus penetration and may represent an important target for antibody neutralization of HIV. Moreover, sera from persons infected with HIV or animals immunized with gp120 were largely unreactive with the peptides by immunoassay. This suggests that when the second conserved domain is presented to the immune system in the context of a larger molecule, it is minimally immunogenic. These findings may be applicable in the design of a vaccine for the acquired immunodeficiency syndrome.

F 406 HUMAN LYMPHOCYTE PROLIFERATION TO A CIRCUMSPOROZOITE PROTEIN T CELL EPITOPE CORRELATES WITH DECREASED SUSCEPTIBILITY TO FALCIPARUM MALARIA, Stephen L. Hoffman, Charles N. Oster, Carl Mason, John C. Beier, James A. Sherwood, W. Ripley Ballou, Mutuma Mugambi, and Jeffrey D. Chulay, Naval Medical Research Institute, Bethesda, MD, Walter Reed Army Institute of Research, Washington, DC, and Kenya Medcial Research Inst., Nairobi, Kenya
This study was designed to delineate non-repeat region T epitopes on the CS protein of P. falciparum that might provide T cell help for production of antibody to the repeats or induce a protective cellular immune response. Eight peptides derived from the CS protein of a Brazilian strain of P. falciparum and a repeat region peptide were selected to stimulate lymphocytes from 28 adults from a malarious area of Kenya. The volunteers were then radically cured, and followed for 126 days. At 15 ug/ml, 8 of 9 peptides, 4 of which were overlapping, induced a proliferative response in one to 5 volunteers. Eleven of the 28 responded to at least one peptide and antibody levels to the repeat region were significantly higher among these 11 individuals. 25 volunteers were reinfected during 125 days; 2 of the 25 responded to peptide 361-380. Three volunteers did not become infected and all 3 responded to 361-380 (P=0.003). The mean stimulation indices to 361-380 of the 25 infected and 3 non-infected volunteers were 1.45 and 12.03 respectively ($P<0.001$). These studies demonstrate at least 5 human T cell epitopes on the CS protein of P. falciparum, suggest that non-repeat epitopes are important for T cell help for human production of antibody to the repeats, and show a significant correlation between proliferative response to peptide 361-380 and decreased susceptibility to malaria. The data provide support for incorporating non-repeat and repeat peptides into new CS protein vaccines.

F 407 IMMUNOGENIC COMPLEXES MADE OF A PEPTIDE ANTIGEN, GLYCOPEPTIDE ADJUVANT AND HIGH MOLECULAR CARRIER, Ivanov V.T., Andronova T.M., Rar V.A., Ivanov B.B., Makarov E.A., Meshcheryakova E.A., Yurovsky V.V. Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, ul. Miklukho-Maklaya 16/10, Moscow, USSR.
A large series of the title triple conjugates was synthesized and subjected to immunochemical study. The 205-213 nonapeptide from the foot and mouth disease VP1 viral protein and the repeating segment $(AsnAlaAsnPro)_3$ from the circumsporozoite protein of the *Plasmodium falciparum* malaria parasite were used as peptide components of the conjugates. Cationic poly(Lys)-poly(DL-Ala) and potentially anionic copolymer of moleic anhydride with vinylpyrrolidone as well as bovine serum albumine were the high molecular carriers and N-acetylglucosamine-β-(1-4)-N-acetylmuramyl-L- Ala-D-Gln-NH_2 or its derivatives were the immunoadjuvants. Immunogenicity of the conjugates obtained was studied by ELISA techniques and recomendations for further development of such constructions are formulated.

F 408 DESIGN, SYNTHESIS AND CHARACTERIZATION OF PREDETERMINED SUPERSECONDARY AMPHIPHILIC PEPTIDES AS CONFORMATIONAL PEPTIDE IMMUNOGENS
Pravin T.P. Kaumaya and Erwin Goldberg, Northwestern University, Evanston IL 60208
Antibodies elicited by immunization with short peptides containing antigenic determinants have been shown, in general, to bind poorly, with greatly reduced affinity to the corresponding region in the native proteins. Thus, such peptides have not proven to be effective immunogens and are poor prospects for vaccines. A novel strategy allowing the design and synthesis of conformational peptides which closely mimic the critical features of the native structure is advanced by using a knowledge-based prediction of protein structure, computer modelling and peptide synthesis. The model antigen being used in this study is the protein lactate dehydrogenase C_4 (LDH-C_4). The three-dimensional structure has been determined to a resolution of 2.9Å and the B cell determinants have been extensively mapped. The immune response to LDH-C_4 is of medical interest because immunization of females with LDH-C_4 has been shown to reduce fertility and is thus a good candidate for a contraceptive vaccine. The design concepts, synthesis and characterization of these novel conformational peptides will be presented. In particular, a linear segment on the surface of LDH-C_4 displaying certain structural features (α helix, β sheet) has been grafted onto a folding unit ($\beta\alpha\beta$, $\beta\alpha$, $\alpha\alpha$) for stabilization into a 3-dimensional architecture representative of the native protein. Preliminary results show that, at their respective PI's, an 18 residue amphiphilic α helical peptide tetramerizes and a 40 residue peptide designed to form an $\alpha\alpha$ fold dimerizes. The goal of this work is to point to a general strategy for tailoring peptide vaccine with more useful antigenic and immunogenic characteristics.

F 409 SYNTHESIS OF PEPTIDES FROM HEPATITIS A VIRUS CAPSID PROTEINS AND THEIR IMMUNOCHEMICAL STUDY.
Kozhich A.T., Ivanov V.S., Tchikin L.D., Ivanov V.T., Nastashenko T.A.,* Kusov Yu.Yu.,* Balayan M.S.* Shemyakin Inst. Bioorg. Chem., USSR Acad. Sci., *Inst. Poliomyelitis and Viral Encephalititis, USSR Acad. Med. Sci., Moscow, USSR.
Hepatitis A virus (HAV) capsid proteins VP1, VP2 and VP3 are exposed at the virion surface and should contain antigenic determinants. Algorithms for hydrophilicity, acrophilicity, antigenicity and flexibility were used to predict probable antigenic sites. Synthesis of 7-23-membered overlapping peptides from seven sites was performed by solution and solid phase methods. These are 1-17, 6-17, 11-25, 73-81, 75-85, 97-108, 97-111, 100-106, 100-107, 107-126, 112-126, 115-126, 112-122, 117-139, 123-139, 126-139, 276-298, 283-298, 288-298 and 290-299 from VP1 as well as 65-85 and 73-85 from VP2. Free peptides and their conjugates with KLH, BSA, gelatine and synthetic polymer were used for immunization and study of antigenicity The resultant sera in all cases contained peptides specific antibodies. Anti-peptide sera were unable to compete for HAV in block-ELISA. Also none of the sera tested was capable of neutralizing 10 $TCID_{50}$ of HAV. However some peptides bind to hyperimmune guinea pig anti-native HAV and anti-denaturated HAV sera. The results obtained do not entirely agree with the data of Emini et al. (J. Virol. 55:1985) concerning the VP1 11-25 peptide. Possible explanations of this discrepancy are discussed.

F 410 INDUCTION OF ANTIBODY PRODUCTION TO SYNTHETIC PEPTIDES VIA PEPTIDE PHOSPHOLIPID COMPLEXES.
Goodman-Snitkoff, P. Heimer, W. Danho, M. Felix and R.J. Mannino. Dept. Micro. and Immunol. Albany Med. Col., Albany, NY 12208. Peptide Research Dept. Hoffmann-LaRoche Inc., Nutley, NJ 02110.
We have found that cross-linking peptides to phospholipid and complexing this with additional phospholipid results in a strong immune reponse to the peptide in the absence of any additional protein carriers or adjuvants. Peptides that are neutral or hydrophilic in a Hopp and Woods analysis do not stimulate antibody production when complexed in this way. However, peptides which are amphipathic can induce significant antibody titers (>25,600) after two immunizations, which increase to more than 100,000 after a third immunization and are predominantly of the IgG_1 and IgG_2 subclasses. These data are indicative of T helper cell involvement. The immunogenicity of the amphipathic peptides is dependent upon their being covalently linked to and complexed with phospholipids. If the peptide is injected in saline or in the presence of phospholipid without covalent linkage to phospholipid, there is no immune response. Furthermore, the covalent linkage of a nonimmunogenic, hydrophilic peptide to the immunogenic, amphipathic peptide stimulates production of antibody to the previously nonimmunogenic peptide. The findings here lead to the following working hypothesis: 1) crosslinking of a peptide to and complexing it with phospholipid allows the peptide to be presented to the immunocompetent cell in a multivalent configuration similar to that on a cell surface; 2) multivalency alone is not sufficient to assure an immune response, but amphipathicity of the peptides is also required. This method of complexing amphipathic peptides and phospholipid molecules allows the presentation of B and T cell determinants in a multivalent complex which stimulates antibody production by providing the components necessary for activation of both cell types.

F 411 IMMUNOGENICITY AND GROUP SPECIFIC NEUTRALIZATION ACTIVITY OF A P17 GAG ANALOGUE (HGP-30),
Paul H. Naylor, Prem S. Sarin, Seiji Wada, Ehud Roffman, Cartha Naylor, Scott Maurer and Allan L. Goldstein, The George Washington University Medical Center, Washington, D.C. 20037, NIH, Bethesda, Maryland 20892.
Antisera directed against p17 gag epitopes effectively neutralize the AIDS-associated virus (HIV) in _in vitro_ assays and the presence of such antibodies in HIV positive individuals correlates with a good clinical prognosis. The antiviral activity of these antisera is due in part to epitopes on the p17 protein contained between positions 92-109. A 30 amino acid synthetic peptide analogue (HGP-30) contains these epitopes, raises neutralizing antisera, and the conserved nature of the epitope results in neutralizing activity which is group specific. Immunization of rabbits with whole p17 results in antibodies which bind HGP-30, demonstrating that the peptide represents an immunodominant region of the p17. The current hypothesis to explain the neutralization activity of such antibodies is that the portion of the p17 to which the antibodies are directed is on the external portion of the virus. Observations supporting this hypothesis include the presence of p17 on the surface of infected cells, myristylation of the N-terminal of the protein, and the existence of an 18 amino acid sequence in the p17 protein which could be a transmembrane region. Based on the results to date, the HGP-30 analogue is predicted to be a useful candidate for an AIDS vaccine, as well as the basis for a clinically useful diagnostic assay to measure protective antibodies.

F 412 T-CELL EPITOPES OF THE MYCOBACTERIAL 65 KD PROTEIN ANTIGEN
Fredrik Oftung, Abu S. Mustafa, Thomas M. Shinnick, Richard A. Houghton and Tore Godal. Lab. for Immunology DNR Oslo, Norway.
A synthetic peptide approach has been used to identify the epitopes recognized by clonal and polyclonal human T-cells reactive to the immunogenic recombinant mycobacterial 65 kD protein antigen. Three of the four epitopes identified were recognized as crossreactive between M.tuberculosis and M.leprae although their amino acid sequence in 2 of 3 cases were not identical. The peptide defining an epitope recognized as specific to the M.tuberculosis complex contains only 2 substitutions compared to the homologous M.leprae region.The peptide reactive T-cell clones showed helper/inducer phenotype (CD4+,CD8-) and secrete both IL 2 and GM-CSF upon antigen stimulation. However,the same clones display cytotoxicity against macrophages pulsed with the relevant peptides or mycobacteria.

F 413 PROCYCLIN: AN IMMUNODOMINANT TRYPANOSOME SURFACE ANTIGEN WITH POTENTIAL FOR USE AS A TRANSMISSION BLOCKING VACCINE, Terry W. Pearson, Jennifer P. Richardson and Isabel Roditi, Department of Biochemistry and Microbiology, University of Victoria, Victoria, BC, Canada, and Institut für Genetik, Kernforschungszentrum Karlsruhe, West Germany.
The procyclic (tsetse fly midgut) stage of African trypanosomes lacks the variant surface glycoprotein (VSG) coat found on bloodstream forms of the parasite. We have purified an immunodominant surface glycoprotein from procyclic trypanosomes using monoclonal antibody affinity chromatography. Microamino acid analysis and gas-phase microsequencing have revealed an unusual acidic polypeptide which contains a large proline-glutamic acid repeat. Synthetic peptides were used in ELISA with 10 monoclonal antibodies to localize several surface epitopes to distinct areas on the procyclin molecule. By gene cloning and expression in E. coli, procyclin was produced in a form which could induce antibodies that bound to epitopes from several parts of the molecule including the proline-glutamic acid repeat and to the surface of living procyclic trypanosomes. The procyclin molecule is a candidate for development of a transmission-blocking vaccine since it has previously been shown that antibodies to procyclic trypanosomes can interfere with tsetse-fly infectivity presumably by affecting trypanosome differentiation in the insect vector.

F 414 DELINEATION OF EPITOPES ON A PLASMODIUM FALCIPARUM MEROZOITE SURFACE ANTIGEN RECOGNIZED BY INHIBITORY MONOCLONAL ANTIBODIES. A. Saul, R. Epping, R. Ramasamy, G. Jones, J. Smythe and M. Geysen. Queensland Institute of Medical Research, Brisbane, Australia, 4006, Walter and Eliza Hall Institute of Medical Research, Melbourne Australia, 3050, and Commonwealth Serum Laboratories, Melbourne, Australia, 3050.
Monoclonal antibodies have been produced which bind to an approximately 45-51kDa glycosylated, myristylated protein located on the surface of P. falciparum merozoites. These antibodies are capable of inhibiting the growth of the parasites in vitro probably by preventing complete release of merozoites from rupturing schizont infected red cells or their invasion into uninfected red cells. A cDNA expression library was screened with the antibodies, positive clone CL2122 isolated and sequenced. Another clone, Ag513, capable of producing a gene product containing the epitope recognized by these antibodies was independently isolated from a library screened with human antisera. Within the coding regions, the two clones show no sequence homology with the exception of a 12 base section coding for the amino acid sequence STNS. Overlapping octapeptides corresponding to the predicted amino acid sequence from each clone were made and tested for their ability to bind antibody. Those peptides from either set, containing the STNS sequence, were positive with 3 of the antibodies. The degree of substitution possible in each position has been tested. An epitope outside this region was found which bound to the another of the antibodies. Synthetic peptides based on the STNS epitope are being investigated as vaccine candidates.

F 415 NOVEL FOOT-AND-MOUTH DISEASE PROTECTIVE PEPTIDES: MECHANISM OF IMMUNOSTIMULATION

Olga Vol'pina, Andrey Surovoy, Vasily Gelfanov, Elisaveta Kahn, Vadim Ivanov, Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, 117871 Moscow, USSR

When designing a synthetic foot-and-mouth disease vaccine, we synthesized a series of overlapping peptides from main immunodominant region 130-160 of viral capsid protein VP_1 144-159, 144-152, 141-152, 141-148, 136-148, 136-152 (strain 0_1K) and 131-149, 131-139, 140-149 (strain A_{22}). Immunization of experimental animals by synthetic peptides 136-152, 136-148 for 0_1K and 131-149, 140-149 for A_{22} induced high virus neutralizing antibody titres and 60-100% protection against foot-and-mouth disease. Immunization by the free peptides gave the same results as in the case of KLH-bound peptides. Fragments 144-159, 141-152 (0_1K) and 131-139 (A_{22}) KLH conjugates were inactive both *in vitro* and *in vivo* virus inhibiting experiments. The active 136-152 (0_1K) free peptide was studied in various tests in detail. It was shown that the 136-152 peptide is the full value immunogen containing agretope, T-epitope and B-epitope. We suppose the B-epitope to be included in the 141-148 peptide. Fragment 136-140 is essential for T-cell activity and the ArgGlyAspLeu (145-148) fragment is a part of the virus cell attachment site.

F 416 22-nm PARTICLES OF THE HEPATITIS SURFACE ANTIGEN CONTINAING EPITOPES OF P190 OF P. FALCIPARUM.

Albrecht V. Brunn, Klaus Fruh and Hermann Bujard ZMBH, Universitat Heidelberg, 69 Heidelberg, FRG.

The hepatitis surface antigen (HBsAg) has the unique property to utilize cellular lipids and to form so called 22-nm particles. HBV group and subtype determinants as well as major -cell epitopes of the HBsAg are located on the surface of these particles. We have constructed a vehicle in which the sequences for the major HBsAg epitopes can be exchanged in a casette-like fashion. The hybrid proteins are expressed via Vaccinia virus and the 22-nm like particles are recovered from the supernatant of infected cells. Using 30 to 40 amino acid long sequences containing - and T-cell epitopes of p190 the precursor of the major surface proteins of P. falciparum merozoites we have redesigned the surface of such particles. The physical and immunological properties of these particles will be discussed.

F 417 EXPRESSION AND IMMUNOGENICITY OF THE HUMAN RESPIRATORY SYNCYTIAL VIRUS F GLYCOPROTEIN USING A BACULOVIRUS VECTOR SYSTEM,

Michael W. Wathen, Roger J. Brideau, and Darrell R. Thomsen, The Upjohn Company, Kalamazoo, MI 49001.

Respiratory syncytial virus (RSV) is a major cause of severe lower respiratory tract disease and hospitalization in children under one year of age. The virus codes for two glycoproteins (F and G) which have been shown to be the major targets for the host antibody cell response. We have expressed the F glycoprotein in insect cells using a baculovirus expression vector system to examine its potential as a subunit vaccine. Two different forms of the F glycoprotein have been expressed: 1) The intact F glycoprotein (F), and 2) Truncated F (Ft) in which the carboxy-terminal anchor region was deleted. The F glycoprotein was retained on infected cell membranes and reached peak levels of expression by 42 h p.i. The Ft glycoprotein was secreted into the media of infected cells and reached peak levels of expression at 56 h p.i. Pulse-chase experiments demonstrated post-translational processing and cleavage of the F_0 precursor into its F_1 and F_2 subunits. A cotton rat animal model showed that both F and Ft were able to induce neutralizing antibodies and protect vaccinated animals from RSV challenge.

F 418 PROTECTIVE EFFECTS OF A SYNTHETIC PEPTIDE FROM THE MURINE MALARIAL CIRCUMSPOROZOITE PROTEIN,
Walter R. Weiss, W. Lee Maloy, Altaf A. Lal, Michael R. Hollingdale, Jay A. Berzofsky, Michael F. Good, and Louis H. Miller, Naval Medical Research Institute, Bethesda MD, Biomedical Research Institute, Rockville MD, National Institutes of Health, Bethesda MD
Studies mapping T cell epitopes of the P. falciparum circumsporozoite (CS) protein led us to synthesize a 21 aminoacid peptide from the corrosponding region of the CS protein of P. yoelii, a murine pathogen. Studies with congenic mice show that this peptide causes T cell proliferation and priming for antibody production in an H-2 restricted fashion. Immunization with the peptide alone does not protect mice. However, priming with the peptide increases the protection given by a single immunization of irradiated sporozoites. This peptide may provide a useful T helper epitope in a synthetic peptide vaccine.

Anti-idiotype Vaccines; Immunological Constraints; Immunological Enhancement with Adjuvants and Biologicals

F 500 INDUCTION OF HIV ANTIBODIES BY Ty: HIV HYBRID VIRUS-LIKE PARTICLES
Sally E Adams, John M Senior, Susan M Kingsman and Alan J Kingsman, Department of Molecular Virology, British Bio-technology Limited, Brook House, Watlington Road, Cowley, Oxford, England.
The yeast retrotransposon Ty moves via an RNA intermediate that is packaged into 60 nm virus-like-particles (Ty-VLP) containing Ty-encoded structural and enzymatic proteins. The major structural proteins are encoded by protein p1 that is encoded by the TYA gene. Overexpression of p1 alone is sufficient to produce high yields of particles and p1 fusion proteins encoded by TYA fusion genes, constructed in vitro produce hybrid Ty-VLPs. We have made a range of TYA:HIV env and gag fusion genes. When these are expressed under the control of an efficient expression system large numbers of hybrid HIV: Ty-VLPs are produced. These are easily purified, react with anti-HIV antibody and elicit the production of anti-HIV antisera in rabbits. The data suggests that presenting HIV antigens in a particulate, polyvalent form may be useful in the development of an AIDS vaccine.

F 501 USE OF A VACCINIA CONSTRUCT EXPRESSING THE CIRCUMSPOROZOITE PROTEIN IN THE ANALYSIS OF PROTECTIVE IMMUNITY TO PLASMODIUM YOELII,
R.L. Beaudoin*, M. Sedegah*, P. De la Vega*, M. Leef*, M.A. Ozcel*, E. Jones**, Y. Charoenvit*, L. Yuan*, M. Gross**, W.R. Marjarian**, W. Weiss*, and S.L. Hoffman*. *Naval Medical Research Institute, Bethesda MD, and **Smith Kline and French Laboratories, King of Prussia, PA.
Mice immunized with irradiated sporozoites produce a strong antibody response against circumsporozoite (CS) protein, however, protection to high dose challenge has been shown to be dependent on CD8+ T cells. Balb/C mice were immunized IP with one to 4 doses of a vaccinia recombinant construct expressing the CS protein of P. yoelii in an attempt to stimulate a protective cellular immune response. The mice were challenged 2 weeks later with 200 or 10,000 sporozoites, and unlike mice vaccinated with irradiated sporozoites which were protected, all of the animals immunized with the recombinant construct became infected on challenge, even though the anti-sporozoite IFA titers of both groups of vaccinated mice were comparable. To determine if antibodies elicited by immunization with irradiated sporozoites protected mice against the low dose challenge, we depleted irradiated sporozoite immunized mice of CD8+ T cells, and found that they were no longer protected against challenge with even 200 sporozoites. It is unclear whether failure to protect mice with vaccinia CS construct results from failure to generate CD8+ immune cells, faulty antigen presentation or the use of the inappropriate antigen. It is clear, however, that antibodies induced by the irradiated sporozoite vaccine are not responsible for the protection observed, irrespective of the size of sporozoite challenge.

F 502 T-CELL HYBRIDS FOR THE DELINEATION OF T-CELL EPITOPES ON PROTEINS OF INFECTIOUS BRONCHITIS VIRUS (IBV).
Mieke Boots, David van Gennep, Hans Kusters, Ben van der Zeijst and Evert Hensen. University of Utrecht, The Netherlands.
IBV, a corona virus, is the causative agent of an acute respiratory disease in chickens. The virus posesses characteristic surface structures, spikes or peplomers, which protrude from the envelope of the virus. The peplomer protein, which consists of some copies of structurally unrelated subunits (S1 and S2), plays an important role in inducing circulating virus neutralizing antibodies in infected birds. S1 and S2 are glycoproteins with a relative molecular mass of 90 and 84 kd respectively.
The aim of our study is to localize T-cell epitopes on the peplomeric protein of IBV and study the role of such T-cell epitopes in the induction of protective immunity. Rats were immunized intraperitoneally with a mixture of FCA and 250 ug of an IBV preparation (strain M41) isolated from infected embryonated chicken eggs. Five weeks later spleen cells showed a more than ten-fold increase in T-cell proliferation after stimulation with the antigen as assayed in the lymphocyte stimulation test (LST). Sera of the immunized rats contained antibodies against IBV strain M41 as detected by western blotting. The immune cells were fused five days after in vitro antigenic stimulation, with a T-cell lymphoma line (W/Fu(C58 NT)D (A.Silva,1983) and hybrids were selected on HAT medium. Virus preparation specific hybrids were selected by measuring monoclonal IL-2 production after stimulation with M41 virus antigen. Twelve out of 36 hybrids showed an antigen specific IL-2 response.
The peplomer gene of IBV strain M41 was cut in 16 overlapping DNA-fragments and subsequently cloned in the bacterial expression plasmid pEX (K.K. Stanley,1984). These 16 different fusion proteins are used to investigate the antigen-specific IL-2 response of the twelve T-cell hybrids.

F 503 VIRAL DELIVERY SYSTEMS FOR HETEROLOGOUS ANTIGENS AND EPITOPES,
Danny Huylebroeck, Geert Maertens[1], Peter Liljeström[2], Henk Stunnenberg, Stephen Fuller and Henrik Garoff[2], EMBL, Heidelberg, Fed. Rep. Germany, [1] Lab. Molecular Biology, State University of Ghent, Belgium, [2] Karolinska Institute, Center for Biotechnology, Huddinge, Sweden.
We are using four approaches to obtain viral delivery systems for expressed epitopes and antigens. The first one exploits the ability of VSV to incorporate foreign viral glycoproteins. Stably and transiently expressed influenza virus H3-HA are used and VSV[HA] virus particles identified by cryo EM and infection of polarized MDCK cells through their apical plasma membrane. The second approach aims at producing a defective virus preparation of an enveloped RNA virus, suitable to be used as a vaccine against different Togaviruses. The basic strategy is to package a replication-incompetent genome into virus particles in a helper cell line that produces the missing gene products needed for viral replication. We are, therefore, constructing an infectious cDNA clone of SFV. We will use this for the generation of chimeric particles containing epitopes from other Togaviridae and Flaviviridae. The third approach exploits the possibility to obtain high-level secretion of HBV 22 nm particles in stably transformed and amplified CHO [dhfr$^+$]-cells. In-frame insertion of identified short viral epitopes in particular regions of the HBsAg might result in export of 22 nm-like particles from the cells. We are also trying to coexpress the hybrid HBsAg with the wt HBsAg. Finally, we are using transient expression systems, including Vaccinia, to map major or minor epitopes on flu HA. These are valuable tools for studying and possibly changing the antibody repertoire (anti-HA) in appropriate animal model systems.

F 504 VACCINATION OF ANIMALS WITH VACCINIA RECOMBINANTS EXPRESSING PSEUDORABIES GLYCOPROTEINS.
Elaine V. Jones, A. Paul Reed, Sharon R. Klepfer, Thomas Kaufman*, and Timothy J. Miller. Department of Molecular Genetics, Smith Kline Beckman Animal Health Products, King of Prussia, PA 19406 and Norden Laboratories, Lincoln, NE 68521.

Pseudorabies (PsR) is a herpesvirus which causes a highly infectious disease in livestock. Swine are the primary host for this virus and piglets under 5 weeks of age are most susceptible. Adult pigs often become latently infected following exposure to PsR, only to have the virus reactivated later in time. The disease is currently controlled by vaccination with inactivated or attenuated strains of PsR virus. A subunit vaccine may provide a safe and effective means of immunizing pigs against PsR while eliminating the potential of latent virus infection. Several PsR glycoproteins have been shown to be protective in mice - gII, gIII, and gp50. Interestingly, the gp50 gene lies immediately upstream of a second glycoprotein of 63 kD and is transcribed from a mRNA species that encodes both glycoproteins. We have expressed this region encoding the gp50 and gp63 genes in vaccinia. The gp50 gene is synthesized as detected by immunoprecipitation with monoclonal antibody specific to gp50. Synthesis of a 63 kD glycoprotein can also be detected in recombinant infected cells reacted with antiserum from immunized animals. Recombinants expressing gp50+gp63 were used to immunize mice by several routes (intracerebral, intraperitoneal, scarification). The mice showed varied levels of protection from lethal PsR challenge depending on the route of inoculation. These results and other host animal studies will be presented.

F 505 DETERMINATION OF MURINE T-CELL EPITOPES ON THE S1 SUBUNIT OF PERTUSSIS TOXIN (PT), K. Jin Kim and Charles R. Manclark, Laboratory of Pertussis, Division of Bacterial Products, Center for Drugs and Biologics, FDA, Bethesda, MD 20892.
Pertussis Toxin is a candidate for inclusion in acellular pertussis vaccines, thus it is important to identify epitopes on PT recognized by antibodies and T-cells. We have studied T-cell epitopes on the enzymatic S1 subunit of PT by the proliferative response of BALB/c lymph node cells to the antigen. The proliferative response was determined by measuring the level of ^{3}H-thymidine incorporated into the cells. Nylon wool column purified T-cells did not respond to S1, but MHC matched irradiated spleen cells restored the response. T-cells recognized heat-denatured S1 as well as native S1. T-cell epitopes were also examined by generating T-cell lines. FACS analysis showed that these T-cell lines belong to the T-helper cell lineage (Thy 1^+, L3T4$^+$, LyT2$^-$, sIg$^-$ and Mac.1$^-$). By using synthetic peptides we have mapped a T-cell epitope (amino acid #65-79) recognized by polyclonal T-cells and are in the process of defining the minimum amino acid sequence coding for this T-cell epitope. Further we are studying whether this particular T-cell epitope is recognized by mice of different H-2 types.

F 506 VACCINES BASED ON FUSIONS IN E COLI FIMBRIAE PROTEINS. Lisbeth Hedegaard, Per Amstrup Pedersen and Per Klemm. Department of Microbiology, Technical Unversity of Denmark, Copenhagen, DK-2800 Lyngby, Denmark. Fimbriae are surface protein polymers found on the surface of many E. coli strains. Our aim has been to investigate the possible use of fimbria protein as a carrier for transport of "foreign" antigenic determinants to the surface of E. coli in the form of protein fusions. Comparison of subunit protein amino acid sequences of related fimbriae indicate hypervariable regions. We assumed that such regions may be suitable for insertions of "foreign" antigenic determinants leading to extracellular expression of fimbriae. We have chosen the K88 and Type 1 fimbria systems of E. coli. Several different fusions with the fimbria subunit genes and foreign DNA fragments have been made. Presently we are investigating these hybrid proteins and some of the constructions have shown to be expressed as extracellular fimbriae.

F 507 ANTIGENIC SPECIFICITY OF HIV-SPECIFIC ADCC RESPONSES IN HIV SEROPOSITIVE SERA, Richard A. Koup, John L. Sullivan, Peter H. Levine, Frank Brewster, Anna Mahr, Gail Mazzara, Sara McKenzie and Dennis Panicali. University of Massachusetts Medical School and Applied Biotechnology Inc. Cambridge, MA USA.
Human immunodeficiency virus (HIV) specific antibody dependent cellular cytotoxicity (ADCC) has been described in HIV-infected individuals. In order to determine the antigenic specificity of this immune response and to define its relationship to disease state, an ADCC assay was developed utilizing Epstein-Barr virus transformed lymphoblastoid cell line (LCL) targets infected with vaccinia virus vectors expressing HIV proteins. The vaccinia virus vectors induced appropriate HIV proteins (envelope glycoprotein gp-160 or core protein p-55) on the surface of infected LCL's demonstrated by indirect immunofluorescence staining, and syncitia formation with c8166 cells. Utilizing sera from HIV seropositive and seronegative hemophilia patients, killer cell-mediated, HIV-specific ADCC was found to be present in sera from HIV seropositive but not HIV seronegative hemophiliacs. This HIV-specific response was directed against gp-160 but completely absent against target cells expressing the p-55 HIV core protein. The gp-160 directed ADCC was present at serum dilutions up to 1/100,000. There was no correlation between serum ADCC titer and the stage of HIV related illness. Individuals showing HIV induced CD4 depletion (CD4<300) demonstrated high titers of ADCC antibodies. These studies clearly implicate gp-160 as the target antigen of HIV specific ADCC activity following natural infection. It is possible that ADCC is an important mechanism responsible for the CD4 depletion seen in HIV infected individuals.

F 508 VACCINE DEVELOPMENT AGAINST SIMIAN IMMUNODEFICIENCY VIRUS AS A MODEL FOR THE HUMAN IMMUNODEFICIENCY VIRUS. LJ Lafrado, G Hunsmann, JC Kelliher, and WC Hobson. Primate Research Institute, Holloman AFB,NM 88330.

Several nonhuman primate retroviruses, related to the human immunodeficiency virus (HIV) have been isolated and characterized. The similarities in these viruses and associated diseases suggests a strong arguement for utilizing the simian immunodeficiency virus (SIV) as model for HIV vaccine development. Employing SIV-infected MOLT-4 cell lines (M Hayami) as a source of viral proteins and whole virus extracts the preliminary immune response of vaccinated rhesus macaques has been studied. Macaques were vaccinated with aggregates of retroviral envelope glycoproteins (gp130) or Tween-ether extract of whole virus through intravenous and intradermal inoculation. Preliminary analysis of antibody titers after one and two vaccination dates have demonstrated the effectiveness of this protocol. Antigenicity of both the whole virus extract and gp130 aggregates induced presumptive protective antibody titers (virus extract:>1:320; gp130:>1:80;). Immunogenicity of the potential vaccines was enhanced by tha covalent coupling of viral antigens to KLH as adjuvant.

F 509 A COMPONENT OF THE IMMUNODOMINANT ANTIGENIC SITE OF HEPATITIS A VIRUS RESIDES ON CAPSID PROTEIN VP3. S.M. Lemon, L-H Ping, R.W. Jansen, J.T. Stapleton, & J. Cohen, University of North Carolina, Chapel Hill, NC, and NIAID, Bethesda, MD, U.S.A.

Hepatitis A virus (HAV), a pathogenic human picornavirus responsible for considerable morbidity worldwide, is distantly related to poliovirus and rhinovirus. Unlike the latter viruses which have multiple antigenic types, HAV demonstrates strict antigenic conservation. The antigenic structure of the virus is relatively simple, as competition studies carried out on solid-phase supports suggest that most neutralizing murine monoclonal antibodies to HAV recognize a small number of closely spaced and mutually overlapping epitopes. A combination of only two monoclonal antibodies completely blocks the attachment of polyclonal human antibody to the virion, suggesting that this array of epitopes is immunodominant in man. *Asp*-70 of capsid protein VP3 was replaced with *his* in three clonally isolated HM-175 strain mutants selected for resistance to neutralization mediated by monoclonal antibody K2-4F2, but not in virus which had reverted to neutralization susceptibility. Virus with this mutation was resistant to neutralization by each of ten monoclonal antibodies tested, suggesting that *asp*-70 of VP3 is involved in the immunodominant antigenic site. This site may be a useful target for peptide vaccine development.

F 510 EXPRESSION OF HUMAN LDH-C_4 FOR DEVELOPMENT OF AN IMMUNOCONTRACEPTIVE VACCINE
Kay M. LeVan and Erwin Goldberg, Northwestern University, Evanston, IL 60208.
The sperm-specific antigen LDH-C_4 has been used to elicit an immune response capable of suppressing fertility by 70 percent in female non-human primates. LDH-C_4 is an isozyme of lactate dehydrogenase unique to males and absent in females. The mouse LDH-C_4 has so far served as a model for development of an immunocontraceptive vaccine. The antigenic determinants of mouse LDH-C_4 have been mapped, and correlated with the three dimensional structure derived from X-ray crystallography. Antigenicity studies with the human protein would be preferable, but because LDH-C_4 is testis-specific, large quantities of the human enzyme are not readily available. Recombinant DNA technologies have been exploited to create a source of the human LDH-C_4. Antibodies to mouse LDH-C_4 were used to screen a λgt11 human testis cDNA expression library. A human *Ldh-c* clone has been identified and sequenced. The 331 amino acid C subunit is encoded by a 999 bp open reading frame. For cloning into expression vectors, the 5' and 3' untranslated sequences of *Ldh-c* have been deleted, followed by addition of linkers adjacent to the translational start and stop codons. Two modified *Ldh-c* genes have been engineered to maximize expression in *Escherichia coli* and baculovirus systems. Purified LDH-C_4, antigenically similar to native LDH-C_4, will be used for immunization. Furthermore, the *Ldh-c* gene has been engineered for expression in a vaccinia viral vector. The recombinant viral system can be expected to stimulate stronger cellular and humoral immune responses more effective in fertility suppression.
Supported by NIH Grant HD 05863.

F 511 VACCINIA VIRUS RECOMBINANTS EXPRESSING EPSTEIN-BARR VIRUS GENES,

M. Mackett and J.R. Arrand, Paterson Institute for Cancer RESEARCH, Christie Hospital and Holt Radium Institute, Manchester M20 9BX, England.

A knowledge of the sequence of Epstein-Barr virus (EBV) DNA together with standard recombinant DNA technology has allowed us to isolate the genes for five different EBV glycoproteins. We have attempted to express the products of the open reading frames BLLF1 (gp340/220) BALF4 and BXRF1 (Herpes simplex virus homologues gB and gH respectively) BILF2 and BDLF3 using vaccinia virus recombinants. Several recombinant viruses containing BALF4 DNA did not give detectable EBV specified protein whereas the other recombinants expressed the appropriate EBV gene products. A gp340 vaccinia recombinant has been shown to produce EBV neutralizing antibodies in vaccinated animals and to protect cotton top tamarins against disease when challenged with EBV (Morgan et al, submitted). In order to investigate the possibility of improving protection against EBV we have expressed gp340 to higher levels and used these other recombinants to immunize animals and to detect antibody production during natural EBV infection.

F 512 T-CELL RESPONSE TO HIV AND HIV-DERIVED PEPTIDES IS SEEN IN NON-INFECTED PARTNERS BUT NOT IN INFECTED HUMAN BEINGS,

S. Mattinen[1], A. Ranki[2,3], and K. Krohn[1,3], [1]Institute of Biomedical Sciences, University of Tampere, Tampere, Finland; [2]Department of Dermatology, University of Helsinki, Helsinki, Finland and [3]Laboratory of Tumor Cell Biology, NCI, NIH, Bethesda, MD.

HIV infection in man is characterized by a progressive course, despite of the presence of group-spesific neutralizing antibodies. We have studied T-cell proliferative responses towards HIV in 58 HIV-infected individuals and in 13 non-infected subjects with verified sexual exposure to HIV. T-cell response was assessed by ^{3}H-thymidine incorporation after stimulation of peripheral blood mononuclear cells with inactivated HIV virions, purified HIV envelope glycoprotein gp120, recombinant envelope proteins gp160 and PB1 (aa. 295-474) and recombinant p24 protein. None of the HIV-infected persons responded to gp120 or to HIV; the occasional response to PB1 or p24 corresponded to a proliferative response to E. Coli-extract. In contrast 4/13 sexual partners to HIV infected persons, who themselves were unequivocally negative with all known tests for HIV infection, responded. No response towards synthetic peptide representing aa. 426-450 of gp120, shown to act as a helper T-cell epitope in gp120 immunized chimpanzees or HIV infected gibbon apes, were seen. The epitopes recognized by human beings may be different from those recognized by immunized animals.

F 513 HUMAN ADENOVIRUS-5 VECTORS: EXPRESSION OF VSV GLYCOPROTEIN IN VITRO AND IN VIVO,

M. Schneider, Graham, F.L., L. Prevec (McMaster University, Hamilton, Ontario, Canada, L8S 4K1) & B. Derbyshire (Guelph, Ontario).

The structural gene for the glycoprotein of the Indiana serotype of vesicular stomatitis virus (VSV) (obtained from J. Rose) was inserted between the promoter and poly A addition signal of the thymidine kinase gene of herpes simplex type 1. This cassette was then inserted in either orientation into the Xba 1 site of an E3-deleted human adenovirus type 5 vector. The adenovirus vectors were capable of replicative growth in human (HeLa) and bovine (MDBK) cells in culture. The VSV glycoprotein, as detected by immunoprecipitation, is expressed only by the vector having the insert in the E3-parallel orientation. The VSV glycoprotein is also expressed by this vector in murine (L) and canine (MDCK) cells in culture even though the vector replicates poorly or not at all in these cell lines. The adenovirus vector, purified by twice banding on CsCl, induced good levels of neutralizing antibody to VSV in mice, calves, pigs and dogs whether administered by injection or intranasally.

F 514 PASSIVE IMMUNIZATION AGAINST HIV: STUDIES IN THE CHIMPANZEE MODEL. **Alfred M. Prince, Jorg Eichberg, Betsy Brotman, Jay Valinsky, Richard Shulman and Bernard Horowitz.**

We have prepared an intravenous hyperimmune globulin (HIVIG) from plasma obtained from donors with the top 10% of HIV neutralizing antibody titers. The globulin is sterilized by a two-step solvent/detergent process in addition to Cohn fractionation. HIVIG has a neutralizing antibody titer of about 1:2,000, an anti-HIV Elisa titer (Dupont) of 1:256,000-1:512,000 and detects all major HIV proteins on Western blots. A safety test in 4 chimpanzees has indicated that the globulin is non-infectious, and without hematological side effects at a dose of 10cc/kilo. The in vivo half-life is 18 days.

A dose of 1 cc/kilo given 24 hours before an I.V. injection of 25 CID_{50} HIV/HTLVIII, did not protect two chimpanzees from developing infection. A second efficacy trial was therefore initiated on September 29, 1987 with a dose of 10 cc HIVIG/kilo. In this trial 1 ml/kilo of is given also at 1, 2 and 3 months after virus challenge. The results of this trial will be reported.

These studies will have implications for the ability of humoral immunity alone to protect against HIV infection. Clinical trials will be required to assess efficacy in perinatal transmission and possibly other settings.

F 515 EPITOPES RECOGNIZED BY HUMAN T LYMPHOCYTES ON MALARIA CIRCUMSPOROZOITE PROTEIN. Francesco Sinigaglia, Maria Guttinger, Dieter Gillessen and Richard Pink, Central Research Units, F. Hoffmann-La Roche & Co. Ltd., 4002 Basel , Switzerland.

The circumsporozoite protein of the malaria parasite *Plasmodium falciparum* contains regions of non-repetitive sequences which are predicted to be T-cell recognition sites. We synthesized peptides corresponding to three of these regions, and tested their ability to stimulate proliferation of peripheral blood lymphocytes from donors living in a malaria-endemic area, or from non-immune donors. Cells from 15 out of 22 donors (including 4 of 6 non-immune individuals) were stimulated by one or more of the peptides. T-cell clones specific for one of the peptides were obtained and shown to recognize the native protein purified from sporozoites. These data help to identify T-cell epitopes which could be incorporated into a malaria vaccine.

F 516 GENERATION OF A HUMAN MONOCLONAL ANTIBODY WITH SPECIFICITY FOR THE TRANSMEMBRANE GLYCOPROTEIN (GP41) OF HIV, Vera J.P.Teeuwsen, Kees H.J. Siebelink, Marij J.Stukart, Fons G.C.M.UytdeHaag and Ab D.M.E.Osterhaus, National Institute of Public Health and Environmental Hygiene, Bilthoven, The Netherlands.

Monoclonal antibodies directed against distinct proteins of human immunodeficiency virus (HIV) are of importance for the development of a HIV vaccine. They can be used for purification of viral proteins, analysis of immunodominant epitopes and for the generation of anti-idiotype antibodies. In order to obtain a human monoclonal antibody with specificity for HIV, B cells from asymptomatic HIV seropositive male homosexuals were immortalized with Epstein-Barr virus. Cultures with anti-HIV reactivity as determined in ELISA were subcultured at low cell density. By this procedure, a stable cell line K14 was obtained. K14 proved to be clonal by analysis of the heavy chain VDJ rearrangements in a Southern blot. The monoclonal antibody (MoAb) secreted by this cell line is directed against an epitope of the transmembrane glycoprotein (gp41) of HIV, as was determined by immunoaffinity chromatography with K14 MoAb and immunoblot analysis of the purified protein. An inhibition ELISA with HIV seropositive sera showed that this epitope is a common immunodominant one. K14 MoAb has no neutralizing capacities. Additional studies on biological activity (ADCC and ACC), and epitope mapping are in progress.

F 517 A CLONE OF POLIOVIRUS TYPE 3 DERIVED FROM THE SABIN TYPE III VACCINE STRAIN IS NON NEUROVIRULENT AND GENETICALLY STABLE AFTER SERIAL HUMAN PASSAGES, Tito R. Ubertini, Constantino Cioni and Luigi Perini, Sclavo S.p.A., 53100 Siena, Italy. A clone of poliovirus type III (TRU-3) was derived from the Sabin-3 vaccine strain, by the cDNA:RNA hybrid molecular cloning method. The virus recovered was found to be considerably less neurovirulent for primates than its progenitor Sabin 3 vaccine strain. Upon serial passages in humans TRU-3 did not increase its neurovirulence (unlike the Sabin strain) and maintained the base sequence of the attenuated strain in a critical part of the 5' region of the genome (unlike the Sabin strain).