Center for Biologics Evaluation and Research
Division of Bacterial Products

Annual report
1991-1992

DIVISION OF BACTERIAL PRODUCTS

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH
FOOD AND DRUG ADMINISTRATION

ANNUAL REPORT

OCTOBER 1, 1991 to September 30, 1992

Summary by Director

DIVISION OF BACTERIAL PRODUCTS
ANNUAL REPORT, OCTOBER 1, 1991-SEPTEMBER 30, 1992

Summary of Director, Division of Bacterial Products

Organization of the Division. During the 1992 fiscal year, the Division underwent significant organizational changes. A new unit, the Laboratory of Molecular and Developmental Immunology, was established and staffed with existing personnel from the Laboratory of Bacterial Polysaccharides to form a critical mass for review and research on bacterial and related products in the field of immunology. This brought the number of laboratories in the Division to seven.

A national search for Chief, Laboratory of Mycobacteria, was concluded with the appointment of a senior scientist from the Laboratory of Pertussis within the Division. If the present research staff and laboratory space remain with the new Laboratory Chief as expected, the effect will amount to a doubling of resources now devoted to review and research of mycobacteria and related products at a time when tuberculosis is reappearing as a major health problem in the United States.

As the fiscal year ends, additional structural changes in the Division are expected from the reorganization of the Center for Biologics Evaluation and Research. As part of that reorganization, the Laboratory of Allergy and Immunochemistry is scheduled for transfer to the (new) Division of Allergy and Immunology in the (new) Office of Vaccines Research and Review. The Laboratory of Molecular and Developmental Immunology is planned to relocate into the (new) Division of Monoclonal Antibodies in the (new) Office of Therapeutics Research and Review. Thus, the Division of Bacterial Products will be comprised of five Laboratories in addition to the office of the Director. Even after allowing for this reorganization, the Division is undergoing a loss of personnel through attrition of >15% of the Division staff. Finally, the Laboratory of Mycoplasma has been recently designated the Laboratory of Enteric and Sexually Transmitted Diseases to reflect increasing research and regulatory needs in these disciplines; however, the full staffing of this laboratory must be delayed because of the hiring freeze in effect at the close of FY 92.

Program reviews were conducted for the Laboratories of Pertussis, Bacterial Polysaccharides, and Molecular and Developmental Immunology. The reports of these reviews and the reviews of the Laboratories of Mycobacteria and Mycoplasma that were conducted in FY 91 were presented to the Vaccines and Related Biological Products Advisory Committee in the course of FY 92.

Regulatory Activities. FY 92 was another busy year. The Division received 64 license actions for review, including three PLAs for new products, compared with 65 actions in FY 91. The Division forwarded 73 recommendations for approval, including six for new products and establishments.

Significant new products were licensed in FY 92. In December 1991, the first acellular pertussis vaccine (Lederle-Takeda) was approved for use as 4th and 5th doses at 18 months and older. In August 1992, a similar license was issued for a second acellular pertussis vaccine (Connaught-Biken). New bioequivalence standards were developed and published for cat allergenic extracts, requiring recalibration of these extracts by manufacturers.

There were 80 original IND submissions, compared with 71 in FY 91.

Research Activities. The basic and clinical research activities of the Division are detailed in the individual reports of the Laboratories and are only highlighted here.

> Laboratory of Allergy and Immunochemistry: Further studies of B.pertussis adhesin molecules, their structure and analogy to other proteins. Standardization of cat extracts.
>
> Laboratory of Bacterial Polysaccharides: Porin proteins as vaccine candidates and type-specific markers for N.meningitidis. Synthesis and secretion of capsular polysaccharides. Role of meningococcal LOS and pneumococcal pneumolysin in virulence and protection.
>
> Laboratory of Bacterial Toxins: Molecular basis for binding, cellular entry and transport of tetanus and diphtheria toxins. Physical map of pertussis genome. Toxicity of vaccines and cytokines.
>
> Laboratory of Molecular and Developmental Immunology: Control of immune responses to meningococcal polysaccharides. VH gene usage in immunologically different strains of mice. Mitogenic activity of components of Haemophilus conjugate vaccines.
>
> Laboratory of Mycobacteria: Immunodominant epitopes of tuberculin and other mycobacterial proteins. Sequence and role of catalase in virulence and drug resistance.
>
> Laboratory of Mycoplasma: Natural history and immunobiology of M.pneumoniae infection in chimpanzees. Antigenic variability of surface proteins of M.hominis.

Special Awards and Recognition.

A number of PHS and FDA awards recognized the accomplishments of DBP staff in FY 92. The DHHS Distinguished Service Award, the highest civilian award of the Department, was presented to Dr. Charles R. Manclark, Chief of the Laboratory of Pertussis, for his outstanding leadership in basic research and vaccine development leading to the new generation of acellular pertussis vaccines.

Dr. Michael F. Barile, Chief of the Laboratory of Mycoplasma, received the FDA Commendable Service Award, the highest civilian award of the agency, in recognition of a distinguished scientific career devoted to understanding the biology of mycoplasma infections.

The PHS Special Recognition Award was presented to the Laboratory of Pertussis for the critical role of that group in characterizing the battery of acellular pertussis vaccines that were evaluated in phase I and II studies and in participating in the planning of pivotal phase III European trials, all under NIAID sponsorship.

Bascom Anthony

Bascom F. Anthony, M.D.

Laboratory Summaries

Laboratory of Allergy and Immunochemistry Summary

Dr. Elizabeth Leininger, a Scientist in the Laboratory of Allergy and Immunochemistry and a Lieutenant Commander in the Public Health Service Commissioned Corp has been a member of the laboratory since November 1987. She has continued her research project directed towards characterizing the mechanism of adherence and invasion of bacteria to mammalian cells, using as a model the human pathogen *Bordetella pertussis*, the causative agent of whooping cough. We have focused on the role of two surface proteins, filamentous hemagglutinin and pertactin, in mediating attachment to mammalian cells and internalization of the bacteria. In collaboration with the Laboratory of Pertussis, our studies suggest that the arginine-glycine-aspartic acid sequence found in the mature pertactin protein plays a role in the attachment and internalization of *B. pertussis*. We are currently investigating protein-protein interactions among the different *B. pertussis* surface proteins and with mammalian cell matrix proteins. In collaboration with Dr. Stephen Barenkamp at St. Louis Children's Hospital in Missouri, we have shown that two high molecular weight surface exposed proteins from nontypeable *Haemophilus influenzae* are cross-reactive with anti-filamentous hemagglutinin monoclonal antibodies. In order to map the epitope of the anti-filamentous hemagglutinin monoclonal antibodies and to determine functional domains on this protein, we have constructed a series of fusion proteins containing specific fragment of the filamentous hemagglutinin protein and maltose binding protein. These fusion protein have been constructed and expressed in *E. coli* thanks to collaborations with other groups. We have also synthesized the complete sequence of filamentous hemagglutinin in 15 amino acid overlapping peptides. A total of 832 peptides were simultaneously synthesized using the Geysen Pin Technique. We have currently mapped the epitope of 2 anti-filamentous hemagglutinin monoclonal antibodies. We have initiated the characterization of specific allergens in allergenic extracts. Immunoblot assays are being developed to investigate the human IgE response to specific allergenic extracts in allergic patients. We have obtained the amino-terminal protein sequence of two specific apple allergens. '

The Hybridoma Facility of the Laboratory of Allergy and Immunochemistry has again increased its characteristic high rate of productivity during this reporting period. A total of nineteen fusion procedures have been initiated or completed this fiscal year. These fusions required screening by ELISA and/or Western blot of approximately 5700 samples of culture fluid. As a result 330 lines and clones have been selected for further study and subsequently expanded in order to allow for frozen storage. Procedures have been initiated for eight additional fusions scheduled during the next reporting period. The Facility has also conducted related procedures from previously completed fusions resulting in 49 cell lines being infused in mice and over a liter of ascites collected. Work has also been conducted to provide myeloma cells to two other laboratories, to grow cell lines in mass culture to collect over 2700 ml of protein free or regular media containing antibodies, determine immunogenicity of a protein in mice and to rescue cell lines damaged in shipment. The Hybridoma Facility collaborates with numerous laboratories in FDA, and other institutions for the production and characterization of monoclonal antibodies (MAbs). We have active and ongoing collaborations with all five of the laboratories in the DBP, two other CBER divisions as well as eight laboratories outside of FDA (three outside of the USA) (a listing of these various collaborations is given in the description of project. Z01-BA-04003-08 LCP). We have received requests from and have provided MAbs to many outside laboratories who have become aware of our antibodies through our publications and presentations at various scientific meetings. All the above mentioned antibodies are playing a vital role in numerous research projects now under way both within and outside of the DBP

Mr. Probst has conducted research in the development of an ELISA system applicable to standardization of allergenic extracts, and of an unrelated system

capable of detecting product specific allergenic adverse reactions to vaccines in patient sera. With respect to allergenic extract standardization we have demonstrated a high degree of sensitivity and reproducibility with respect to most grass pollens, and have recently demonstrated the system to be applicable to mites. Successful system development could lead to the illimination of more costly and labor intensive methods currently employed. In promising initial studies, on analysis of allergenic adverse reactions detection of plague vaccine specific IgE was indicated in serum from an immunization subject who died within 18 hours of receiving a booster dose of the vaccine. Through Mr. Probst the laboratory continues to supply DBP with synthetic oligonucleotides, which have been incorporated into the research programs of eight investigators in six of the division's laboratories. This year the laboratory supplied 60 oligonucleotides which varied between 10 and 71 bases in size

The Clinical Research Program of the LAI has continued clinical evaluation of reference preparations, reference methods, and samples submitted for standardization.

Pharmacodynamic Studies-Biological Standardization: The clinical evaluation of the potency of standardized cat extracts has been completed. Assignment of Bioequivalent Allergy Units based on clinical testing as a potency measure rather than the arbitrary Allergy Units previously assigned to standardized cat extracts have been implemented with the approval of the FDA Allergenics Advisory Committee. The assignment of Bioequivalent Allergy Units will more accurately reflect the clinical activity of these products.

The clinical evaluation of the compositional differences of standardized cat extracts submitted for licensure has been completed. Clinical testing confirmed laboratory studies indicating that the products submitted could be classified as either primarily containing Fel D I identified as Standardized Cat Hair Extract or as containing Fel d I plus non-Fel d I allergens identified as Standardized Cat Pelt Extract. The two compositionally different cat extracts were designated as CBER cat references. Clinical studies confirmed the applicability of using IEF to detect these compositional differences. The identification of these clinically important compositional differences among cat extracts has resulted in new labeling requirements to more accurately identify these products. This will improve their safe and effective use.

Clinical evaluation of the potency of proposed CBER references of pollen extracts is continuing. Clinical evaluation of Bermuda grass and giant ragweed indicated a decrease in the number of arbitrary potency units previously assigned to these products to Bioequivalent Allergy Units is required to more accurately reflect the potency of these references.

Clinical evaluation of the potency of standardized short ragweed extracts is completed. The data confirm that the number of Allergy Units arbitrarily assigned to selected Licensed products based on their Amb a I content will not require change when they are labeled as Bioequivalent Allergy Units. These data more precisely define the Amb a I content required for assignment of Bioequivalent Allergy Units.

Clinical studies of the stability of short ragweed extract is continuing. Highly dilute solutions of short ragweed (0.0016 Amb a I content) when stored in FDA approved HSA diluent at 2-8^{0}C extract were found to be stable for 7 weeks.

Evaluation of the clinical relevance of the in vitro methods used by LAI for estimating potency of allergenic products is continuing. Relative potency estimated by major allergen content (Fel d I and Amb a I) and Rast Inhibiton were found to be highly predictive of the relative potency estimated by parallel line skin test confirming the clinical predictive value of CBER potency assays.

The application of biological standardization methods requires proficiency methods to demonstrate the method is being performed with acceptable accuracy, precision, and sensitivity. This has applicability to clinical practice where skin testing is used for diagnosis. A simplified method, applicable to clinical

practice, for evaluating the proficiency of an individual performing intradermal skin testing was developed. This approach has applicability to evaluation of the proficiency of an individual performing puncture testing as well as to evaluating the analytical precision of the puncture test device itself.

Epidemiologic Studies: The prevalence of allergic and nonallergic respiratory conditions, the prevalence of immediate cutaneous hypersensitivity responses to FDA licensed allergenic extracts, and the association of allergen skin reactivity with respiratory disease in the US population based on the second National health and Nutrition Examination Survey have been published. These data demonstrate a significant association between skin reactivity to allergens and allergic rhinitis and asthma. Allergen skin reactivity was not predictive of sinusitis, chronic rhinitis, chronic bronchitis, or chronic cough in the US population. The diagnostic utility of specific allergens differed depending whether asthma occurred in combination with allergic rhinitis or singly.

The prognostic influence of the age of asthma onset on the future course of asthma was determined in a cross-sectional sample of the US population aged 6-24 years using the second National Health and Nutrition Examination Survey. In 6-14 year olds, late onset asthmatics (after 2-4 years of age) were more likely to be allergic based on a higher frequency of positive allergy skin tests and more likely to report problems with active asthma and wheezing in the past 12 months compared to early onset asthmatics. This difference was not detectable in 15-24 year olds, suggesting recall of asthma onset in older asthmatics may be inaccurate or nonallergic factors more important in asthma etiology in older asthmatics.

Pharmacoepidemiology: A survey of the membership of the American Academy of Allergy and Immunology for reports of fatalities following exposure to allergenic extracts elicited 10 reports of fatalities for the years 1990-1991. No fatalities were associated with skin testing. Fatalities most often occurred in asthmatics. The age range of fatalities was 12 to 73 years. Most fatalities were of high or moderate allergen sensitivity. Doses associated with fatal overdose ranged over 10,000-fold. When reported, time of onset was less than 20 minutes. Additional precautions in the dosing of allergenic extracts to asthmatics need to be exercised.

REGULATORY ACTIVITIES:

I. A total of 131 INDs have been reviewed by 3 members of the laboratory.
  E. Leininger: 75 INDs involving monoclonal antibodies as therapeutic agents.
  P. Turkeltaub and J. Kenimer: 56 INDs involving allergenic products

II. There are currently a total of 116 active PLA/ELA files being reviewed by the Laboratory. Six members of the laboratory are involved in this review and are chairpersons of the various review committees:
  A. Karpas: Chair of 12 review committees
  P. Probst: Chair of 10 review committees
  J. Kenimer: Chair of 33 review committees
  C. Anderson: Chair of 15 review committees
  P. Turkeltaub: Chair of 32 review committees
  K. Boyle: Chair of 4 review committees

III. Product Labeling Review

  P. Turkeltaub: 121 label submissions reviewed

III. A total of 12 licensed establishments will be inspected by members of the laboratory.
  J. Kenimer: 5 establishments
  E. Leininger: 3 establishments
  A. Karpas: 1 establishment

P. Probst: 2 establishments
M.C. Anderson: 3 establishments
J. Mathews: 2 establishments
P. Turkeltaub: 4 establishments

SCIENTIFIC MEETINGS:

Pathogenesis Conference, Oakland, CA. October 18-20, 1991. (Dr. Elizabeth Leininger)

Conference on Emerging Microbes and Microbial Diseases, Washington, D.C. November 13-15, 1991. (Dr. Elizabeth Leininger)

Meeting on Generating Designer Ligands for Biological Targets, Rockville, MD. March 1-2, 1992. (Dr. Elizabeth Leininger and Dr. Li-Shan Hsieh)

48th Annual Meeting American Academy of Allergy and Immunology, Orlando, FLA., March 6-10, 1992 (Kenimer, Turkeltaub, Mathews, Boyle, Anderson)

New Approaches for the Treatment of Allergic Diseases and Asthma, IBC USA Conference, Phila., PA, April 27-28, 1992 (Turkeltaub)

1992 American Society for Microbiology General Meeting, New Orleans, LA. May 26-30, 1992. (Dr. Elizabeth Leininger)

World Congress of Tissue Culture June 20-25, 1992, Washington D.C. (Dr. Karpas)

Therapeutics Update-1992, American College of Physicians, Ontario, Canada, July 10-11, 1992 (Turkeltaub)

TRAINING:

Interpersonal Skills Workshop on Communication, Baltimore, MD. February 25-26, 1992. (Dr. Elizabeth Leininger)

Sexual Harrassment Awareness Course, Bethesda, MD. (Dr. Leininger, Turkeltaub)

NIH Radiation Safety Refresher Course (All as needed)

Scientific Computing, Biomedical Research Macintosh Users Group, 11/22/91 (Dr. Karpas)

35th Annual Education Conference (Food and Drug Law Institute), 12/10-11/91 (Dr. Karpas, Mr. Probst, Dr. Leininger, Turkeltaub)

CBER Inspector Training, 12/16/91 (Dr. Karpas)

Medline for the Health Professional, NIH Library, 12/5/91 (Turkeltaub)

Computer Training Course entitled *All-In-One*, 1217/91 (Dr. Karpas)

CBER Workshop on Pre Clinical Testing of Monoclonal Antibodies, 1/9-10/92 (Dr. Karpas)

Workshop on Clinical trial monitoring and interim analysis in the pharmaceutical industry, PMA-FDA, Feb. 24-25, 1992 (Turkeltaub)

Radiation Safety Refresher Course, 3/9/92 (Dr. Karpas, Ms. Febus)

CBER Seminar on Potency Testing, 5/1/92 (Dr. Karpas)

Pharmaceutical Update-92, Food and Drug Law Institute, May 7-8, 1992 (Turkeltaub)

FDA film on computer Awareness, 5/12/92 (ALL staff members)

Clinical Trials: Issues in the design, conduct, and analysis. CBER and the Johns Hopkins Center for Clinical Trials, 6/1-6/2/92 (Turkeltaub)

Biologics Update '92, The Food and Drug Law Institute. Bethesda, MD. July 13-14, 1992. (Dr. Leininger, Dr. Karpas, Dr. Turkeltaub, Mr. Probst and Ms. Delasko)

CBER film on Inspector Training, 7/15 and 7/17/92 (Dr. Karpas)

CBER Seminar on the IND Process, 7/17/92 (Dr. Karpas)

INVITED SPEAKER:

August 24, 1992. Invited speaker: "Women's Equality Day: Breaking Through the Glass Ceiling", EEO, CBER, Lipsett Auditorium, Bethesda, MD. (Dr. Elizabeth Leininger)

Proper use of standardized and non-standardized extracts, now and in the near future, Fellows-in-Training Allergen Immunotherapy Course, Annual Meeting American Academy of Allergy and Imunology, Orlando, FLA, March 6, 1992 (Turkeltaub)

Allergen Standardization, Clinical Conference, Johns Hopkins Asthma and Allergy Center, Baltimore, MD, April 24, 1992 (Turkeltaub).

OTHER ACTIVITIES:

Hispanic Employees Representative, Division of Equal Employment Opportunities (EEO), CBER. (Dr. Elizabeth Leininger)

EEO Recruitment trip; Hispanic Enterprise Annual Federal Government Job Fair, San Juan, P. R. November 20-23, 1991. (Dr. Elizabeth Leininger)

EEO Recruitment trip to interview candidates from the Hispanic Enterprise Annual Federal Government Job Fair for possible hires. San Juan, P.R. April 2-4, 1992. (Dr. Elizabeth Leininger)

FDA Ad Hoc Review Group for Scientific Conference Grant Applications. (Dr. Elizabeth Leininger)

HONORS:

April 1992. *PHS Unit Commendation* from The Department of Health and Human Services, Public Health Service Commissioned Corp, Bethesda, MD. (Dr. Leininger)

FDA Commissioner's Special Citation to members of the Integrase Monoclonal Antibody Production Group for outstanding group effort., 5/29/92 (Dr. Karpas).

Laboratory of Molecular and Developmental Immunology Summary

Studies of the regulation of the immune response to bacterial polysaccharides involved a number of different approaches including regulation of antibody isotype and diversity, immunoglobulin heavy chain variable regions (V_H) gene expression in normal and polysaccharide unresponsive mice. Studies were continued on the immune response to the capsular polysaccharide of *Neisseria meningitidis* group C (MCPS) and the thymus dependent form of the antigen, MCPS coupled to tetanus toxoid (MCPS-TT). We have generated two panels of monoclonal antibodies (mAb) from mice immunized with MCPS-TT and boosted with MCPS-TT (C2 mAb) or fixed bacteria (CP mAb). The anti-MCPS mAb are primarily of the IgG3 (53%) and IgM (27%) isotypes, whereas the anti-C2 and anti-CP mAb are mainly IgG1 (87% and 88% respectively). The data indicate a change in fine specificity as assessed on native MCPS and a naturally occurring non-O-acetylated form, OAc-. As reported earlier, the anti-MCPS mAb are predominantly of two specificities, MCPS specific (47%) or OAc- >> MCPS (20%). The anti-C2 mAb are more diverse, none are MCPS only specific, 27% are MCPS ≈ OAc-, 20% are MCPS >OAc- and 20% are OAC- >> MCPS. The anti-CP specificities were more similar to the anti-C2 mAbs suggesting that the secondary antibody repertoire is determined by the primary immunization. Moreover, the data also suggest the anti-C2 and anti-CP mAbs are of higher avidity than the anti-MCPS mAbs. The [OD 2] (concentration of Ab that results in fluorescence units = 2 on a scale of 0-4 in a direct binding ELISA) for the majority of the anti-C2 and anti-CP mAbs are 10-100 fold lower than the [OD 2] for most of the anti-MCPS mAbs. To examine differences in affinity maturation between the TI and TD forms of the antigen, we are currently establishing methods to measure affinity constants. We also will test the IgG1 anti-C2 and anti-CP mAbs for bactericidal activity using mouse complement.

Antibody diversity is also being studied by examining the V_H gene usage in panels of mAb from CBA/Ca and BALB/c mice following one or two immunizations with bacterial levan (BL), a β (2 6) linked polyfructosan with β (2 1) (inulin determinant) linked branch points. A panel of 102 monoclonal antibodies (mAb) from BALB/cAnN and CBA/CAHN mice (which are closely related at the immunoglobulin locus) was generated following one or two doses of 10 mcg BL. V_H gene analysis of CBA mAb showed a biased usage of J606 and 36-60 families with an expected frequency of J558 in response to single or multiple injections. In BALB/c mAb there is a pronounced V_H restriction to the J606 family (84%) following a single injection of BL, but a more normal V_H profile in response to two injections. V_L usage is also restricted. There is a correlation in both strains (with only one exception in CBA) between VK11 usage and inulin reactivity of the mAb. Sequence data from three BALB/c J606/VK11 mAb show the use of one J606 gene, which has the same derived amino acid sequence as that of the J606 myeloma protein. 12 mAb have been generated from BL-injected CXBG/B4 mice which is a BALB/c by C57BL/6 recombinant inbred strain that expresses the BALB/c immunoglobulin heavy chain gene locus and the C57BL/6 *Sr-1* gene, a diversity gene previously shown to influence fine specificity of the response to BL. CXBG/4 mAb are a mix of IgM, IgA, and IgG3, do not cross-react with inulin, and are currently being analyzed for V region gene usage. We conclude that CBA and BALB/c mAb specific for BL differ markedly in V_H gene family usage and fine specificity, that light chain usage correlates with fine specificity, and that the BALB/c immunoglobulin heavy chain locus is, itself, not enough to explain these differences. Molecular studies are in progress to further define the basis for these differences.

Studies are continuing on V_H gene expression in CBA/N mice, a strain which fails to respond to polysaccharide antigens, as compared to the normal parent strain, CBA/Ca. Using in situ hybridization on B cell colonies, we determined the expression of 9 V_H gene families in CBA/CaHN females (genotypically normal), CBA/N males (*xid*) and females (*xid*) and (CBA/N X CBA/CaHN) F1 males (*xid*) and females (phenotypically normal). We found that CBA/N female mice, but not CBA/N males or F1 males, showed marked individual variation which persisted over time. V_H gene

family expression in CBA/N males and F1 males is similar to that of CBA/CaHN and F1 females with predominant expression of J558, the largest gene family, in all individuals. These results suggested a possible gene dosage effect on VH gene expression. To further explore this possibility, mice with the *xid* defect on a BALB/c background were examined. In these mice both males and females were found to exhibit individual variation in which gene family was predominantly expressed. Moreover, the non-J558 families that were predominantly expressed in individual mice differed somewhat, depending on the background strain. We conclude that mice with the *xid* defect do exhibit abnormal VH gene expression and the extent of the abnormality is depended on background gene(s). Studies are continuing to determine the molecular basis for this defect.

Finally, the mitogenic activity of lipooligosaccharides from the human pathogens *Haemophilus influenzae*, *Neisseria meningitidis*, and *Bordetella pertussis* was studied in order to determine if these molecules, containing short O-side chains, were more potent than lipopolysaccharides. The results indicated that if the various preparations are compared on an equal molar basis they have similar mitogenic activities. These preparations were similar in activity to Lipid A, itself, the active moiety in LPS and LOS. Thus, no correlation was found, positive or negative, between O-side chain length and mitogenic activity, comparing LOS from these pathogens to LPS of *Escherichia coli*, used as the positive control. These studies have now been extended to examine the relative mitogenic capacities of *Salmonella minnesota* wild type LPS and various LPS mutants having different length core oligosaccharide structures. Again, no correlation was found between O-side chain length and mitogenic activity.

Regulatory Responsibilities, Laboratory of Molecular and Developmental Immunology

1. **IND submissions reviewed** A total of 62 IND submissions were reviewed by one member of the LMDI (KES). These submissions included, but were not limited to, polysaccharide-conjugate vaccines and monoclonal antibodies.

2. **Product License Applications reviewed** A total of 93 PLA submissions were reviewed by one member of the LMDI (KES). Of these, 23 were vaccines or miscellaneous products and 70 were monoclonal antibodies.

3. **Inspections** One inspection was made by one member of the LMDI (KES).

Scientific Activities, Laboratory of Molecular and Developmental Immunology

1. **Meetings**
   a. ICAAC - 1
   b. FASEB - 4

2. **Grants and Manuscripts** One grant and five manuscripts were reviewed by one member of the LMDI(KES).

Laboratory of Bacterial Polysaccharides Summary

Members of the Laboratory of Bacterial Polysaccharides were involved in a diverse array of research projects including capsule biosynthesis, lipopolysaccharide chemistry, outer membrane protein structure, conjugate vaccines for meningococci and pneumococci, as well as studies on the immune response to capsular polysaccharides.

Strains of *Neisseria meningitidis* express one of two porin proteins. These proteins have been identified as the class 2 and class 3 proteins, and express serotype specific epitopes. They have been evaluated as vaccine constituents. We obtained the gene for the class 3 protein by PCR from a serotype 4 strain as a 1025 bp fragment. The sequence of this gene was obtained and compared to two recently published sequences. Based upon this comparison we identified two possible variable regions that may be associated with serotype specificity. Primers were prepared to obtain sequences in the VR1 and VR2 regions from 5 additional group B *N. meningitidis* strains of serotypes 1, 4, 8, 12, and 15, all expressing class 3 proteins. The VR1 and VR2 regions were hypervariable and flanked by highly conserved regions among eight different class 3 sequences. These two hypervariable regions of 15 and 9 amino acids are predicted to be in surface exposed loops.

The synthesis of controlled size oligosaccharides is being pursued in order to provide material for the preparation of conjugates. These conjugates will make it possible to determine the effect of oligosaccharide size on immunogenicity of a conjugate vaccine. An appropriate solid phase support has been prepared. This support incorporates a four-carbon spacer arm which is attached to the support by a selectively cleavable disulfide bond. The support has been found to bear derivatizable groups at a concentration of 50 mmoles/gm. It was determined that the conventional b-cyanoethyl protected phosphitylating reagent, required to complete the synthesis of the activated monomer, would not be optimal for this synthesis, therefore a benzyl protected phosphitylating reagent was prepared. This reagent was preferable because it uses the same protective group as is already present in the carbohydrate moiety of the monomer. In contrast to the case of the b-cyanoethyl protected intermediate, the phosphite enantiomers of the a-anomer of the benzyl protected intermediate copurify. This makes the essential separation of anomers much less labor intensive. The required monomer, 2-acetamido-3-0-acetyl-4-0-benzyl-2-deoxy6-fluorenylmethoxycarbonyl-a-D -mannopyranosyl-N, N-diisopropy-O-benzylphosphoramidite, was prepared, purified and characterized. This monomer has been used to prepare a tetrasaccharide attached to the solid phase. Measurement of coupling efficiency indicates that the synthesis can be readily extended to produce a 20-mer. Work is now progressing on the efficient removal of the saccharide from the support.

Wild type *N. meningitidis* elaborate lipopolysaccharides without long O polysaccharide side chains, and are referred to as lipooligosaccharides (LOS). Others have shown that antibodies to meningococcal LOS determinants can be bactericidal. Studies have also shown that changes in the molecular size of the LOS in *Haemophilus inf/uenzae* type b result in changes from serum resistance to serum sensitivity. Naturally occurring antibodies to the saccharide determinants of meningococcal LOS are present in the sera of healthy adults. Using changes in colony opacity and SDS-PAGE with silver staining, we selected a series of LOS variants from group B (BB-1 and 44/76) and group C (BB-305) *N. meningitidis* strains. We obtained sera from 4 healthy adults that had not worked with Neisseria. These sera were quickly frozen at -70°C to preserve complement activity. The sera were tested for bactericidal activity against each LOS variant by measuring Log decrease in viable count during 1 hour at 37°C. We found the BB-305 wild type to be serum resistant (SR) in all four sera, while variant 1 (V1) was serum sensitive (SS) in all four sera. V2 was SR in two sera and SS in the other two . BB-305 wildtype LOS was 4800 daltons, V1 was 4300 daltons, and V2 was 3600 daltons. Loss of about two sugars from the wildtype resulted in from SR to

SS, possibly due to exposure of a cross-reactive epitope. We are now using rabbit anti-LOS typing sera and monoclonals to determine epitope differences may account for the differences in serum sensitivity.

N. meniningitidis LOS mimic human glycolipids in having lacto-N- neotetraose (LNnT, Galβ31-4GlcNAcβ1-3Galβ1-4Glc) sequence in the oligosaccharides. The oligosaccharides of *N. meniningitidis* LOS have been reported to have a triatennary structure with LNnT at the nonreducing end of the longest antenna in the branched oligosaccharides. We have used a mouse monoclonal antibody (anti- My-28) which recognizes LNnT, to investigate this sequence in the oligosaccharides of the LOS. Eight of the twelve meningococcal LOS immunotypes, all except types 1, 6, 11, and 12, bound the antibody as measured by ELISA, immunodot and immunoblot assays. N-Acetyllactosamine inhibited the binding of the antibody to all eight reactive LOS. The antibody binding to a represntative LOS (type 2) was best inhibited by LNnT, next by Nacetyllactosamine, but not inhibited by galactose, Galβ1-3GlcNAc, and lacto-Ntetraose, Galβ31-3GlcNAcβ1- 3Galβ31-4Glc. These results suggest that the LNnT sequence is present in 8 of 12 LOS immunotypes. The presence of the LNnT sequence in the LOS, which is found in paragloboside (LNnT- ceramide) and its related glycolipids in a variety of human cells, may play a role in the virulence of *N. meningitidis* by enabling the organism to evade host immune defenses. The expression of the antibody-reactive epitope in the LOS was influenced by growth conditions and the LNnT epitope could be masked by a sialic acid which was identified as N-acetyl-neuraminic acid. We are currently investigating the linkage of the sialic acid to LNnT in meningococcal LOS and its possible function.

Oligosaccharide-protein conjugates are being prepared as a vaccine that may prevent meningococcal disease. The OS was isolated from strain A1 LOS which has no LNnT sequence, by acetic acid hydrolysis of the LOS and purified through P-4 chromatography. The carboxylic acid group of KDO in the OS was linked to a bifunctional adipic acid dihydrazide (ADH) using carbodiimide (EDC) and N-hydroxysulfosuccinimide. This ADH derivatized OS was further coupled to tetanus toxoid (TT) or other protein carriers using EDC. Antigenicity of the conjugates in vitro was similar to that of LOS as determined by ELISA using a rabbit immune serum against whole cells as a binding antibody. Immunogenicity of the conjugates showed that the conjugates induced the specific IgG antibody to LOS in mice, and also induced the higher antibody response in rabbits. MPL enhanced the immunogenicity of the OS-TT. The binding reactivities of rabbit sera elicited by the OS- TT to 12 immunotype LOS showed that M978 (B,L8), BB431 (B), 44/76 (B,L3,8), and A1 (A) LOS had the strongest reactivity which indicated that the A1 OS-TT conjugate induced immunotype 8 specific antisera against several group B strains including two disease isolated strains 44/76 and BB431 .

The immunological defense mechanisms that resist pneumococcal infection involve complex interactions among various host cells, humoral components and the bacteria. The pneumococcus has a number of virulence factors including capsular polysaccharide (PS), pneumolysin (Ply) and other cell-surface antigens. The pneumococcal PS vaccine-induced antibodies confer protective immunity and resistance to disease caused by these organisms. Some PSs contained in the pneumococcal vaccine, e.g. type 9V and 1 9F are less immunogenic in young children. Several research projects have been conducted regarding (a) molecular cloning and characteristics of pneumolysin gene from pneumococcal group 19, (b) protective immunity induced by pneumococcal PS-protein conjugates, and (c) effect of the molecular size of the saccharide on immune response to pneumococcal PS-protein conjugate.

Molecular cloning of the group 19 pneumolysin genes were done to examine the relationship of pneumolysin to virulence. Genomic DNA that contained the ~y from 1 9F, 1 9A, 1 9B and 1 9C strains were examined by the polymerase chain reaction (PCR). The isolated *ply* gene was inserted into a vector and cloned into *E. coli*. Recombinant clones 19A31 and 19F5 contained the *ply* gene as confirmed by restriction enzyme analysis and Southern blot. The dna sequence showed that the type 19A and type 2 *ply* proteins differed in only two amino acids (type 2 Arg226 -->19A Asp; type 2 Asp380 -->19A Asn). The deduced amino acid sequence from 19A *ply* gene showed 24.6% homologous with other cytolytic toxins. Studies on mutation of

ply gene have been conducted to produce truncation of the pneumolysin molecule with elimination of hemolytic activity and retention of the antibody binding properties.

Inactivated pneumolysin molecules have been evaluated as potential protein carriers for pneumococcal PS to prepare a conjugate vaccine. A new conjugation method was applied to link the pneumococcal PS, such as type 9V or type 19F, to an inactivated pneumolysin. The 19F PS-PdB conjugate was given to female mice during gestation and/or lactation and an additional immunogen was given to young mice after birth. Later, the young immunized mice were challenged with 19F pneumococci. Almost all animals the in nonimmunized control group died within first 3 days after challenge, while most mice in immunized group survived. Young mice from mothers immunized with the conjugate and received an additional dose of conjugate produced significantly higher 19F antibody and anti-pneumolysin levels as well as removed 19F cells in blood more rapidly than the non-immunized controls. Thus, injection of mice with 19F PS conjugated with inactivated pneumolysin conferred protective immunity against pneumococcal infection.

To study the effect of molecualr size of pneumococcal PS on the immune response of mice to PS- protein conjugates, the type 19F PS was cleaved into different sized PS or oligosaccharide, then conjugated to the inactivated 19F pneumolysin. The serum levels of 19F IgG1 and IgG3 antibodies were significantly higher in mice immunized with a large molecular size PS-protein conjugate compared to the group injected with a small sized OS conjugate.

Escherichia coli N-acetylneuraminic acid cytidyltransferase (CMP-NeuAc synthetase), catalyses the formation of cytidine 5' monophospho-Nacetylneuraminic acid (CMP-NeuAc) from N-acetylneuraminic acid (NeuAc) and cytidine triphosphate (CTP). This enzyme forms an important intermediate in the *in vivo* biosynthesis of glycoconjugates. The availability and specificity of purified sugar activating enzymes and glycosyltransferases make these enzymes convenient tools for the synthesis of biologically relevant oligosaccharides. Very little information has been published on the nature of the active site residues of CMP-neuAc synthetase or the structure and stability of the protein. The role of the cysteines in the structural and catalytic properties of the enzyme has been examined by site directed mutagenesis and chemical modification. Chemical modification experiments with the sulfhydro specific reagent 4,4-dithiodipyridine (DTDP) suggests that one cysteine residue is involved in catalysis, since the enzyme can be completely inactivated by titration of one cysteine residue with DTDP. The enzyme can be protected from inactivation in the presence of the substrate CTP. Site directed mutagenesis demonstrates that cysteine 129 and cysteine 329 are not essential for catalysis despite the sensitivity of the enzyme to the sulfhydro reagent DTDP, since both residues can be substituted by selected amino acids without complete loss of activity. Chemical inactivation with N-ethylmaleimide (NEM) at room temperature demonstrates that cysteine 329 and cysteine 129 are inaccessible to NEM at room temperature. Cysteine 329 can be thermally exposed to chemical modification with NEM at 42°C but cysteine 129 is inaccessible to chemical modification with NEM and probably buried. We have purified and sequenced the amino terminus of the CMP NeuAc synthetase from *Neisseria Meningitidis* group B which synthesizes the same a 2-8 NeuAc capsule of *E. coli* K 1. The amino terminus sequences were compared and a concensus was obtained. Arginine and Iysine residues were identified as common amino acids in the compared sequences. We have mutated 14 arginines in *E. coli* CMP NeuAc synthetase to Glycine residues and found that mutation of the identified common arginine completely inactivates CMP NeuAc synthetase. This arginine has been further changed to alanine and Iysine residues. Changing arginine to alanine still results in a completely inactivated enzyme but a change to Iysine restores the specific activity to wild type values. Km determinations are being conducted at different pH to determine the involvement of this arginine in catalysis.

Laboratory of Bacterial Toxins Summary

During this fiscal year, the Laboratory staff changed only slightly; one Staff Fellow, Dr. Halpern, was converted to a tenured position and our stay-in-school pharmacy student, Tuan Lam, left after graduation. The current staff consists of the Laboratory Chief, Dr. Halpern, three Senior Staff Fellows, and three technical support personnel. One Fellow has had no technical support.

The Laboratory conducted a productive research program in four separate areas, corresponding to the programs of each of the senior investigators.
Each of these programs is related to mission of the Laboratory, with emphasis on toxins and virulence factors.

Dr. Jane Halpern has been preparing and characterizing mutants of the heavy chain of tetanus toxin in order to better understand the structural requirements for binding, entry, and axonal transport of the toxin. Her findings indicate that up to 263 amino acids can be deleted from the amino terminus before ganglioside binding is markedly reduced. In contrast, the deletion of only ten residues from the carboxyl terminus results in major reduction in receptor binding.

Dr. Virginia Johnson is studying the influence of structure on the binding and translocation of diphtheria toxin. She has shown that substitution of a proline_{345} with a Glu or Gly residue has no effect on enzymatic activity but reduces the toxicity of the molecule by about 100-fold due to a comparable reduction in translocation activity. Drs. Halpern and Johnson are also working on the construction of hybrid toxins using in vitro methods to avoid violation of regulations prohibiting the cloning of neurotoxins.

Dr Scott Stibitz has completed a physical map of the *Bordetella pertussis* which has demonstrated that the known virulence determinants of this organism are unlinked. The map will enable the genetic mapping and isolation of genes which affect the level of production of pertussis toxin, pertactin, FHA, and other vaccine components. The map has also facilitated the analysis of mutations which affect the rate of phase-variation in *B. pertussis* and thus may lead to the development of more stable vaccine production strains which switch to the avirulent phase at a lower frequency.

Dr. Sherry Ansher has investigated models for assessing the toxicity of cytokines and vaccines (primarily DTP vaccine). Additional projects are directed at understanding the impact of other factors such as acetaminophen administration or infection with murine AIDS on hepatotoxicity. Current results indicate that LPS is responsible for some but not all of the toxicity seen in animal models with DTP vaccine. Cytokines have been shown to exhibit synergistic toxicity when administered in certain combinations and may play a role in vaccine toxicity.

Future research in the Laboratory will expand on the interesting findings of the present work. The synthesis and characterization of chimeric molecules, containing components of both tetanus and diphtheria toxins, will be performed as appropriate using both cloning techniques and *in vitro* synthesis. Work on the pertussis *vir* gene will exploit the methodologic options provided by the genetic map to study evolutionary relationships between Bordetella species. Studies on the hepatotoxicity of DTP vaccine are being expanded to include the analysis of cytokine levels in infants after immunization. Some of the completed and planned work has been supported by the National Vaccine Program.

In the regulatory area, the Laboratory is responsible for bacterial toxins, toxoids and many other products. Areas of considerable regulatory activity this past year have included asparaginases, products related to needs identified by Desert Storm (e.g. enteric vaccines, anthrax and botulism), botulinum toxins, acellular DTP etc. During this FY the Laboratory staff reviewed approximately 200

IND submissions and 50 ELA and PLA submissions, many label reviews (especially related to the Section 314 review of pediatric vaccine labeling) and performed inspections of several licensed establishments. The Staff Fellows provided invaluable contributions to the regulatory mission.

Laboratory of Mycobacteria Summary

Primarily because of the AIDS epidemic, the incidence of mycobacterial disease has been dramatically increasing. *Mycobacterium avium, Mycobacterium intracellulare* complex (MAC) infections are the most frequent bacterial complication of AIDS and occur in over 50% of advanced cases. The WHO estimates that internationally 40% of HIV-infected individuals are co-infected with *M. tuberculosis*. The emergence of multiple drug-resistant strains of *M. tuberculosis* in institutional settings has intensified concerns about the HIV/tuberculosis co-epidemic in the United States.

Because of the increasing importance of mycobacterial diseases, research in the Laboratory of Mycobacteria has focused on (1) the development of better methodologies for diagnosing mycobacterial disease and (2) the improved molecular definition of basic mycobacterial biology and immunopathology.

(1) Serological and PCR protocols to accelerate the detection of MAC infections are being developed. We have demonstrated that the recombinant mycobacterial protein MK35 may be a useful indicator of MAC disease because MK35 is seroreactive with sera from 95% of MAC-infected AIDS patients. We have also shown that MAC antigen, detected in AIDS sera using our MAC-specific MAb 4004, may be a viable serological marker of MAC disease. PCR protocols based on mycobacterial 16s RNA sequences have been developed to specifically and rapidly detect nontuberculous mycobacterial infections. The capacity of these PCR protocols to detect mycobacterial bacilli in clinical specimens is currently being evaluated.

(2) At present, mycobacterial biology and immunopathology are not well understood. A better understanding of the biology of the disease process - particularily the antigens involved in pathogenesis - should facilitate the design of improved chemotherapeutic and immunotherapeutic regimens for managing mycobacterial infections.

To better define mycobacterial biology and immunopathology, the Laboratory of Mycobacteria has continued its evaluation of the important mycobacterial antigens - MI43, MK35, and MI85. Nucleotide sequence analysis, detergent phase separations, and metabolic labeling has shown that MI43 is a 27 kDa lipoprotein. Serological analysis has demonstrated that MI43 is recognized by sera from patients with MAC disease and tuberculosis patients. Completion of the nucleotide sequence of the gene encoding MK35 has revealed that MK35 is a 27 kDa lipoprotein. Using PCR, *M. tuberculosis* and *M. avium* genes encoding MK35 homologs have been identified and are being characterized. Previously, we have demonstrated that MI85 encodes a catalase-peroxidase, an enzyme that may be critical for the intracellular survival of mycobacteria. During this year, the induction of MI85 by peroxide and the structure of the MI85 promoter was evaluated. In addition, the catalase genes from *M. tuberculosis* and *M. avium* have been identified and cloned.

To define immunodominant epitopes and to develop monospecific skin test antigens, T cell epitopes from homologous 19 kDa proteins from *M. intracellulare* and *M. tuberculosis* were defined using overlapping synthetic peptides. Six *M. tuberculosis* peptides and four*M. intracellulare* peptides induced T cell proliferation *in vitro*. Six *M. tuberculosis* and nine *M. intracellulare* peptides elicted DTH reactions in sensitized guinea pigs. Since one of the reactive *M. intracellulare* peptides evoked a specific DTH response, the development of monospecific skin test reagents from synthetic peptides may be possible.

Because of the emergence of drug resistant *M. tuberculosis* strains in clinical isolates, our laboratory has begun examining the molecular biology of drug resistance in *M. tuberculosis*. Resistance to isoniazid (INH), the primary drug for the treatment of tuberculosis, has been frequently correlated with a loss of catalase activity. We have demonstrated by Southern hybridization analysis that the catalase gene of a INH^r *M. tuberculosis* strain is deleted. Other resistant strains are currently being evaluated to elucidate the role of catalase in INH resistance. Resistance to streptomyocin (SM), another primary tuberculosis drug, often results from mutations in the ribosomal S12 protein genes. Nucleotide sequence analysis has shown that two different SM resistant M. tubreculosis strains have identical mutations in the S12 protein gene. This molecular

defintion of mutations conferring drug resistance should permit the rational design of PCR protocols for the rapid detection ofdrug resistant *M. tuberculosis* strains.

Regulatory Activities

Product License Applications and Amendments: The Laboratory of Mycobacteria personnel have been the chairman or a member on eight PLA committees.

Investigational New Drugs: The laboratory has reviewed 70 INDs.

Protocol Releases: The laboratory has reviewed and released over 80 protocols.

BCG Potency Assay: The laboratory has tested and passed several lots of BCG vaccine.

Inspections: Laboratory personnel have inspected five CBER regulated facilities.

Review of Promotional Materials and Product Labeling: Laboratory personnel have reviewed numerous promotional materials, package linserts, and product labels relating to BCG and tuberculins.

Complaint Reports: The laboratory has reviewed adverse reaction reports for tuberculins and BCG and has reviewded false positive reaction reports for tuberculins.

Contract Activities: Dr. Morris was the Project Officer on contract #223-89-1200 (characterization of the new tuberculin standard, PPD-S2).

Meetings and Workshops

1991 - NIH Special Grant Review Committee - National Cooperative Drug Discovery Group for the Treatment of Opportunistic Infections Associated with the Acquired Immunodeficiency Syndrome

1991 - FDA Grant Review

1992 - NIH Contract Review - RFP-AI-92-04, Reference Laboratory for Nontuberculous Mycobacteria Isolated from AIDS Patients

1992 - FDA Grant Review

1992 - BCG Vaccine Workshop

1992 - American Society for Microbiology, New Orleans, LA

1992 - International AIDS Conference, Amsterdam, Netherlands

1992 - AIDS and Tuberculosis Conference, Bethesda, MD

1992 - Bacterial Vectors for AIDS Vaccines, Bethesda, MD

Extracurricular Related Activies:

Personnel of the Laboratory of Mycobacteria were involved in:

Peer review of grants and contracts submitted to NIAID
Review of the National Task Force on Multi-drug Resistant Tuberculosis proposals and the BCG Vaccine Workshop summary
Review of manuscripts submitted to refereed journals

Regulatory Activities

Product License Applications and Amendments:
The Laboratory of Mycobacteria personnel have been the chairman or a member on eight PLA committees.

Investigational New Drugs:
The laboratory has reviewed 70 INDs.

Protocol Releases:
The laboratory has reviewed and released over 80 protocols.

BCG Potency Assay:
The laboratory has tested and passed four lots of BCG vaccine.

Inspections:
Laboratory personnel have inspected five CBER regulated facilities.

Review of Promotional Materials and Product Labeling:
Laboratory personnel have reviewed numerous promotional materials, package linserts, and product labels relating to BCG and tuberculins.

Contract Activities:
Dr. Morris was the Project Officer on contract #223-89-1200 (characterization of the new tuberculin standard, PPD-S2).

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1992 - Bacterial Vectors for AIDS Vaccines, Bethesda, MD

Laboratory of Mycoplasma Summary

The Mycoplasma Laboratory has two major responsibilities: One deals with mycoplasma contamination of biologic products produced in cell substrates, such as viral vaccines, immunologic modifiers, and monoclonal antibodies. The second deals with the potency, efficacy, and safety of biologic products produced from mycoplasmas and similar organisms. Accordingly, research programs have been developed to evaluate animal models to investigate the infectious processes and host immune responses to mycoplasma diseases. The goals include identification of the immune mechanisms that protect against respiratory mucosal infections and the protective immunogens as they relate to their inclusion in vaccines and the evaluation of vaccine potency. Attempts are also being made to identify and characterize the genes which encode for the protective immunogenic components. A summary of the results of these studies during the past year follow:

STUDIES ON MYCOPLASMA PNEUMONIAE: Mycoplasma pneumoniae-Induced Pneumonia in Chimpanzees: Six sero-negative, culture-negative young adult chimpanzees were inoculated intratracheally with an early passage of a virulent *Mycoplasma pneumoniae* strain PI-1428, which has been shown to produce disease in human volunteers, monkeys, hamsters and guinea pigs. Each of the six chimpanzees became colonized with peak respiratory mycoplasma titers of 10^6 to 10^8 ccu/ml. The oropharyngeal and tracheal tissues remained colonized for up to 70 days and lungs remained colonized for up to 26 days. Animals showed overt signs of clinical disease within two weeks post-inoculation which corresponded with the onset of positive X-ray findings, the development of cold agglutinins and peak colonization of lungs. All animals developed a persistent cough and some developed rhinitis, inflamed oropharyngeal tissues, diarrhea, and loss of appetite. Low grade fever was seen in three of the infected animals. Lung lavage fluids and sera contained *M. pneumoniae*-specific IgA and IgG immunoglobulins with up to five-fold higher values in the lung washings than in the serum, as determined by a radioimmunoassay. Two control chimpanzees maintained in individual cages and in rooms separate and distant from the infected animals became infected on days 19 and 48, respectively. Although we were unable to determine the mode of transmission, this represents the first report of *M. pneumoniae* disease transmission in an animal model.

Unlike all other animals tested, chimpanzees become overtly ill and develop positive X-ray findings and cold agglutinin titers. Thus, the *M. pneumoniae* pneumonias induced in chimpanzees are remarkably similar to naturally occurring primary atypical pneumonia in humans and the infection in the chimpanzee animal model best reflects and parallels pneumonia in humans.

Serum immunoblot patterns of infected chimpanzees: In this study, the sera of these chimpanzees were examined using immunoblot analyses to strain PI-1428 whole cell lysates. Infected chimpanzee reacted with 17 to 20 protein bands and produced immunoblot patterns remarkably similar to the patient's convalescent sera. The most prominent immunogens were the 169 kDa protein band which comigrated with the P1 adhesin, and protein bands at 117, 86, 35 and 30 kDa. The 86 and 35 kDa protein bands may represent important immunogens because these two bands were present in the pathogenic, cytadsorbing strains PI-1428, M129 and FH, but not the non-pathogenic, non-cytadsorbing strain B176.

Immunization with a formalin-inactivated or acellular vaccine did not induce a specific serum immunoblot response, but all these animals seroconverted after challenge. The immunoblot patterns of the three groups were similar, but variations were noted among individuals receiving the same treatment and among individuals receiving different treatments. Previously infected chimpanzees who were protected on challenge showed the most pronounced immunologic responses. These studies provided more evidence that the P1 protein is an important immunogen and indicated that the 86 and 35 kDa proteins may also be important immunogens.

Identification of New Adhesins from *Mycoplasma pneumoniae*: Based on the attachment studies, affinity columns of dextran sulfate and fetuin are being used to identify *M. pneumoniae* adhesins other than P1. Several membrane-localized proteins, as determined by their susceptibility to surface proteolysis, have been

identified that have specific and avid binding activity to the affinity columns ligands. These results suggest surface proteins other than the best known adhesin, P1, might also be involved in the attachment of the pathogen to host tissues. These include the 90, 40 and 32 kDa proteins.

Development of a Mycoplasma pneumoniae-Adenovirus Hybrid Live Oral Vaccine: Presently, there is no known vaccine fully protective against mucosal infections and the failure to protect is due mainly to our inability to effectively stimulate protective mucosal immunity. The Division of Bacterial Products has supported a contract deals with developing an oral *Mycoplasma pneumoniae*-adenovirus hybrid vaccine using recombinant DNA technology. The P1 attachment protein is a major protective immunogen and monoclonal antibodies specific to P1 inhibit attachment of the organisms to the respiratory epithelium. Since the P1 antibody can inhibit attachment, then immunization might inhibit attachment, and subsequently colonization and disease. Adenovirus replication in the gut permits direct and continuous mucosal stimulation by the expressed excreted immunogen. Protection is presumably due to the migration of activated plasma cells from the gut to the respiratory tract. In addition, immunization by this live, orally administrated vaccine has been given to tens of thousands of immunized military personnel and was shown to be safe and efficacious. Future efforts will identify the protective immunogens of other mucosal pathogens for gene insertion, in combination, to develop a polyvalent oral recombinant vaccine capable of protecting humans against various mucosal tract infections. Thus far, the contractor has inserted portions of the *Mycoplasma pneumoniae* P1 adhesin protein gene which encode immunodominant epitopes into the E3 region of the virus as well as into a chimeric hexon protein of the adenovirus which he has constructed. These mycoplasmal epitopes are expressed by the virus in both the *in vitro* cell culture system and in immunized animals as determined by immunofluorescent staining and Western blotting techniques.

STUDIES ON MYCOPLASMA HOMINIS: Antigenic Variability of Surface Proteins of arthrogenic *Mycoplasma hominis*: *Mycoplasma hominis* strain 1620 was isolated from the synovial exudates of a patient with severe recurring septic arthritis. Three monoclonal antibodies raised to this pathogenic isolate and specific for surface proteins were used to demonstrate that these M. hominis proteins exhibit antigenic variability. The Mabs are being used as probes of *M. hominis* gene banks to isolate, clone and characterize a gene or genes that encode the protein portion(s) of the membrane lipoproteins. The phenotype associated with antigenic switching of is also under study by analysis of clones from plated cultures.

Binding Specificity of arthrogenic *M. hominis* Strain 1620: *Mycoplasma hominis* strain 1620, isolated from the synovial fluid of a chronically infected septic arthritis patient, was shown to adhere to sulfatides but not to gangliosides or other neutral glycolipids or to many of the common glycoproteins.

STUDIES ON SEPTIC ARTHRITIS: An acute septic arthritis was experimentally induced in chimpanzees inoculated intraarticularly with strains of *Mycoplasma hominis* and *Ureaplasma urealyticum*, each isolated from a different patient with septic arthritis. The disease induced was similar to that observed in patients with regard to clinical signs of disease, the degree of colonization, and the extent of the inflammatory and antibody responses produced. A very large inoculum of the arthrogenic *Mycoplasma hominis* strain 1620 given intravenously or of the non-cytadsorbing laboratory reference type strain PG-21 given intraarticularly produced no overt clinical disease. *U. urealyticum* serovar SVII strain 2010B isolated from the septic joint of a patient with hypogammaglobulinemia and in low broth passage produced severe disease, whereas the laboratory reference Serovar SVII type strain CO [urethral] in high broth passage produced mild disease. Whereas the arthrogenic ureaplasma strain produced arthritis, an intense inflammatory response, but a weak metabolism inhibition antibody response, the high passage laboratory type strain produced a mild arthritis and minimal inflammation but a greater metabolism inhibition antibody response. The low level of antibody detected in the infected exudates may have been a result of antibody bound to antigen resulting in the antigen-antibody complex which could have

activated complement contributing to the inflammatory response. Thus, the source of the original isolate, attenuation of virulence by continuous broth passage and the ability to attach may all be important factors in determining the ability of mycoplasmas to experimentally induce arthritic disease. These finding may be significant in our understanding of the pathogenesis of mycoplasmal arthritic infections, and that the chimpanzee provides an excellent model to study known as well as reputed mycoplasma pathogens of man.

STUDIES ON MYCOPLASMA FERMENTANS AND AIDS: Dr. S.-C. Lo and coworkers [Washington, D. C.] reported the intracellular detection of *M. fermentans* (strain *incognitus*) in organ biopsies of AIDS patients. Dr Luc Montagnier believes that co-infecting mycoplasmas which he and colleagues have recovered from circulation leukocytes of AIDS patients are co-factors responsible for the potentiation of HIV infections leading to an accelerated disease process. Rhesus monkeys are on test to try to confirm the pathogenic nature of *M. fermentans*. A previous study involved the intraperitoneal inoculation of monkeys with broth grown cultures of Lo's *M. fermentans* VLIA agent as well as the type strain, PG18. All four monkeys inoculated with Lo's agent lost weight within the first three months but then gained weight and had no other discernible symptoms. The monkeys that received the type strain were apparently unaffected. The current study utilized cell culture-grown mycoplasmas as the inoculum. Four monkeys received only the cell culture (3T3 cells) in the intraperitoneal injection. Four groups of four monkeys each received different strains of *M. fermentans*, while a fifth group of four monkeys were injected with tissue culture-grown *M. hominis*. The animals, on test since November 1991, are currently being assessed.

Regulatory Responsibilities

1. Protocol Review and release: October 1, 1991 to present:250

2. Product License Applications: 10
PLA Amendments: 12

3. IND Reviews: 15

4. Telephone conversations regarding mycoplasma regulations primarily for the generation of new productions: 14

5. Recommended procedures for mycoplasma testing sent out: 2

6. Inspections of licensed establishments:
Three companies (L.D. Olson)

7. Manuscripts:
Published: 3
Accepted and In Press: 3
Abstracts 7

8. Abstracts submitted and accepted and papers presented:
i. American Society for Microbiology (ASM): 3 (ldo 2, mfb 1)
ii. International Organization of Mycoplasmology: 2
iii. Convener: IOM Workshop (L.D. Olson)
iv. Abstracts 3

9. Attendance at Scientific meetings

- i. Technical Staff: C.A. Renshaw to national ASM meeting
  A.A. Gilbert to international IOM meeting
- ii. Staff Fellows: L.D. Olson and H.M. Marcus to ASM
  L.D. Olson to IOM meeting
- iv. Lab Chief: M.F. Barile to ASM meeting
  M.F. Barile to IOM meeting

10. Guest Lecturer:

11. Contract Review:

- i. University of North Caroline, Dept. of Pediatrics: M.F. Barile, Project Officer, L.D. Olson, Assistant Project Officer

- ii. Frederick Cancer Research Center, Frederick MD. Interagency Agreement NCI #YOI-CO-07A04 and FDA #224-76-1202. M.F. Barile, Project Officer

12. Courses:

Food and Drug Law Institute Biologics Update 1992: A.A. Gilbert, H.M. Marcus, C.A. Renshaw

Using Animals in Intramural Research: H.M. Marcus

Laboratory of Pertussis Summary

The Laboratory of Pertussis is responsible for a wide range of problems concerned with basic and developmental research and the regulation of bacterial vaccines in general and pertussis vaccines in particular. The Laboratory initiates, directs, and participates in research on the immunochemistry of *Bordetella pertussis* and the host parasite interactions in pertussis prerequisite to the development of improved pertussis vaccines and their control and clinical use. In addition, the Laboratory of Pertussis is the World Health Organization Collaborating Center for Research on Pertussis Vaccines.

Current research programs are concerned with the mechanisms by which *Bordetella pertussis* attaches to and infects susceptible tissues and the cellular components responsible for local and systemic symptoms of whooping cough. The Laboratory of Pertussis has been instrumental in the identification and purification of seven of the important virulence factors of *B. pertussis* (lymphocytosis promoting factor or pertussis toxin, heat labile or dermonecrotic toxin, filamentous hemagglutinin, agglutinogen 2 fimbriae, agglutinogen 3/6 fimbriae, pertactin (69k outer membrane protein), and the lipooligosaccharide of agglutinogen 1). A number of monoclonal antibodies directed against agglutinogens of *B. pertussis* have been produced in the Laboratory and are being used to identify serotype specific antigens and to study the potential protective role of these antigens. Other research studies include the isolation and characterization of virulence factors and the development of *in vitro* and animal laboratory models to study disease and evaluate potential immunogens.

Control testing. The Laboratory tests the potency and freedom from toxicity of all pertussis vaccines produced by US licensed manufacturers and submitted to CBER for release plays a crucial role in the characterization of acellular vaccines to be evaluated in the clinic, and is actively involved in the development and standardization of tests that assess the safety and potency of acellular pertussis vaccines. The Laboratory serves as a reference to the World Health Organization for the evaluation of pertussis vaccines, and assists in the standardization of reference vaccines for national control laboratories. One of the goals of the Laboratory is to develop methods to evaluate pertussis vaccine potency and freedom from toxicity by physical-chemical methods and innovative *in vitro* and *in vivo* systems that are more specific, less costly, and less variable than classical animal systems.

Animal models. An aerosol-induced respiratory infection of mice has been developed to evaluate the ability of antigens to protect by active immunization and of specific antibodies to passively protect against disease. Pertussis toxoid, the B oligomer of pertussis toxin,filamentous hemagglutinin, purified type 2 and type 6 fimbriae, pertactin, and genetically engineered non-toxic mutants of pertussis toxin have been evaluated and shown to protect in the aerosol challenge model. Monoclonal antibodies to B oligomer, the carbohydrate moiety of lipooligosaccharide, and pertactin passively protect against respiratory challenge as well. The identification of monoclonal antibodies that passively protect in this model is the first step in the elucidation of protective epitopes on these antigens. The aerosol-challenge model has been employed in ongoing studies on mucosal immunity to *B. pertussis* and should be useful for the evaluation of new pertussis vaccines and potential protective antigens.

Agglutinogens of *Bordetella pertussis*. The only available monoclonal antibodies have been developed that specifically recognize agglutinogens on *B. pertussis*. Monoclonal antibodies that agglutinate serotype 1 strains have been shown to recognize lipooligosaccharide A of *B. pertussis*. These antibodies are being used for studies on the structure and function of *B. pertussis* lipooligosaccharide.

The fimbrial agglutinogens of *B. pertussis* are thought to be important protective antigens and are being evaluated for possible inclusion in acellular pertussis vaccines. Methods for the purification of type 2 and type 6 fimbriae were developed in the Laboratory. Preliminary studies have suggested that immunization with purified fimbriae can protect mice from lethal respiratory

infection with *B. pertussis*. Monoclonal antibodies produced against type 2 and type 6 fimbriae are being used to characterize these fimbrial antigens. Hybridomas reactive with serotype 3 strains recognize a 69 kDa nonfimbrial outer membrane protein (pertactin). These monoclonal antibodies passively protect mice against respiratory challenge. Pertactin has been purified and its role as a protective antigen is under investigation.

Adherence of *Bordetella pertussis* to mammalian cells. Attachment of *B. pertussis* to respiratory epithelial cells is an essential step in the colonization of the respiratory tract and in the development of disease. The identification and isolation of bacterial adhesins may prove important in the development of an acellular pertussis vaccine that prevents both infection and disease.

We have provided evidence that filamentous hemagglutinin mediates the adherence of *B. pertussis* cells to human intestinal carcinoma cells *in vitro*. Studies on the potential role of pertactin as a bacterial adhesin are ongoing. Currently we are attempting to identify cell surface receptors for *B. pertussis* as well as *B. pertussis* adhesins.

Purification and characterization of *Bordetella pertussis* porin. Porin is a channel-forming outer membrane protein of *Bordetella* species. Our previous studies have demonstrated that on certain *B. pertussis* strains the porin exists as a naturally crystalline trimer. Methods have been developed to purify the porin from cell envelope preparations and characterization of the porin protein is ongoing. Using oligonucleotide probes, the porin gene has been cloned and sequenced. The DNA sequence related to the porin gene is unique for *B. pertussis* and has been used to design primers for the PCR diagnosis of pertussis.

Characterization of a new *bvg*-regulated *Bordetella pertussis* outer membrane protein. Mutants of *B. pertussis* generated using the transposon Tn*phoA* have been tested in the mouse aerosol challenge model. One mutant, defective in a 95kDa outer membrane protein was unable to induce lymphocytosis or persist in the lungs. Another mutant is defective in a 30kDa protein. Currently we are cloning and sequencing the gene for these proteins with the aim of establishing its role in pathogenesis. An additional vag products identified by TrphoA mutagenesis appears toobe a 30 kDa outer membrane protein. The gene has been cloned and sequenced and shows considerable homology with the sequence of pertactin

Purification of lymphocytosis promoting factor (pertussis toxin). Two procedures for the purification of pertussis toxin from culture supernatants of *Bordetella pertussis* have been developed and refined in the Laboratory and have had commercial application in the manufacture of acellular vaccines. The first method uses chromatography on hydroxyapatite and haptoglobin Sepharose and the second method employs Affi-gel Blue and fetuin-Sepharose affinity matrices. Amino acid analysis of toxin purified from strain 165 suggests that the toxin is very similar if not identical to the pertussis toxin isolated from strains Tohama and 114.

A panel of monoclonal antibodies which are specific for various determinants on the pertussis toxin molecule have been produced and extensively characterized. Four of the antibodies react with different epitopes on the S1 subunit of pertussis toxin (enzymatic subunit) and another reacts predominately with the S4 subunit (part of the binding subunit). Only the antibody which is reactive with the S1 subunit shows toxin neutralizing activity when assayed in the CHO-cell assay. The antibodies have been used to develop a sensitive ELISA assay for pertussis toxin. Preliminary results indicate that the antibodies also will be extremely useful in the identification of the membrane receptor for pertussis toxin and in the study of toxin internalization by cells.

Although much is known about the ADP-ribosylation activity of the A subunit little is known about the eukaryotic cell receptor(s) for pertussis toxin. We have demonstrated that pertussis toxin specifically binds to a 165 kDa CHO cell glycoprotein which is absent in a lectin-resistant line of CHO cells that cannot fully process N-linked oligosaccharides on glycoprotein. The studies suggest that a glycoprotein serves as a cell surface receptor for pertussis toxin and that the

receptor interacts with a lectin-binding site located on the B oligomer.

Structural characterization of pertussis toxin. Pertussis toxin is an important vaccine candidate which must be properly inactivated before it can be included in a vaccine. Studies are under way to understand the mechanism of action of the toxin and how structure relates to function so that the molecule's toxicity can be eliminated while retaining immunogenicity .

The toxin is composed of an enzymatically active A subunit and a B oligomer which interacts with the eukaryotic cell receptor. The A subunit was readily separated from the B oligomer in the presence of ATP and detergents. The N-terminal 200 amino acids of the A subunit were found to contain the NAD glycohydrolase activity whereas the 40-50 amino acids at the C-terminal end of the molecule were shown to be important in maintaining the oligomeric interactions of the toxin. Chemical modification of Cys41 results in inactivation of the enzymatic activity of the toxin. The isolated B oligomer, as well as its dimeric subunits, have been shown to be an immunogen which can protect mice against challenge with pertussis toxin. The B oligomer was found to be as protective as a genetically attenuated holotoxin molecule in animal studies. B-oligomer assembled *in vivo* from recomginant subunits expressed in *Escherichia coli* possessed teh mitogenic and hemagglutinating activities characteristic of the native B-oligomer and immunization of mice with the recombinant B-oligomer elicited toxin-neutralizing antibodies and provided protetion *in vivo*. Since the B oligomer does not have the biological activities of the holotoxin molecule, the B oligomer is a potential vaccine candidate.

Current studies are aimed at examining the entry of pertussis toxin into eukaryotic cells, as well as residues which play a role in the binding of the toxin to the eukaryotic cell. Modification of these residues should yield a biologically inert toxin molecule. Additionally, studies are underway to examine the secretion of pertussis toxin from *Bordetella pertussis*. Investigation in the Laboratory demonstrated that highly purified pertussis toxin profoundly inhibited the migration of macrophages in both *in vivo* and *in vitro* models.

Other investigators subsequently demonstrated similar affects on neutrophils and lymphocytes, suggesting that pertussis toxin released systemically from bacteria colonizing the respiratory tract could interfere with the host immune response to infection.

Molecular Chaperones. While much progress has been made concerning the cloning and expression of foreign proteins in *Escherichia coli* or other common hosts used for the large-scale production of proteins in the biotechnology industry, the proteins produced by these organisms are often found in the form of inclusion bodies which are large aggregates of improperly folded proteins. The next advance in this field will involve an understanding of the mechanism by which proteins properly fold and assemble and a knowledge of the accessory proteins, termed molecular chaperones, which are involved in this process. The Laboratory of Pertussis is currently studying the molecular chaperones of *B. pertussis* in the hope of gaining information which will aid in the large scale production of properly folded and assembled recombinant pertussis antigens in *E. coli*. Knowledge gained from these studies may be useful for pertussis vaccine production and would likely be applicable to large-scale production of a variety of other proteins. Four monoclonal antibodies that recognize the 63 kDa subunit of the *B. pertussis* chaperonin have been produced.

Serological methods. Methods and reagents for a standardized microagglutination procedure have been developed and provided to laboratories throughout the world. In addition, sensitive and specific ELISA procedures have been developed which employ highly purified antigens and antibodies. Included are ELISAs to detect isotype-specific antibodies to pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin, fimbriae, lipooligosaccharide (LOS), and whole pertussis cells in sera and secretions following infection with *Bordetella pertussis* or immunization with whole-cell or acellular pertussis vaccines. Statistical procedures for calculating ELISA unitage are being developed, evaluated, and standardized.

The Laboratory has made considerable contributions to the international

memory population of immune cells in the respiratory tract. We have demonstrated that fewer *B. pertussis* cells are recovered from the lungs and tracheas of mice immunized wither intranasally or via the gut mucosal with filamentous hemagglutinin prior to respiratory challenge with *B. pertussis* in comparison to unimmunized infected control animals. The demonstration of cross-protection between the gut and the respiratory mucosa is the basis for future studies using live oral vaccine vectors that express protection antigens of *B. pertussis*, as well as novel formulations of pertussis antigens, including biodegradable microspheres and liposomes. The immune response to pertussis antigens in the respiratory mucosa following experimental *b. pertussis* infection has been studied by measuring specific antibodies in the serum and secretions as well as by using assays that enumerate antigen specific B cells. We have found that specific antibody to filamentous hemagglutinin in the respiratory tract is produced many weeks after a single *B. pertussis* infection. The long-lived antibody response that we describe in mice following a single respiratory infection with *B. pertussis* is also observed in infants and may be a paradigm for the specific immune response in infants following pertussis infection. Further study of the chronic interactions of bacterial antigens with the immune system is ongoing.

T-cell epitopes of the S1 subunit of lymphocytosis promoting factor (pertussis toxin). A model has been established to study murine T-cell epitopes by measuring the proliferative response of lymphocytes to pertussis toxin. Several T-cell lines have been generated so that the epitopes can be studied at the single-cell level and progress has been made in cloning these T-cell lines. Several peptides encoding various regions of the S1 subunit have been synthesized and the peptide containing amino acid residues 65-69 has been shown to contain an epitope recognized by our T-cell lines. Studies are underway to define the minimum amino acid sequences in this peptide coding for the T-cell epitope. Studies of the epitopes on the S1 subunit recognized by human and murine T-cells are under investigation.

Future studies. There will be continuing efforts to isolate and characterize virulence factors from *B. pertussis* and evaluate their role in immunity and their possible candidacy for inclusion in a vaccine. Present issues are concerned with FHA, LPF and the fimbrial and nonfimbrial agglutinogens with increasing attention given to adenylate cyclase, heat labile toxin, and cell surface antigens as these materials become available for study. For those prospective vaccine components which are toxins, studies of structure, function, and mode of action will be done and specific methods to modify the molecule to abolish toxicity but retain immunogenicity will be pursued.

As the antigens are identified, the structural genes for the antigen will be defined, linked to various promoters, and transformed into different hosts to determine conditions for maximum expression. Studies on the folding and assembly of these antigens, both *in vitro* and *in vivo*, should help in achieving the goal of obtaining the antigens in their native form. Such materials could be used to produce reagents as well as vaccine components. Inactivated toxins of *B. pertussis* could be produced by deleting or substituting the nucleic acid sequences encoding the active or binding sites for the molecules.

Monoclonal antibodies have been developed which recognize agglutinogens on *B. pertussis* that correlate with US Reference serotypes 1, 2, 3 and 6. Studies are being conducted to further characterize these agglutinogens and to establish their relationship with previously recognized Eldering serotype antigens. Assays including immunofluorescence tests are ongoing to determine if the monoclonal antibodies are potentially useful for the diagnosis of infection with *B. pertussis*.

Our studies indicate that pertactin and FHA are protective antigens. The role of pertactin and FHA as protective antigens will be further elucidated using both active and passive immunization trials in the respiratory challenge assay. Studies on the function of pertactin and FHA, particularly as bacterial adhesins, will continue along with the identification and characterization of cell surface molecules on eucaryotic cells that bind pertactin and FHA and function as receptors for *B. pertussis*. Emphasis also will be given to structural and

standardization of pertussis ELISA procedures. In collaboration with scientists at the Statens Bakteriologiska Laboratorium in Sweden, a standardized ELISA procedure has been proposed and distributed. The Laboratory of Pertussis has procured and tested control reagents including FHA, PT, and enzyme-conjugated anti-human IgG. These control reagents, along with assay protocols and calculation software have been sent to control and research laboratories throughout the world. Studies have been performed to demonstrate the intra- and inter-laboratory reproducibility of the standardized ELISA procedures.

An assay based on the morphological change induced in Chinese Hamster Ovary (CHO) cells by pertussis toxin has been established for the assay of pertussis toxin and for the assay of toxin-neutralizing antibodies. The CHO cell assay has been used to measure antibodies in human serum samples following vaccination and/or disease. The assay has also proved useful in the measurement of toxin in vaccine preparations before toxoiding, can be used to validate the methods for toxoiding pertussis toxin, and provides a sensitive measure of the residual active pertussis toxin in pertussis toxoid preparations.

Clinical studies. The Laboratory has employed the standardized assay procedures in a variety of clinical studies to evaluate the antibody response to infection and/or immunization. In collaboration with investigators at the Centers for Disease Control and the Children's National Medical Center, the IgG, IgA, and IgM antibody response to PT, FHA, pertactin, fimbriae, LOS, and whole bacteria have been measured in serum and mucosal secretions of pertussis cases and household contacts. These results indicate that the measurement of serum IgA and IgG antibodies to the protein antigens can assist in the serodiagnosis of pertussis infection. In addition, a strong correlation between serum agglutinins and antibodies to purified fimbriae has been observed, indicating that antibodies to fimbriae are important agglutinins. IgA antibodies to pertussis components were routinely detected in saliva and nasopharyngeal aspirates of infected individuals, supporting the concept that mucosal antibodies may play an important role in immunity to pertussis and should be investigated.

The ELISA, CHO-cell, and agglutination assays have been employed to evaluate the immune response to vaccination with either whole-cell or acellular pertussis vaccines. The Laboratory is the principal serological laboratory involved in a NIAID-sponsored safety and immunogenicity trial in which 13 acellular and two whole-cell vaccines are being studied in the clinic. In addition to developing and performing the serologic assays, the Laboratory is providing guidance on analytical procedures for the serologic data.

Human Serological response to *Bordetella pertussis* antigens The human serological response to several envelope-associated proteins and adenylate cyclase toxin of *B. pertussis* was examined using immunoblot techniques. Antigens recognized by sera from individuals with culture-confirmed pertussis as well as by sera from infants immunized with three doses of conventional whole-cell pertussis vaccine included a 63,000 Da protein that was shown to be antigenically-related to a mycobacterial heat-shock protein and structurally related to the chaperonin family of proteins involved in protein folding and assembly. *Bordetella pertussis* lipooligosaccharide was also recognized by antibodies in certain of these sera. A 29,000 Da species reacted with sera from convalescent individuals whereas a 91,000 Da species reacted with sera from vaccinated individuals. Antibodies to adenylate cyclase toxin were common in sera from individuals diagnosed with pertussis, and were found to be prevalent in the sera of neonates. The neonatal antibodies are likely of maternal origin passively transferred through the placenta. Maternal antibodies may have been elicited by previous exposure of mothers to *B. pertussis* infection or by vaccination with pertussis vaccine, since all four whole-cell pertussis vaccines currently in use in the United States elicited antibodies in mice which were reactive with adenylate cyclase toxin. The data suggest that some of these antigens may play a role in immunity to pertussis and that further investigation is warranted.

Mucosal immunity to pertussis. Current efforts are focused on comparing different routes of immunization (oral, respiratory, and parenteral) and different formulations of antigen in the ability to protect from disease and to prime a

functional analysis of the newly identified bvg-regulated outer membrane proteins of *B. pertussis*.

Further biochemical and ultrastructural characterization of the porin protein is ongoing. Physical analysis of the porin protein by electromicroscopy and crystallography in collaboration with other laboratories continues; the gene for porin has been cloned and will allow further structural and functional analysis of this protein, including the development of a shared primer PCR system which can identify and differentiate *B. pertussis* and *B. parapertussis*. This molecular technique is being evaluated with clinical specimens.

Studies on the mammalian cell receptor for pertussis toxin will continue along with further characterization of the cell binding site on the B oligomer. Identification of putative receptors for pertussis toxin on other mammalian cells such as lymphocytes are planned as are the identification of receptors for other *B. pertussis* virulence factors including FHA, fimbriae type 2 and 3, and pertactin.

As prospective immunogens are identified their ability to protect in laboratory models will be evaluated, as will their ability to generate mucosal and systemic immune responses. A key goal is to delineate the mechanisms of protective immunity using well characterized monoclonal antibodies to defined epitopes. As *B. pertussis* antigens are cloned and sequenced, and protective epitopes are identified, we anticipate the development of bacterial strains which express these determinants and are able to persistently colonize the host's mucosa. One such vaccine would be a genetically manipulated, attenuated live oral vaccine that would colonize the small bowel. The Laboratory is investigating the potential use of nontoxigenic *Vibrio cholerae and Salmonella* strains for the delivery of *B. pertussis* antigens to the mucosal immune system. We have obtained expression of cloned FHA in *V. cholerae* and are evaluating the resultant live vaccine vector in the mouse aerosol challenge model. The research is being done in collaboration with John J. Mekalanos at Harvard University and Samuel Miller at Massachusetts General Hospital. Intestinal priming has been shown to generate a memory B cell population resident at distal mucosal sites, such as lungs. In addition to providing protection in the mucosa of the respiratory tract, such a vaccine would also provide herd immunity, e.g., between mother and infant, between siblings and between schoolmates; thus decreasing infection.

A number of clinical studies to evaluate acellular pertussis vaccines are in progress. These studies will establish the safety and efficacy of various vaccine components. To date, no laboratory procedure has been shown to correlate with clinical efficacy of acellular pertussis vaccines. Development of such assays is essential both for manufacturers and regulatory authorities for licensure of new vaccines as well as lot release of licensed vaccines. Defining a laboratory correlate of immunity requires a better understanding of the mechanisms of immunity to pertussis and the development of laboratory assays that adequately assess these mechanisms. Therefore the Laboratory has placed a high priority on the development of new assays that evaluate the functional role of antibodies to individual pertussis components. Using samples from completed or future efficacy trials, both new and established assays will be evaluated to determine if they correlate with clinical protection. It is likely that the Laboratory of Pertussis will be engaged in a series of studies to optimize the time of immunization, dose, interval, schedules, etc. Of particular interest would be studies to investigate the use of maternal passive immunity to protect infants coupled with active immunization started at six months of age and continued later in life.

The evaluation of vaccine efficacy usually is based on protection from disease. The ideal pertussis vaccine would protect both from infection and disease. If a vaccine is to be evaluated for its ability to prevent infection/carriage, techniques would need to be developed to diagnose and detect inapparent infection. Research to elucidate the mechanisms of infection of mucosal surfaces and the basis for mucosal immunity in pertussis would be necessary. Detection of *B. pertussis* or its products is of more diagnostic value early in the disease, whereas antibody responses are more important later in the disease process.

Refinement of serological methods already in place in the Laboratory may permit diagnosis of established pertussis infections by examining serum and secretions, while information gained from studies of the immunochemistry, monoclonal antibodies, and genetics of *B. pertussis* may provide potent and specific reagents to detect organisms by fluorescence microscopy or with genetic probes.

Studies on the mechanism of action of pertussis toxin continue. Currently, we are examining the mechanism by which pertussis toxin is secreted from *Bordetella pertussis* and how the toxin binds and enters mammalian cells. Information gained from such studies will be useful in determining the molecular mechanism by which proteins cross membrane barriers and may be useful in devising methods for delivering proteins such as immunotoxins to the interior of eucaryotic cells.

REGULATORY RESPONSIBILITIES

Regulatory Assays Performed in the Laboratory, Oct. 1, 1991 to July 24, 1992:

Potency Tests:	
Test whole cell pertussis vaccines and standards	119
Test acellular pertussis vaccines and standards	14
Virulence Controls	69
Total	202

Mouse Toxicity Tests:	
Test whole-cell pertussis vaccines	41
Test acellular pertussis vaccines	30
Controls	24
Total	95

Investigational New Drugs:

Total number of submissions received	125 for a total of approximately 465 reviews
Original Submissions	6
New License amendments/applications	8
Master Files	0

From October 1991 to September 1992 there were ten reviewers: Dr. Brennan, Dr. Burns, Dr. Finn, Ms. Gould-Kostka, Ms. Hannah, Ms. Hausman, Ms. Jansen, Dr. Meade, Dr. Shahin and Dr. Manclark.

Other Activities:

Meade, Bruce D. Invited participant to a "Workshop on Serological Testing of Component Pertussis Vaccine Antibodies in Human Sera". Toronto, Canada, May 12-13, 1992

Lynn, Freyja V.C. Invited participant to a "Workshop on Serological Testing of Component Pertussis Vaccine Antibodies in Human Sera". Toronto, Canada, May 12-13, 1992.

Finn, Theresa M. Participant in the "Conference on Emerging Microbes and Microbial Diseases". Washington, D.C., November 13-15, 1992.

Finn, Theresa M. Participant in the "Third Conference on Microbial Virulence Factors and the Human Immune Response". Oakland, CA, October 18-20, 1991.

Manclark, Charles R. Temporary Advisor to the World Health Organization and the Government of Mexico to present a course on the production and control of bacterial vaccines. Mexico City, Mexico, April 26 - May 1, 1992.

Manclark, Charles R. Invited participant, Temporary advisory to the World Health Orgnaication and Rappateur for a joint meeting of the World Health Organization and the International Task Force on Hepatitis Immuinzation to discuss Quadrivalent Diphtheria, Tetanus, Pertussis and Hepatitis B Vaccines. Geneva, Switzerland. May 7-8, 1992.

Manclark, Charles R. Consultant to the Rijtesinstituut voor Volksgezondheid en Milieuhygiene, toolve problems with the manufacture and control of traditional whole-cell and the new acellular pertussis vaccines. Bilthoven, The Netherlands, September 15-17, 1992.

Manclark, Charles R. Invited participant and panel discussant at a meeting on DTP Vaccines: Development, Clinical Trials and USage. Lansing, Michigan, September 21, 1992.

Invited Lectures:

Burns, DL. 1991. Molecular investigation of pertussis. Public Health Service Technology Transfer Symposium, National Institutes of Health, Bethesda, Maryland.

Burns, DL. 1991. Mechanism of action of pertussis toxin. Center for Vaccine Development, University of Maryland, Baltimore, Maryland.

Meade, BD. 1992. The National Institute of Allergy and Infectious Diseases Phase 2 study of acelluler pertussis vaccines. Workshop on Serological Testing of Component Pertussis Vaccine Antibodies in Human Sera. Toronto, Canada, May 12-13, 1992.

Lynn, FVC. 1992. Quality Control of ELISA Testing Workshop on Serological Tsting of Component Pertussis Vaccine Antibodies in Human Sera. Toronto, Canada, May 12-13, 1992.

Brennan, MJ. 1991. Adherence of *Bordetella pertussis* to Mammalian Cells. Microbial Pathogenesis lecture series sponsored by Centers for Disease Control, Atlanta, GA, February, 1992.

Brennan, MJ. 1991. Multifactorial Interactions of *Bordetella pertussis* with Mammalian Cells. Gordon Research Conference on Microbial Adhesion. Newport, RI, July 1991.

Brennan, MJ. 1991. Department of Microbiology Lecture Series: Mechanisms of Adherence of *Bordetella pertussis*. University of Maryland, College Park, MD October 1991.

Brennan, MJ. 1992. Adhesins of *Bordetella pertussis* that Mimic Eukaryotic Attachment Proteins. Department of Infectious Diseases, St. Louis University Medical Center, St. Louis, MO, January 1992.

Brennan, MJ. 1992. Molecular Mimicry in *Bordetella pertussis*. UA/UC Conference in Infectious Diseases, Kananaskis Village, Alberta, Canads, June 1992.

Shahin, RD. 1991. Genetically Engineered VAccines: Prospects for Oral Disease Prevention. National Institute of Dental Research Workshop. November 6-8, 1991.

Shahin, RD. 1991. Protective Immunity to *Bordetella pertussis*y Tract Infection.

Bureau for Microbiology, Canadian Laboratory Centre for Disease Control, Ottawa, Canada. December 10, 1991.

Manclark, CR. 1992. The control testing of pertussis vaccines. Laboratorio Nacional de Salud Publica, Mexico City, Mexico, April 29, 1992.

Manclark, CR. 1992. Scientific and technical considerations of the formulation of diphtheria and tetanus toxoids and pertussis vaccine adsorbed when combined with hepatitis B vaccine. Meeting on Quadrivalent Diphtheria-Tetanus-Pertussis-Hepatitis B Vaccine. World Health Organization and the International Task Force on Hepatitis B Immunization. World Health Organization, Geneva, Switzerland. May 7-8, 1992.

Manclark, CR. 1992. Growth of *Bordetella pertussis* in submerged culture: development and validation of a manufacturing process. Rijksinstituut vook Volksgeqondheid en Milieuhygine, Biltholven, The Netherlands. September 16, 1992.

Manclark, CR. 1992. Pharmacology and development of DTP vaccines. DTP Vaccines: Development, CLinical Trials and Usage. Michigan Department of Public Health, Lanswn, September 21, 1992.

Presentations:

Shahin, RD, Hamel J, Brodeur B and Leef MF. 1992. analysis of protective and non-protective monoclonal antibodies specific for *Bordetella pertussis* Lipooligosaccharide. Abstracts of the American Society for Microbiology, New Orleans.

Amsbaugh, D, Manclark CR and Shahin RD. 1992. Long-lived antibody response to filamentous hemagglutinin following *Bordetella pertussis* infection. Abstracts of the American Society for Microbiology, New Orleans.

Shahin, R, Leef M, Amsbaugh D, Eldridge J, Hudson M and Gilley R. 1992. Analysis of protective mucosal immunity ot respiratory *Bordetella pertussis* infection. Abstracts of the 7th International Congress of Mucosal Immunology, Prague, Czechoslovakia.

Hannah JH, Leininger E, Bhargava A, Brennan MJ. 1992. Nonfimbrial adhesins of *Bordetella pertussis* that mimic eukaryotic adhesive proteins. Annual Meeting of the American Society for Microbiology, New Orleans, LA.

Leininger E, Renauld GA, Bowen SH, Hannah JH, Stibitz ES, Locht C, Kenimer JG, Brennan MJ. 1992. Identification of functional domains on *Bordetella pertussis* filamentous hemagglutinin. Annual Meeting of the American Society for Microbiology, New Orleans, LA.
Li ZM, Halperin SA, Jansen DL, Finn TM, Manclark CR, Brennan, MJ. 1992. Identification of pertussis in children using molecular techniques. Annual Meeting of the American Society for Microbiology, New Orleans, LA.

Patents/Patents Pending:

Patent pending: Brennan MJ, Li ZM, Cowell JL, Manclark CR. US patent 07/312.097: Hybridomas and resulting monoclonal antibodies directed against antigens of *Bordetella pertussis*, February 17, 1989.

Patent pending: Shahin RD, Manclark CR. US patent pending: A Protective Vaccine.

Patent awarded: Burns DL, Brennan MJ, Gould-Koskta JL, Manclark CR. Process for the purification of a 69,000 Da outer membrane protein of *Bordetella pertussis*.

Patent No. 5,101,014, March 31, 1992.

Honors and Awards:

FDA Group Recognition Award
Acellular Pertussis Vaccine Characterization Group
Laboratory of Pertussis Staff Members
Drusilla Burns, Ph.D., Jeanine Gould-Kostka, Julie Hannah, Sally Hausman, Deborah Jansen, Frederick Johnson, Mary Leef

FDA Commendable Service Award
Michael Brennan, Ph.D.

PHS Superior Service Award
Laboratory of Pertussis Staff Members
Diana Amsbaugh, Michael Brennan, Ph.D., Drusilla Burns, Ph.D., Jeanine Gould-Kostka, Julie Hannah, Sally Hausman, Deborah Jansen, Frederick Johnson, Mary Leef, Bruce Meade, Ph.D., Theresa Romani, Roberta Shahin, Ph.D.

DHHS Distinguished Service Award - Biomedical Research
Charles R. Manclark, Ph.D.

PUBLICATIONS

DIVISION OF BACTERIAL PRODUCTS
ANNUAL REPORT
OCTOBER 1, 1991 TO SEPTEMBER 30, 1992

PUBLICATIONS

Pavliak, V., Pozsgay, V., Kovac, P., Karpas, A., Chu, C., Schneerson, R., Robbins, J.B., Glaudemans, C.P.J. 1991. Mapping the Binding Mode of the O-Antigen of *Shigella dysenteriae* Type 1 to a Monoclonal Murine Antibody. Submitted to J. Biol Chem.

Gu, X-X., Tsai, C-M., Karpas, A. B. 1991. Production and Characterization of Monoclonal Antibodies to Type 8 Lopooligosaccharide of *Neiseria meningitidis*. Accepted, J. Clin Micro. 30(8)

Klutch, M., Karpas, A., Woerner, A., Zhang, P-f., Marcus-Sekura, C.J. 1991. Production of Type-Specific Anti-Human Immunodificiency Virus Type I Monoclonal Antibodies Reactiec with Epitopes in the C-Termini ofIntegrase and Reverse Transcriptase. Submitted to J.Vir.

Pavliak, V., Pozsgay, V., Kovac, P., Karpas, A., Chu, C., Schneerson, R., Robbins, J.B., Glaudemans, C.P.J. 1991. Mapping the Binding Epitope of the O-Antigen of *Shigella dysenteriae* Type 1. The Gordon Conference, June 24-28. New Hampshire, Presentation.

Klutch, M., Karpas, A., Woerner, A., Marcus-Sekura, C. 1991. Characterization of Anti-HIV-1 Integrese Hybridomas. NIH Research Festival. Sept 23-24. Poster L8.

Leininger, Elizabeth, Carol A. Ewanowich, Amit Bhargava, Mark S. Peppler, James G. Kenimer, and Michael J. Brennan. 1992. Comparative Role of the Arg-Gly-Asp Sequence Present in the *Bordetella pertussis* Adhesins, Pertactin and Filamentous Hemaggluitinin. Infect. Immun. **60**:2380-2385.

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Ansher, S., Thompson, W. and Watson,R. The Effects of Cytokines and Murine

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Ansher, S., Thompson, W., Snoy, P. and Habig, W. Role of Endotoxin in Alterations of Hepatic Drug Metabolism by Diphtheria and Tetanus Toxoids and Pertussis Vaccine Adsorbed. Infection and Immunity: 60: Sept. 1992.

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Morris S, Nair J, and Rouse DA. The catalase-peroxidase of *Mycobacterium intracellulare*: Nucleotide sequence analysis and expression in *Escherichia coli.* J. Gen. Microbiol. 1992 (in press).

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Li, C. M., Loechel S, Huang CH, Taylor J, Miller L, Clyde, Jr. WA, Barile MF and Hu P-C. Prospects for development of Mycoplasma pneumoniae vaccines. IOM Proceedings, Ames, Iowa. Aug 2-7, 1992.

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Z01 BA 07001-24 LP

Meade DB, Deforest A, Romani TA, O'Brien CH, Swartz CB, Bradley AT, Palmer PS, Hager C, Holland KL, Deloria MA, Reed GF, Edwards KM, and the NIAID Acellular Pertussis Vaccine Study Group. 1992. Evaluation of the serologic methods employed in a multicenter trial of acellular pertussis vaccines: Demonstration of intra- and inter-laboratory reproducibility. Annual Meeting of the Society for Pediatric Research, Baltimore, Maryland (Abstract)

Arciniega JL, Hewlett EL, Edwards KM, Meade B. 1992. Antibodies to *Bordetella pertussis* adenylate cyclase toxin in neonatal sera. ASM Annual Meeting (Abstract)

SEMINAR SERIES

DIVISION OF BACTERIAL PRODUCTS SEMINAR SERIES
OCTOBER 1, 1991 through September 30, 1992

Date, (Sponsor), Speaker and Affiliation, Title of Presentation

October 10, 1991 (Jeanine Gould-Kostka)
Dr. Sue Wickner, Laboratory of Molecular Biology, National Cencer Institute National Institutes of Health.
"The function of DnaJ and DnaK in plasmid P1 replication."

October 24, 1991 (Clara Lin, Ph.D.)
Dr. Kurt Klimpel, Laboratory of Microbial Ecology, Intramural Research Program, National Institute of Dental Research.
"Proteolytic activation of bacterial toxins."

October 31, 1991 (Virginia Johnson, Ph.D.)
Dr. Pierre HenKart, Immunology Branch, National Cancer Institute
"Granule exocytotic model of lymphocyte-mediated gytotoxicity."

November 7, 1991 (Zhong-ming Li, M.D.)
Dr. Mei G. Lei, Ph.D., Department of Microbiology, Molecular Genetics and Immunity, School of Medicine, University of Kansas Medical Center.
"Identification and characterization of LPS Binding Proteins on mammalian cells."

November 12, 1991 (Carl Frasch, Ph.D.)
Dr. Gregg Silvermann, Department of Medicine, University of California at San Diego.
"Variable region diversity in human antibodies to *haemophilus influenzae* type b and pneumoccal capsular antibodies."

November 21, 1991 (Kathryn Stein, Ph.D.)
Dr. Donald Marcus, Department of Internal Medicine, Baylor College of Medicine, Houston, Texas.
"Structure-function studies of anti-carbohydrate antibodies."

November 21, 1991 (Jane Halpern, Ph.D.)
Dr. David Neville, Laboratory of Molecular Biology, National Institute of Mental Health.
"In vivo T cell ablation by an anti-CD3 immunotoxin based on a diphtheria toxin binding site mutant. Potential therapy for graft vs. host disease and AIDS."

December 5, 1991 (William H. Habig, Ph.D.)
Ulrich Eisel, Ph.D. (Visiting Associate, Laboratory of Cell Biology, National Institite of Mental Health.
"Molecular biology of the tetanus toxin gene."

December 11, 1991 (William H. Habig, Ph.D.)
T. Jacob John, Ph.D., Christian Medical College and Hospital, Department of Virology and Immunology, Vellore, India.
"Diagnostic antigen detection tests for *salmonella typhi*."

December 12, 1991 (Jane Halpern, Ph.D.)
Dr. Dennis Kopecko, Department of Bacterial Immunology, Walter Reed Army Institute of Research.
"Travel expands the mind and often loosens the bowel: How invasive enteric bacteria initiate disease."

December 19, 1991 (Kathryn Stein, Ph.D.)

Dr. David Zopf, Washington, D.C.
"Isolation of oligosaccharide andtigens by weak affinity chromatography."

January 15, 1992 (Kathryn Stein, Ph.D.)
John Schreiber, M.D., Department of Pediatrics, Case Western Reserve Medical School, Cleveland, Ohio.
"Anti-idiotypes as alternative vaccines for bacterial polysaccharides."

January 16, 1992 (Jane Halpern, Ph.D.)
Dr. Alison O'Brien, Department of Microbiology, Uniformed Services University of the Health Sciences.
"Pathogenic mechanisms of shiga-like toxin producing *E. coli*."

January 23, 1992 (Chi-Jen Lee, Ph.D.)
Dr. David E. Briles, Department of Microbiology, University of Alabama at Birmingham.
"Protective immunity to non-capsular antibens of *Streptococcus pneumoniae*."

January 30, 1992 (Roberta Shahin, Ph.D.)
Dr. Carl Alving, Department of Biochemistry, Walter Reed Army Institute of Research.
"Liposomes as carriers of vaccines."

February 6, 1992 (Theresa Finn, Ph.D.)
Dr. David Beattie, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA.
"Signal transduction and gene expression in *Bordetella pertussis*."

February 13, 1992 (Kathryn Stein, Ph.D.)
Dr. Aino Takala, Department of International Health, Johns Hopkins University, School of Hygiene and Public Health, Baltimore, MD.
"Reduction of oropharyngeal carriage of *Haemophilur influenzae* type b (Hib) with Hib Conjugate Vaccines."

February 20, 1992 (Bascom F. Anthony, M.D.)
Dr. Barry Gray, Department of Pediatrics, University of Alabama, Birmingham, AL.
"Group B Streptococci: New approaches to pathogenic mechanisms and prevention."

February 27, 1992 (Bascom F. Anthony, M.D.)
Dr. John Clemens, Chief, Epidemiology Branch, National Institute of Child Health and Human Development, National Institutes of Health.
"Interactive relationships between PRP-T and DTP vaccines."

March 5, 1992 (Jane Halpern, Ph.D.)
Dr. Ken Stover, Med Immune, Inc., Gaithersburg, MD
"Progress on the development of BCG as a recombinant vaccine vehicle."

March 19, 1992 (Michael Brennan, Ph.D.)
Dr. David G. Russell, Associate Professor, Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri.
"Leishmania nd the taming of the macrophage."

March 25, 1992 (Kathryn Stein, Ph.D.)
Pablo A. Garcia, Ph.D.
Department of Chemistry, University of North Carolina at Chapel Hill.
"Revertanta that Suppress Mating-type Regulation of CYC7-H2 Gene in Yeast."

April 2, 1991 (Lyn Olson, Ph.D.)
Dr. Barry Cole, Department of Internal Medicine, University of Internal Medicine, University of Utah College of Medicine, Salt Lake City, Utah.
"Mycoplasma superantigen MAM: Immunoreguation and Role in Autoimmune Disease."

April 9, 1992 (Willie E. Vann, Ph.D.)
Dr. Jeffrey D. Esko, Department of Biochemistry, University of Alabama at Birmingham.
"Biological activity of heparan sulfate."

April 16, 1992 (Elizabeth Leininger, Ph.D.)
Dr. Stephen J. Barenkamp, Associate Professor of Pediatrics, St. Louis University School of Medicine, St. Louis, Missouri.
"Haemophilus high molecular weight proteins related to *Bordetella pertussis* FHA."

April 23, 1992 (Bascom F. Anthony, M.D.)
Dr. Sandra Holmes, Department of Pediatrics, Washington University School of Medicine, St. Louis, Missouri.
Haemophilus influenzae b Conjugate vaccines: Studiees of immunogenicity and vaccine failures."

May 7, 1992 (Micheal Brennan, Ph.D.)
Dr. Richard J. Weber, Division of Cytokine Biology, Center for Biologics Evaluation and Research, FDA.
"Neural Control of Natural Cytotoxic Lymphocytes."

May 8, 1992 (Bascom F. Anthony, M.D.)
Dr. Juhani Eskola, National Public Health Institute, Department of Infectious Disease Epidemiology, Helsinki, Finland.
"*Haemophilus b* vaccine and disease in Finland: A status report."

May 14, 1992 (William H. Habig, Ph.D.)
Dr. Karen Elkins, Walter Reed Army Insitute of Research
"*In vivo* delivery of cytokines via recombinant viruses for immunomodulation of bacterial diseases and cancer: From *Francisella tularensis* to tumor regression."

May 21, 1992 (James Kenimer, Ph.D.)
Dr. Chris Taylor, Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, NIH.
"Effects of cytokines on the antibody response to bacterial polysaccharide antigens."

May 21, 1992 (Bascom F. Anthony, M.D.)
Dr. Karen Farizo, Division of HIV/AIDS, Centers for Disease Control, Atlanta, GA.
"Epidemiological features of pertussis in the United STates, 1980-1989."

June 4, 1992 (Sherry Ansher, Ph.D.
Dr. Ronald Watson, University of Arixonia Health Sciences Center, College of Medicine, Department of Family and Community Medicine.
"Effect of Alcohol on Disease and Cancer Resistance in Murine AIDS."

June 10, 1992 (James Kenimer, Ph.D.)

Dr. Kathleen Barnes, University of Florida, Department of Anthropology.
"The role of household pests in the epiidemiology transition of human allergy: Human behavior and domestic environment in Barbados."

June 11, 1992 (Roberta Shahin, Ph.D.)
Dr. Michael Cole, Department of Microbiology, Georgetown Medical School, Washington, D.C.
"Humoral immunity to commensal oral bacteria."

June 18, 1992 (Bascom F. Anthony, M.D.)
Dr. Frank Collins, Trudeau Institute, Saranac Lake, New York.
"Mycobacterial infections in a model of immuonsuppression."

June 24, 1992 (Carl E. Frasch, Ph.D.)
Dr. Paula Kuzemenska, Institute of Hygine and Epidemiology, Prague, Czechoslovakia.
"Bacterial infections in the Czech Republic aquired through airborne transmission."

June 25, 1992 (Willie Vann, Ph.D.)
Dr. Ajit Varki, Division of Hematology/Oncology, Department of Medicine, La Jolla, California.
"Sialic acids on cell surfaces: Exploring the outer fringes of glycobiology."

July 22, 1992 (Bascom F. Anthony, M.D./William H. Habig, Ph.D.)
Dr. Raymond Daynes, Division of Cell Biology and Immunology, University of Utah School of Medicine, Salt Lake City, Utah.
"Enhancement of vaccination efficiency employing natural regulators of the immune response."

PROJECTS

ANNUAL REPORT

OCTOBER 1, 1991 TO SEPTEMBER 30, 1992

DIVISION OF BACTERIAL PRODUCTS

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH
FOOD AND DRUG ADMINISTRATION

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-04001-07 LAIC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Identification of Neutralizing Epitopes on Pertussis Toxin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: James G. Kenimer, Ph.D., Chief, LAIC, DBP, CBER

Peter G. Probst, Biologist, LAIC, DBP, CBER
H.R. Kaslow, Dept. of Physiology and Biophysics, USC, Los Angeles, CA

COOPERATING UNITS *(if any)*

Univ. of Southern California, Los Angeles, CA

LAB/BRANCH

Laboratory of Allergy and Immunochemistry, DBP, CBER

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The laboratory continues to provide investigators in many research laboratories within and oustide of the FDA with monoclonal antibodies (mABs) generated against pertussis toxin(PT). These mABs have been useful in research directed towards identification of epitopes within the PT molecule, which are a significant factor in acellular vaccine design.

PHS 6040 (Rev 1/84)

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 BA-04003-09 LAIC

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Production and Characterization of Monoclonal Antibodies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*
PI: Arthur B. Karpas, Ph.D., Coordinator Hybridoma Facility, LAIC, DBP, CBER
James G. Kenimer, Ph.D., Chief, LAIC, DBP, CBER
Peter G. Probst, Biologist, LAIC, DBP, CBER
Mona F. Febus, Microbiologist, LAIC, DBP, CBER
Jennifer Bridgewater, Microbiologist, LAI, DBP, CBER

COOPERATING UNITS *(if any)*
DBB and DV, CBER; Lab of Developmental and Molecular Immunity, NICHD, NIH; Arkansas Childrens Hospital, Little Rock, AR; Federal Univ. of Minas Gerias, Brazil; Kyushu Univ, Fukuoka, Japan; and 5 other Labs in DBP, CBER

LAB/BRANCH
Laboratory of Allergy and Immunochemistry, DBP, CBER

SECTION

INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
2.2	0.9	1.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

The Hybridoma Facility of the Laboratory of Allergy and Immunochemistry collaborates with numerous laboratories in FDA, NIH and other institutions in the production and characterization of monoclonal antibodies (MAbs). During this reporting period, the Facility has conducted or assisted in nineteen hybridoma fusions. These fusions required screening by ELISA or Western blot of approximately 5700 wells of culture fluid as a result 330 lines and clones have been selected for further study and subsequently expanded in order to allow for frozen storage. Procedures have been initiated for eithg additional fusions scheduled during the next reporting period.

The Facility has also conducted related procudures from previously completed fusions resulting in 49 lines being infused in mice and over a liter of ascites collected. Work has also been conducted to provide myeloma cells to two other laboratories, to grow cell lines in mass culture to collect over 2700 ml of protein free or regular media to determine immunogenicity of a protein in mice and to rescue cell lines damaged in shipment.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-04004-04 LAIC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Characterization of Bacterial Adhesins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Elizabeth Leininger, Ph. D., Scientist, LAIC, DBP, CBER
James G. Kenimer, Ph. D., Chief, LAIC, DBP, CBER
Michael J. Brennan, Ph.D., Biologist, LP, DBP, CBER
Mark S. Peppler, Ph.D., University of Alberta
Carol S. Ewanowich, Ph.D., University of Alberta

COOPERATING UNITS *(if any)*

Laboratory of Pertussis, DBP, CBER
University of Alberta, Edmonton, Alberta, Canada

LAB/BRANCH

Laboratory of Allergy and Immunochemistry, DBP, CBER

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS 0.5	PROFESSIONAL 0.5	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The importance of various putative *Bordetella pertussis* cell adhesins which mediate the adherence of the bacteria to mammalian cells is being investigated. We have demonstrated that pertactin, a 69 kDa surface protein, can promote the adherence of Chinese hamster ovary (CHO) cells. We have also shown that another surface protein, filamentous hemagglutinin (FHA) can also independently promote the adherence of mammalian cells. To investigate the mechanism of attachment of these protein to their cell receptor, we have studied the role of the sequence Arginine-Glycine-Aspartic Acid (RGD), which is a sequence found in pertactin and FHA, in mediating cell adherence. The adherence of CHO cells to pertactin occurs via an RGD binging site on the protein. The RGD sequence does not seem to be involved in the interaction of CHO cells to FHA, a lectin-like interaction seems more probable to be to mechanism of cell interaction used by this protein. It has recently been shown that *B. pertussis* can invade and survive within mammalian cells. Peptides homologous to the RGD sequence from pertactin can inhibit invasion of the bacteria. The peptides derived from the sequence of FHA with RGD had no effect on invasion. We have further demonstrated the role of FHA and pertactin in attachment and invasion by coating the noninvasive *Staphilococus aureus* bacteria with purified pertactin or FHA and quantitating the amount of colony forming units found internalized in HeLa cells. Specific RGD-containing peptide inhibhtion of invasion was achieved with pertactin-coated *S. Aureus*.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-04005-03 LAIC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Characterization of Bacterial Mutants in Cell Adhesins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Elizabeth Leininger, Ph.D., Scientist, LAIC, DBP, CBER
James G. Kenimer, Ph.D., Chief, LAIC, DBP, CBER
Michael J. Brennan, Ph.D., Biologist, LP, DBP, CBER
Amit Bhargava, M.D., Visiting Scientist, LP, DBP, CBER
Ian Charles, Ph.D., Wellcome Biotech, Kent, UK

COOPERATING UNITS *(if any)*

Wellcome Biotech, Kent, UK
Laboratory of Pertussis, DBP, CBER

LAB/BRANCH

Laboratory of Allergy and Immunochemistry, DBP, CBER

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In order to investigate the role of various surface proteins from the bacteria *B. pertussis* in the mechanism of attachment of the whole bacteria to mammalian cells, mutants have been constructed which do not express specific proteins normally localized on the surface of the bacteria. By use of antibiotic markers and double recombination events, mutants deficient in the expression of pertactin, FHA or both proteins have been constructed. We have characterized these mutants in terms of the expression or lack of expression and activity of various proteins including pertactin, FHA, pertussis toxin, adenylate cyclase, fimbriae, and olipooligosaccharide. We have also characterized these mutants in terms of their ability to promote cell attachment. A decrease in the ability to adhere to mammalian cells has been seen with all three mutants, and a synergistic effect of decrease adherence is observed with mutant lacking both pertactin and FHA. With the use of the pertussis suicide vector pRTP1, mutants lacking the expression of pertussis toxin and other virulent associated proteins are being constructed to determine their possible role in bacterial adherence.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-04006-03 LAIC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Monoclonal Antibodies Directed Against Filamentous Hemaglutinins.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Elizabeth Leininger, Ph.D., Scientist, LAIC, DBP, CBER
James G. Kenimer, Ph.D., Chief, LAIC, DBP, CBER
Stephen H. Bowen, Biologist, LAIC, DBP, CBER
Peter G. Probst, Biologist, LAIC, DBP, CBER
Stephen J. Barenkamp, M.D., Washington University School of Medicine

COOPERATING UNITS *(if any)*

Washington University School of Medicine, Div. of Infectious Diseases, St. Louis, Missouri.

LAB/BRANCH

Laboratory of Allergy and Immunochemistry, DBP, CBER

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER.
0.4	0.4	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Three monoclonal antibodies (MO8-X3C, MO8-X3E and MO8-X4B) directed against filamentous hemagglutinin (FHA) have been produced. We have characterized these monoclonal antibodies and investigated their role in inhibiting attachment of mammalian cells to FHA. The monoclonal antibodies have been purified by HPLC and used in ELISA and Western Blots to screen for FHA. MO8-X3C and MO8-X3E, both IgG1 subtype, can inhibit the attachment of Chinese hamster ovary (CHO) cells to purified FHA when this protein is coated on plastic wells. MO8-X3C has also been shown to inhibit the attachment of the whole bacteria, *B. pertussis*, to CHO cell monolayers. Furthermore, this monoclonal antibody, MO8-X3C, can also inhibit the invasion of HeLa cells.by *B. pertussis*. These antibodies should be useful reagents for screening *B. pertussis* mutants for expression of FHA and in regulation and characteriazation of pertussis acellular vaccines. FHA is one of the primary candidates being considered for future pertussis acellular vaccines. Determining the binding epitope for these MAbs on FHA should help elucidate the cell attachment site of FHA. We are using the epitope scanning pin technology, where 15 amino acid peptides are synthesized on activated pins and with increments of 4 amino acids along the FHA protein sequence, the entire sequence is covered. We will also study the role of these mAb directed against FHA in passive immunization of mice against *B. pertussis* infection to further understand the pathogenesis of B. pertussis and the different mechanisms of protection. We have also recently found cross reactivity with these anti-FHA MAbs and two high molecular weight outer membrane proteins from nontypable *Hemophilus influenza*. We are further characterizing these proteins which contain some homology with FHA at the protein level.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BA-04007-02 LAIC

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Characterization of Bacterial Receptors on Mammalian Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Elizabeth Leininger, Ph.D., Scientist, LAIC, DBP, CBER
James G. Kenimer, Ph.D., Chief, LAIC, DBP, CBER
Michael J. Brennan, Ph.D., Biologist, LP, DBP, CBER
Julie H. Hannah, M.P.H., Microbiologist, LP, DBP, CBER

COOPERATING UNITS *(if any)*

Laboratory of Pertussis, DBP, CBER

LAB/BRANCH

Laboratory of Allergy and Immunochemistry, DBP, CBER

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

We are investigating the nature of the cell receptor for various bacterial adhesins. Experiments with thin layer chromatography (TLC) of glycolipid extracts indicate that virulent strains *B. pertussis* bind to certain glycolipids present in lung tissues. We are currently investigating the structure of the glycolipids involved in this adhesion. Using purified glycolipids separated on thin layer chromatography, we have demonstrated that the virulent strains of *B. pertussis* bind to sulfatides and to asialo GM1. Avirulent strains of this bacteria were found to also bind to asialo GM1 but not to sulfatides. We are also trying to identify which bacterial adhesins are interacting with the glycolipids. Since pertactin and FHA, bacterial adhesins, both contain the sequence Arginine-Glycine-Aspartic Acid (RGD), we are also investigating the role of integrin receptors in bacterial adherence to mammalian cells.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 BA-04008-02 LAIC

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Synthesis of Oligonucleotides.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Peter G. Probst, Biologist, LAIC, DBP, CBER
James G. Kenimer, Ph.D., Chief, LAIC, DBP, CBER

COOPERATING UNITS *(if any)*
Five Laboratories within DBP, CBER

LAB/BRANCH
Laboratory of Allergy and Immunochemistry, DBP, CBER

SECTION

INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Synthesis of oligonucleotides by our laboratory in collaborative efforts with several laboratories within the Division of Bacterial Products has proven invaluable to the following research programs: A PCR approach to clone and express a potential surface antigen from Mycoplasma pneumonia, and to modify existing cloning vectors. Development of a method to allow for direct cloning of PCR generated products, and as templates for DNA sequencing in Mycoplasma. Completion of the nucleotide sequence analysis of mycobacterial genes encoding several immunoreactive antigens. Isolation of the pneumococcal pneumolysin gene from two types of genomic DNA, to produce trunicated point mutations of the pneumolynsin gene, and for conjugation of a single polysaccharide. Continued research in the production of selective mutation and amplification of bacterial toxin genes for use in examining the effect of mutation on toxin function. Primers were also utilized in the development of PCT protocols to rapidly detect non tuberculosos mycobacterial infections.

Research in our laboratory is being conducted in the use of cloned overlapping strnads, to develop an in vitro transcription probe for allergenic screening of apple to determine and isolate the allergenic component. Studies are also continuing to include the use of oligonucleotides as tools for the creation of an epitope library.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-04010-01 LAIC

PERIOD COVERED

December 15, 1991 - September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Epitope Library.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Li-Shan Hsieh, Ph.D., Molecular Biologist, LAIC, DBP, CBER
James G. Kenimer, Ph.D., Chief, LAIC, DBP, CBER

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COOPERATING UNITS *(if any)*

-

LAB/BRANCH

Laboratory of Allergy and Immunochemistry, DBP, CBER

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS: 1.0	**PROFESSIONAL:** 1.0	**OTHER:**

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A new type of synthetic peptide library for identifying ligand-binding activity was reported last year (Nature 354:82-84, 1991). The method involves the chemical synthesis of a random peptide library on polyacrylamide beads using a 'pool & split synthesis approach'. A peptide library generated by this method results in each bead containing only a single peptide. The purpose of this experiment was to utilize this new technology to determine epitopes of the correspondent antibodies or allergens.

The experiment involves: (1) designing an organic and water compatible linker, which could stand the organic acidity and basicity during the synthesis as well as the aqueous basicity during the antibody recognition; (2) using standard Fmoc-chemistry to perform peptide synthesis on polyacrylamide beads; (3) incubating the peptide library with a reporter coupled molecules; (4) sequencing the oligopeptide on corresponding beads. The preliminary result looks successful. The color indication shows clear positive and negative differences using a monoclonal antibody which binds to a known amino acid sequence.

We have now synthesized a random peptide library on beads, screened it with monoclonal antibody with unknown epitopes specificity, and are subsequently sequencing the positive beads.

With an apple fruit cDNA library in hand we can also clone the apple allergenic proteins using the peptide sequence information which are obtained from epitope library when screen with patient serum.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-04011-01 LAIC

PERIOD COVERED

January 1, 1992 - September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Standardization of Allergenic Extracts Using ELISA.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Peter G. Probst, Biologist, LAIC, DBP, CBER
Catherine A. Miller, Medical Technologist, LAIC, DBP, CBER
James G. Kenimer, Ph.D., Chief, LAIC, DBP, CBER

COOPERATING UNITS *(if any)*

-

LAB/BRANCH

Laboratory of Allergy and Immunochemistry, DBP, CBER

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 1.0	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Research is being conducted toward the development of an ELISA system for standardization of allergenic extracts. Current standardization testing of most allergenic products is accomplished by way of the RAST inhibition assay. Successful development of an ELISA system would greatly reduce the expense and personnel time incurred by this laboratory in the testing of allergenic products prior to their release for sale. Initial studies involving capture antigen and antigen/serum inhibition dilution parameters indicated that the system is applicable to grass pollens. Currently, studies are proceeding with mites.

PHS 6040 (Rev 1/84)

U.S. GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-04012-01 LAIC

PERIOD COVERED

January 1, 1992 - September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Adverse Allergic Reactions to Vaccines.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Peter G. Probst, Biologist, LAIC, DBP, CBER
Jennifer Bridgewater, Biologist LAIC, DBP, CBER
James G. Kenimer, Ph.D., Chief, LAIC, DBP, CBER

COOPERATING UNITS *(if any)*

-

LAB/BRANCH

Laboratory of Allergy and Immunochemistry, DBP, CBER

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An enzyme linked immunosorbant assay (ELISA) incorporating mouse anti-human IgE monoclonals as capture and secondary antibodies has been tested for measurement of total IgE in human serum samples. Reagents tested have provided accurate, reproducible readings using an in-house standard human serum pool with known IgE content. Sera obtained from JEV reactors participating in the U.S. Navy immunization program and from JEV reactors immunized in Denmark have been examined for total IgE content. Pre and post-immunization sera obtained from plague vaccine immunized individuals have also been examined for total IgE. Assay development for detection of specific IgE to vaccines and vaccine components has been initiated. Detection of plague vaccine specific IgE has been indicated in limited assays.

PHS 6040 (Rev 1/84)

U.S. GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 BA-04013-01 LAIC

PERIOD COVERED
January 1, 1992 - September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Characterization of Specific Allergens.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Elizabeth Leininger, Ph.D., Staff Fellow, LAIC, DBP, CBER
Li-Shan Hsieh, Ph.D., Molecular Biologist, LAIC, DBP, CBER
Stephen H. Bowen, Biologist, LAIC, DBP, CBER
James G. Kenimer, Ph.D., Chief, LAIC, DBP, CBER

COOPERATING UNITS *(if any)*
-

LAB/BRANCH
Laboratory of Allergy and Immunochemistry, DBP, CBER

SECTION

INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.0	1.0	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The identification of specific allergens in allergenic extracts is of great importance to further understand the mechanism of allergic reactions and to allow proper standardization of commercially available allergenic extracts. We are investigating the human IgE response to specific protein bands by separating the crude extracts using electrophoresis, followed by electroblotting the proteins on to nitrocellulose and immunoclotting with serum from allergic patients. We have found several reactive bands in apple extracts and we have obtained the amino-terminal protein sequence from two specific reactive bands. We are in the process of cloning the genes from an apple cDNA library that code for these specific apple allergens.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 BA-01001-11 LAIC

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Potency Levels in Standardized Allergenic Extracts

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: M.C. Anderson, M.S., Microbiologist, LAIC, DBP, CBER

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COOPERATING UNITS *(if any)*
-

LAB/BRANCH
Laboratory of Allergenic Products, DBP

SECTION

INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER:
0.05	0.05	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Lot-by-Lot release continues for extracts containing Antigen E (Amb a 1) and Cat allergen I (Fel d I). Data bases have also been created for each mite species, as each lot of standardized mite is now released by CBER. The efforts to standardize cat extract have added valuable information to this database. Venoms have also been monitored for product consistency.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA 01002-16 LAIC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Standardization of Allergenic Extracts of Pollens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.C. Anderson, M.S., Microbiologist, LAIC, DBP, CBER
Kelly T. Boyle, B.S., Biologist, LAIC, DBP, CBER

—

COOPERATING UNITS *(If any)*

-

LAB/BRANCH

Laboratory of Allergenic Products, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS 0.1	PROFESSIONAL 0.1	OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Standardization efforts have focused on Timothy, June, Perennial Rye, Bermuda, Redtop and Orchard grass extracts with a total of 21 applications and 52 lots now under review. Each extract submitted by a company is analyzed for its IsoElectric focusing pattern, IgE binding activity by Blotted RadioImmuno ElectroFocusing (BRIEF), total protein content, and RAST relative potency compared to CBER reference extracts. Some of these products have also been submitted for Clinical testing under IND.

In order to facilitate the standardization of pollen extracts, LAP currently supplies all testing reagents and references to the companies. Ongoing testing is needed to replenish laboratory supplies for each of these items. Candidate references are constantly being evaluated in the laboratory for concurrent clinical testing. The standardization of other pollen extracts including Sweet Vernal, Giant Ragweed and Meadow Fescue are in progress, and Reference preparations have been selected, tested and ordered for the next phase of standardization.

PHS 6040 (Rev 1/84)

U.S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 BA 01003-07 LAIC

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Studies and Standardization of House Dust Mite Extracts

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: M.C. Anderson, M.S., Microbiologist, LAIC, DBP, CBER
Kelly T. Boyle, B.S., Biologist, LAIC, DBP, CBER

COOPERATING UNITS *(if any)*
-

LAB/BRANCH
Laboratory of Allergenic Products, DBP

SECTION

INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER.
0.05	0.05	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Surveillance of potency testing has continued on lots of Mite extracts produced by the eleven manufacturers holding appropriate product licenses. The Laboratory continues to evaluate mite extracts produced from mites that have been raised under different culture conditions, to attempt to detect possible compositional or quantitative differences in these products. Tests to evaluate these extracts and media components include immunoblotting, RAST inhibition, assays for total protein, and clinical testing.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA 01004-05 LAIC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Continuing Studies of Cat Allergy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.C. Anderson, M.S., Microbiologist, LAIC, DBP, CBER
Kelly T. Boyle, B.S., Biologist, LAIC, DBP, CBER

COOPERATING UNITS *(if any)*

-

LAB/BRANCH

Laboratory of Allergenic Products, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS: 0.2	**PROFESSIONAL:** 0.2	**OTHER:**

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Cat extracts may contain pelt, hair, dander, concentrated cat wash, saliva, or any mix of these materials. Due to variability in source material, differences in protein and non-Fel d I allergen levels have been detected. The standardization cat extracts has resulted in the development and implementation of an additional laboratory method and accompanying reference preparations to assist in the characterization of these extracts.

The current release test for potency of cat extracts is a radial immunodiffusion test that measures Fel d I content, one of the primary allergens of cat. Blotted RadioImmuno ElectroFocusing (BRIEF) demonstrated the value of simple IsoElectric Focusing (IEF) in the qualitative detection of non-Fel d I allergens in standardized cat extracts. The characterization cat extracts by IEF has proven to be a reliable indicator of clinical activity in patients that are sensitive to non-Fel d I allergens. Activities are in progress to designate standardized cat extracts as cat hair or cat pelt products. Accompanying labeling will indicate the presence of additional non-Fel d I in the pelt products, thereby enhancing the safety of these new products and eliminating confusion related to source material terminology.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA 01005-03 LAIC

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Isoelectric Focusing Procedures for Allergenic Extracts

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: M.C. Anderson, M.S., Microbiologist, LAIC, DBP, CBER
Kelly Thompson, BS, Biologist, LAIC, DBP, CBER

COOPERATING UNITS *(if any)*

-

LAB/BRANCH

Laboratory of Allergenic Products, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS: 0.2	PROFESSIONAL 0.2	OTHER.

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Isoelectric Focusing has proven to be a useful tool in the evaluation of allergenic extracts. This type of electrophoresis serves as a basis for examining the immunological activity of the extract, and identifies the IgE binding components in the products. Differences in the behavior of IgE binding in the sera of allergic individuals may be surveyed, and sera may be selected for testing pools with these methods, thereby assuring a fair representation of IgE binding activity in quantitative tests. Preparative IEF, using a new apparatus , has permitted the isolation of single components from allergenic extracts, separating non-IgE binding and IgE binding components in sufficient quantities for analysis and further testing.

The development of a 2D electrophoresis method using IEF as the first dimension has been initiated this year. This method has the potential to assist in the isolation and sequencing of IgE binding extract components.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-01007-03 LAIC

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Clinical Application of Allergy Units

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J. Matthews, R.N., M.S., Clinical Nurse Specialist, LAIC, DBP, CBER
P.C. Turkeltaub, M.D., Medical Officer, LAIC, DBP, CBER

COOPERATING UNITS *(if any)*

J. Murray, M.D., Ph.D., Vanderbelt University, Nashville, TE

LAB/BRANCH

Laboratory of Allergenic Products, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

When standardized cat extracts were licensed, they were arbitrarily assigned 100,000 Allergy Units (AU)/ml if they contained 10-19.9 Fel d I u/ml. With the introduction of the ID50EAL method, biological Allergy Units are currently assigned based on skin test potency determined in highly skin reactive cat allergic subjects. Two standardized cat extracts (standardized cat pelt and standardized cat hair extracts) widely differing in composition of non-Fel d I allergens, but containing equal Fel d I contents of 10 and 20 Fel d I u/ml were assayed to estimate the number of Bioequivalent Allergy Units (BAU) to be assigned to each. Both the 10 and 20 Fel d I containing extracts had mean D50s' between 11 and 12.9 and therefore merit assignment of 10,000 BAU/ml rather than the 100,000 AU/ml arbitrarily assigned. Manufacturers have been notified of this change in labeling.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-01008-03 LAIC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Prevalence of Allergic and Non Allergic Respiratory Disease in U.S. Population

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: P. C. Turkeltaub, M.D., Medical Officer, LAIC, DBP, CBER
P. J. Gergen, M.D., M.P.H., DHES, NCHS

COOPERATING UNITS *(if any)*

DHES, National Center for Health Statistics

LAB/BRANCH

Laboratory of Allergenic Products, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.05	0.05	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Information on allergic rhinitis (AR) and asthma (A) was obtained from a sample of the US population aged 6 years to 24 years in the second National Health and Nutrition Examination Survey 1976-1980 (NHANES II). Analyses were limited to 4,877 whites. Eighty-six percent had complete data. Catarrhal symptoms related to both season and pollen were labeled AR and wheezing was labeled A. The overall prevalent of AR was: 6-12: 6.2%; 12-17: 7%; 18-24: 9%. The overall prevalence of A was: 6-12: 6.2%; 12-17: 3.9%; 18-24: 4.5%. A peaked in the age range 6-12 and AR peaked in the age range 17-24. Eight, 1:20 wt/vol, 50% glycerol, unstandardized extracts were administered by prick puncture. Allergen reactivity was reported as the percent with a mean erythema diameter 10.5 mm or greater at 20 minutes. Only the prevalence of asthma and allergic rhinitis increased with the increasing number for positive allergen skin tests. The independent association of individual allergen reactivity with respiratory disease was quantified with logistic models that included other allergen reactivity, age, sex, smoking and region. Asthma was associated with reactivity to house dust (odds ratio, 2.9; 95% confidence interval [CI] 1.7 to 5) and Alternaria (odds ratio 5.1; 95% CI: 2.9 to 8.9). Allergic rhinitis was associated with reactivity to ragweed (odds ratio, 2.3; 95% CI: 1.5 to 3.3); ryegrass (odds ratio, 2.8; 95% CI: 1.8 to 4.3); house dust (odds ratio, 2.5; 95% CI 1.6 to 3.9); Alternaria (odds ratio, 2.3; 95% CI: 1.5 to 3.4). Asthma only (without allergic rhinitis) was associated with dust and Alternaria. Allergic rhinitis only (without asthma) was associated with ryegrass, ragweed, and house dust. When both A and AR were present only house dust and Alternaria remained associated. These findings highlight the association of specific allergens with upper and lower respiratory diseases and the interactions among coexisting respiratory disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-01010-03 LAIC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Accuracy and Precision of Parallel-line Skin Test Estimates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J. Matthews, R.N., M.S., Clinical Nurse Specialist, LAIC, DBP, CBER
P.C. Turkeltaub, M.D., Medical Officer, LAIC, DBP, CBER
M.C. Anderson, M.S., Microbiologist, LAIC, DBP, CBER
K T. B, B.S., Biologist, LAIC, DBP, CBER

COOPERATING UNITS *(if any)*

-

LAB/BRANCH

Laboratory of Allergenic Products, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.05	0.05	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The relative potency of standardized grass extracts (orchard, redtop, Bermuda, perennial rye, and timothy) with respect to their respective FDA reference extracts were estimated by both *in vivo* PLST and *in vitro* RAST. The concordance between PLST and RAST relative potency was found to be 90.6%. For each grass except timothy, the PLST and RAST relative potency estimates fell within their respective 95% confidence interval demonstrating equipotence by each assay. For timothy grass, the PLST relative potency was just below the 95% lower limit while the RAST relative potency was just above the 95% lower limit. Nonetheless, the concordance of relative potency for timothy between PLST and RAST was 92%. There was no statistically significant difference found between the RAST and PLST relative potency estimates for all the grasses tested. These data indicate that RAST relative potency estimates for standardized grass extracts are highly predictive of their *in vivo* relative potency. The relative potency of standardized short ragweed extracts estimated based on Amb a 1 content with respect to the CBER reference was compared with the relative potency based on parallel-line skin test. The correlation coefficient of the two estimates was 0.995, p<0.001. The skin test estimate of RP was 105% of the Amb a 1 estimate underrating that potency based on Amb a 1 is highly predictive of clinical activity.

The relative potency of standardized cat extracts based on Fel d 1 content with respect to the CBER reference was compared with the relative potency in Fel d 1 reactors estimated by parallel line skin test. The coefficient was 0.85, p=0.0002. The mean in vivo relative potency estimate was 91.4$ of the in vitro estimate confirming the high predictive value in estimating clinical potency based on Fel d 1.

PHS 6040 (Rev 1/84)

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-01011-03 LAIC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

The Effect of Early *vs.* Late Onset of Childhood Asthma

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: P.C. Turkeltaub, M.D., Medical Officer, LAIC, DBP, CBER
P. J. Gergen, M.D., M.P.H., DHES, NCHS

COOPERATING UNITS *(if any)*

DHES, National Center for Health Statistics, Hyattsville, MD

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INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS: 0.1	**PROFESSIONAL:** 0.1	**OTHER.**

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

There is controversy over the role of age of asthma onset in childhood asthma. Data collected on self-reported physician-diagnosed asthmatic children and young adults aged 6-24 years (N=352), who participated in the second National Health and Nutritional Examination, 1976-1980 (NHANES II), a national sample, were examined to see whether reported age at onset was associated with the future course of the asthma. Three definitions were used for early-onset asthma: asthma beginning before the second birthday, before the third birthday, and before the fourth birthday. Late-onset asthma was defined as asthma beginning on or after the second birthday, the third birthday, and the fourth birthday, respectively. Among 6-14 year olds, late-onset asthmatic subjects as compared with early-onset asthmatic subjects using the three definitions reported more allergic rhinitis OR = 3/79 (95% CI 1.53, 9.41), 3.06 (1.33, 7.07), 2.71 (1.18, 6.22), and were more likely to have at least one positive allergen skin test OR = 2.21 (95% CI 1.02, 4.79), 2.90 (1.29, 6.49), 3.41 (1.50, 7.75). Late-onset asthmatic subjects tended to report that their asthma was active, have more problems during the past 12 months with wheezing, and have lower values for predicted FVC and EFV1. No difference was found in reported chronic rhinitis, sinusitis, other allergies, problems within the last 12 months with cough attacks, or during the past 3 years a period of cough and phlegm lasting more than 3 weeks. Among 15-24 year olds, the age of asthma onset was not associated with the factors examined. In this group the association may be obscured by inaccurate recall of asthma onset or no longer be present due to the dominant role of other factors. The role allergy plays among 6-14 year old late-onset asthmatic subjects requires additional investigation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-01012-03 LAIC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Allergen Skin Test Reactivity and Respiratory Disease in the U.S. Population

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: P. C. Turkeltaub, M.D., Medical Officer, LAIC, DBP, CBER
P. J. Gergen, M.D., M.P.H., DHES, NCHS

COOPERATING UNITS *(if any)*

DHES, National Center for Health Statistics, Hyattsville, MD

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Laboratory of Allergenic Products, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER.
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Data collected on 12- to 74-year-old whites (N=10,854) during the second National Health and Nutrition Examination Survey, 1976 to 1980, a sample of the US population, were used to determine the association between various respiratory symptoms and the degree of allergen skin test reactivity. Prick-puncture testing using eight unstandardized allergens was performed. Allergen skin test reactivity was classified by means of the mean diameter of the erythema reaction at the 20-minute reading. Non reactors were the comparison group. The prevalence of allergic rhinitis increased as allergen skin test reactivity increased, with the odds ratio exceeding 8 for the group with two or more positive test results. The prevalence of asthma increased with the increasing allergen skin test reactivity only in nonsmokers. The odds ratio for allergic rhinitis with allergen skin test reactivity was higher with outdoor than indoor allergens. The association of allergic rhinitis with allergen skin test reactivity was higher when a physician had previously diagnosed allergic rhinitis. Chronic rhinitis, chronic bronchitis and chronic cough was not associated with allergen skin test reactivity.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA 01014-08 LAIC

PERIOD COVERED
October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Fatalities from Immunotherapy and Skin Testing with Allergenic Extracts

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: P. C. Turkeltaub, M.D., Medical Officer, LAIC, DBP, CBER
R. F. Lockey, M.D., Prof. of Medicine, U. of S. Florida
M. J. Reid, M.D., Medical Corp, USN
T. A. E. Platts-Mills, M.D., Prof. of Medicine, U. of Virginia
S. B. Lehrer, Ph.D., Tulene University

COOPERATING UNITS *(if any)*
University of South Florida, Tampa, FL; Medical Corp, U.S. Navy, Oakland, CA University of Virginia, Charlottesville, VA; American Academy of Allergy and Immunology (AAAI), Tulene University

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Laboratory of Allergenic Products, DBP

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INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.10	0.10	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The Committee on Allergen Standardization has been surveying fatalities from IT. Prior reports presented data up to 1984, and 1285-89. This study reports 10 for 1990-91. Ten fatalities were reported associated with IT, none associated with skin testing (ST). Three males and 5 females were reported with 2 unreported. Ages were 12 to 73 with a mean of 49.1 years. Seven were asthmatic and 3 were not reported. Three were non-steroid dependent and stable, 1 was steroid dependent, 1 reported an ER visit, and the status of 5 was not reported. Allergic stnsitivity was reported as high or moderate in 6 and unreported in 4. None had cardiovascular disease and 6 were unreported. Six extracts contained pollens, 3 mold, 3 dust mite, 4 house dust, 2 cat, and 4 were unreported. Four contained standardized extracts, 2 did not, 4 were not reported. Three patients were reported on build-up, 3 on maintenance, and 4 were unreported. Doses associated with fatal reactions ranged from 0.4 ml 1/100,000 pollen, mold, dust mite, house dust to 0.3 ml 1/50 pollen mix. None were using beta-blockers or ACE inhibitors. One has a prior systemic reaction, 4 did not and 5 unreported. Two received inaccurate doses. An M.D. was present in 4, was not present in 2, and 4 were unreported. Onset of symptoms was <20 minutes in 5 and unreported in 5. Cause of death was asthma in 5 and 5 unreported. Fatal reactions associated with IT were reported to have occurred in 10 patients from January 1990 to the present. None were associated with ST. Although overall risk is low, asthmatics werw at the greatest risk for a fatal reaction. IT should be given by trained physicians aware of the risks and skilled in the treatment of anaphylaxis. Patients should be screened before each injection.

PHS 6040 (Rev 1/84)

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 BA-01017-02 LAIC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

In vivo determination of compositional differences in standardized cat extracts

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J. Matthews, R.N., M.S., Clinical Nurse Specialist, LAIC, DBP, CBER
P.C. Turkeltaub, M.D., Medical Officer, LAIC, DBP, CBER

—

COOPERATING UNITS *(if any)*

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LAB/BRANCH

Laboratory of Allergenic Products

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.10	0.10	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study used in vivo assays to detect DC among SCEs. Cat extracts are currently standardized based on Fel d 1 (F). Nine SCEs were tested with Fs of 6.2 to 17.8 U/ml and with F to protein ratios (F/P) of 1.31 to 21.5. SCEs with low F/P were suspected to contain significant non-Fel d 1 (NF) allergen. Two cat sensitive populations were identified by skin testing: F+/NF- subjects; puncture reactive to SCE, but not to cat serum (no detectable F), and F+/NF+ subjects; reactive to both SCE and cat serum. Relative potencies (RP) of the various SCEs were determined by parallel-line skin test (PLST) against a SCE reference which contained significant NF allergen (F=11.8, F/P=1.93). There was a significant correlation ($r=0.95$, $P<0.001$) between RP based on PLST and RP based on F in F+/NF- subjects, but not in F+/NF+ subjects ($r=0.32$, $P=0.4$). The RP of two SCEs with F content similar to the reference, but with higher F/P ratios (10.6 and 21.5) were only 13-16% of the reference in F+NF- subjects (p=NS). These two SCEs were CD from the reference as were three other SCEs with higher F/Ps than the reference. Three SCEs with F/Ps equal or less than the reference were equipotent to the reference in F+/NF+ and F+/NF- subjects demonstrating they were compositionally similar (CS) to the reference. Because there is a subgroup (~20%) of cat allergic patients who are sensitive to NF allergens, an assay to detect non-F allergens in addition to F is needed for standardization of cat extracts. This will insure lot to lot consistency in F and NF allergen composition, thus permitting labeling to accurately differentiate CD from CS cat extracts.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-01018-02 LAIC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

In vivo determination of stability of standardized grass extracts

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J. Matthews, R.N., M.S., Clinical Nurse Specialist, LAIC, DBP, CBER
P.C. Turkeltaub, M.D., Medical Officer, LAIC, DBP, CBER
J. Delasko, R.N., M.S., Clinical Nurse Specialist, LAIC, DBP, CBER

COOPERATING UNITS *(if any)*

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Laboratory of Allergenic Products, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The in vivo stability of standardized short ragweed concentrate (3.5 Amb a 1 u/ml) and dilutions were determined by means of parallel-line skin test. The freezer-line concentrates were reconstituted 50% glycerol and diluted using FDA approved HSA diluent. The mean dilution tested for stability was 0.0016 Amb a 1 u/ml (range 0.03 to 0.00016 or 1:2 hundred thousand, 1:7 thousand to 1:2 million). These dilutions were equipotent to the reference for up to 7 weeks. The concentrate was also stable at 7 weeks.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-01019-02 LAIC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Study of the Allergenicity of Latex

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.C. Anderson, M.S., Microbiologist, LAIC, DBP, CBER
Kelly T. Boyle, B.S., Biologist, LAIC, DBP, CBER

COOPERATING UNITS *(if any)*

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Laboratory of Allergenic Products, DBP

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INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS. 0.01	PROFESSIONAL 0.01	OTHER.

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Increased contact with latex products has resulted in an increased number of allergic reactions in health care workers and patients. Reactions range from contact urticaria to systemic anaphylaxis. Studies have been initiated to identify and possibly isolate the component(s) causing these reactions, which are mediated in many cases by latex component specific IgE.
Comparative extractions of Ammoniated and Buffered latex purchased from various sources have been evaluated by Isoelectric Focusing(IEF), Blotted RadioImmuno ElectroFocusing (BRIEF), SDS PAGE, Preparative IEF, and Modified Ninhydrin procedures. A Laboratory Reference preparation of Latex has been prepared and shared with other investigators. Extracts prepared form various devices are also under study. Review of these data is still in progress.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-01020-02 LAIC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Study of Cockroach allergens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.C. Anderson, M.S., Microbiologist, LAIC, DBP, CBER

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Allergenic Products, DBP

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INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS: 0.01	PROFESSIONAL 0.01	OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Studies of cockroach allergy have expanded to include studies of extracts prepared for single sexes of roaches, as well as different strains of roaches. Samples have also been analyzed from the crop, mid and hind gut of roaches, and the effect of various changes in rearing methods is under investigation. Acquisition of an allergic serum pool has been delayed, although the initiation of contact with pesticide manufacturers may provide additional sources of serum for analysis.

PHS 6040 (Rev 1/84)

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-01021-02 LAIC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Alternate Test Method for Assay of Hyaluronidase in Hymenoptera Venoms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.C. Anderson, M.S., Microbiologist, LAIC, DBP, CBER

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COOPERATING UNITS *(if any)*

-

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Laboratory of Allergenic Products, DBP

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DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS: 0.01	PROFESSIONAL 0.01	OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Alternate test method for assay of Hyalurondase in Insect Venoms using a synthetic substrate has been introduced to the two manufacturers currently licensed for venom products. Preliminary data form the manufacturers indicate that the use of the synthetic substrate has eliminated the particular problems seen in assays of yellow jacket venom with the natural substrate.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 BA-01022-01 LAIC

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Mite extract surveillance and testing

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: M.C. Anderson, M.S., Microbiologist, LAIC, DBP, CBER

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COOPERATING UNITS *(if any)*
-

LAB/BRANCH
Laboratory of Allergenic Products, DBP

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INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS: 0.01	**PROFESSIONAL** 0.01	**OTHER:**

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Surveillance of potency testing has continued on lots of Mite extracts produced by the eleven manufacturers holding appropriate product licenses. the Laboratory continues to evaluate mite extracts produced from mites that have been raised under different culture conditions, to attempt to detect possible compositional or quantitative differences in these products. Tests to evaluate these extracts and media components include immunoblotting, RAST inhibition, assays for total protein, and clinical testing.

U.S. GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-01023-01 LAIC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Evaluating Proficency of Skin Testing

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Paul C. Turkeltaub, M.D., Medical Officer, LAIC, DBP, CBER
Jacqueline Matthews, R.N., M.S., Clinical Nurse Specialist, LAIC, DBP, CBER
Jeanne Delasko, R.N., M.S., Clinical Nurse Specialist, LAIC, DBP, CBER

COOPERATING UNITS *(if any)*

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LAB/BRANCH

Laboratory of Allergenic Products, DBP

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INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS: 0.01	**PROFESSIONAL** 0.01	**OTHER.**

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To be useful clinically, diagnostic tests require evaluation of their accuracy, precision, and sensitivity. Once these test properties have been specified, then personnel can be evaluated for proficiency by determining whether their test resulsts meet these specifications. This study addressed whether an FDA proficiency method for ID testing could be modified for use in practice. The modified method employs two doses of FDA licensed histamine base (H): 1.8 mg/ml and 0.1 mg/ml. The relative potency (RP) of these two H concentrations is 18 (1.8/0.1). The RP by ID test was determined as follows: The endpoint dilution and the next 3 serial 3 fold dilutions of each H were determined in each subject after injection of 0.05 ml. The endpoint was the first dilution from the concentrate (Dilution #0) in which an erythema response was absent or equal to the wheal. Acceptable titrations had graded erythema responses with dilutions bracketing 50 mm sum of erythema (SE). The Dilution# producing a SE between 45-55mm or by interpolating between the 2 dilutions bracketing 50mm is the estimated D50. The difference between the two estimated D50s of each H is the RP. The mean difference in estimated D50 in six subjects is 2.5 (2 to 3). This is the estimate of test accuracy. The standard deviation of this mean is less then 0.6. This is an estimate of test precision. The mean estimated D50 of 1.8H is 6 (5.5-7). The mean estimated D50 of 0.1 H is 4 (3-4.5). These latter two means are test sensitivity specifications. The proposed method provides an approach tossessment of proficiency of ID testing in practice.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-01024-01 LAIC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Evaluation of Adverse Allergic Reactions to Vaccines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Paul C. Turkeltaub, M.D., Medical Officer, LAIC, DBP, CBER

COOPERATING UNITS *(if any)*

S.C. Rastogi, Ph.D. and Robert Wise, M.D., M.P.H., Office of Epidemiology and Biostatistics, CBER

LAB/BRANCH

Laboratory of Allergenic Products, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Influenza vaccine is recommended for asthmatics regardless of age. Recent correspondence in Lancet indicates some concern among physicians of the safety of administering influenza vaccine to asthmatics. A review of FDA AERs will be carried out to evaluate the frequency of allergic/asthmatic reactions following influenza immunization.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-01025-01 LAIC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Assignment of Bioequivalent Allergy Units to Pollen Extract

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Paul C. Turkeltaub, M.D., Medical Officer, LAIC, DBP, CBER

COOPERATING UNITS *(if any)*

S.C. Rastogi, Ph.D. and Robert Wise, M.D., M.P.H., Office of Epidemiology and Biostatistics, CBER

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

When standardized weed and grass pollen extracts were licensed they were arbitrarily assigned 100,00 AU/ml if they were equipotent to the reference. With the introduction of the ID50EAL method, Bioequivalent Allergy Units are currently assigned based on skin test potency determined in highly skin reactive rhinitis subjects.

Using the ID50AEAL method, proposed FDA reference preparations of giant ragweed and Bermuda grass were evaluated clinically. In each reference the mean D50 fell between 11-12.9, indicating they should be assigned 10,000 BAU/ml rather then the 100,000 AU/ml arbitrarily assigned. The Bermuda grass reference was also evaluated in another region of the U.S. to determine whether the CBER estimate was replaceble. When sugjects equal in reactivity were selected in Nashville, no significant difference with the Nashville D50 estimate with respect to the CBERmuda D50 estimate was found confirming the assignment of 10,000 BAU/ml to the Bermuda reference.

When standardized short ragweed extracts were licensed and labelled in Allergy Units, they were arbitrarily assigned 100,000 AU/ml, if they contained between 200 to 400 Amb a 1 units/ml. Estimation of the D50 of standardized short ragweed extracts at 250, 300, 315, 500 and 900 Amb a 1 u/ml indicated that extracts with Amb a 1 contents of 250 to 450 u/ml warranted assignment of 100,000 BAU/ml.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-02007-10 LBP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biosynthesis of Capsular Polysaccharides of Pathogenic Bacteria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: W.F. Vann, Ph.D. Research Chemist, LBP, DBP, CBER
G. Zapata, Ph.D., Sr. Staff Fellow, LPB, DBP, CBER
J. Crowley, B.S., Microbiologist, LBP, DBP, CBER
S.J. Freese, Ph.D., Staff Fellow, LBP, DBP, CBER
R.P. Silver, Ph.D., Assoc. Prof. Dept. Microbiol, Univ. Rochester, NY
G.B. Boulnois, Prof. Dept. Microbiol. Uuiv. Leicester, England

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Department of Microbiology, Univ Leicester, Leicester, England

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Laboratory of Bacterial Polysaccharides, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Escherichia coli CMP-NeuAc synthetase catalyses formation of cytidine 5' monophospho-N-acetylneuraminic acid (CMP-NeuAc) from N-acetylneuraminic acid (NeuAc) and cytidine triphosphate (CTP). Availability and specificity of purified sugar activating enzymes and glycosyltransferases make these enzymes convenient tools for synthesis of biologically relevant oligosaccharides. The role of the cysteines in the structural and catalytic properties of the enzyme has been examined by site directed mutagenesis and chemical modification. Chemical modification with the sulfhydro specific reagent DTDP suggests that one cysteine residue is involved in catalysis, since the enzyme can be completely inactivated by titration of one cysteine residue with DTDP. The enzyme can be protected from inactivation in the presence of the substrate CTP. Site directed mutagenesis demonstrates that cysteine 129 and cysteine 329 are not essential for catalysis, since both can be substituted by selected amino acids without complete loss of activity. Chemical inactivation with N-ethylmaleimide (NEM) demonstrates that cysteine 329 and cysteine 129 are inaccessible to NEM at room temperature. Cysteine 329 can be thermally exposed to chemical modification with NEM at 42 °C but cysteine 129 is inaccessible to chemical modification with NEM. We have sequenced the amino terminus of the CMP NeuAc synthetase from meningococcus group B which synthesizes the same a 2-8 NeuAc capsule of E. coli K 1. The N terminal sequences were compared and a concensus obtained. Arginine and lysine residues were identified as common amino acids in the compared sequences. We have mutated 14 arginines in E. coli CMP NeuAc synthetase to Glycine and mutation of the common arginine completely inactivates CMP NeuAc synthetase. Changing arginine to alanine results in an inactivated enzyme, but a change to lysine restores the specific activity to wild type values. Km determinations are being done at different pHs to determine involvement of arginine in catalysis.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-02010-09 LBP

PERIOD COVERED

October 1, 1991 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunochemical studies of meningococcal lipooligosaccharide antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: C-M Tsai, Ph.D., Research Chemist, LBP, DBP, CBER
P. A. Balakonis, B.S. Chemist, LBP, DBP, CBER
I. Hodge, Biological Technician, LBP, DBP, CBER
C. I. Civin, M. D., Director, Pediatric Oncology, Johns Hopkins Univ.

COOPERATING UNITS *(if any)*

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Laboratory of Bacterial Polysaccharides, DBP

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INSTITUTE AND LOCATION

LBP, DBP, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

N. meniningitidis LOS mimic human glycolipids in having lacto-N-neotetraose (LNnT, Galß1-4GlcNAcß1-3Galß1-4Glc) sequence in the oligosaccharides.

The oligosaccharides of N. meniningitidis LOS have been reported to have a triatennary structure with LNnT at the nonreducing end of the longest antenna in the branched oligosaccharides. We have used a mouse monoclonal antibody (anti-My-28) which recognizes LNnT, to investigate this sequence in the oligosaccharides of the LOS. Eight of the twelve immunotype LOS, all but types 1, 6, 11, and 12, bound the antibody as measured by ELISA, immunodot and immunoblot assays. N-Acetyllactosamine inhibited the binding of the antibody to all eight reactive LOS. The antibody binding to a represntative LOS (type 2) was best inhibited by LNnT, next by N-acetyllactosamine, but not inhibited by galactose, Galß1-3GlcNAc, and lacto-N-tetraose, Galß1-3GlcNAcß1-3Galß1-4Glc. These results suggest that the LNnT sequence is present in 8 of 12 immunotype LOS. The presence of the LNnT sequence in the LOS, which is found in paragloboside (LNnT-ceramide) and its related glycolipids in a variety of human cells, may play a role in the virulence of N. meningitidis by enabling the organism to evade host immune defenses. The expression of the antibody-reactive epitope in the LOS was influenced by growth conditions and the LNnT epitope could be masked by a sialic acid which was identified as N-acetyl-neuraminic acid. We are currently investigating the linkage of the sialic acid to LNnT in meningococcal LOS and its possible function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-02011-04 LBP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Quantitation of polysaccharide (PS) content in Hib conjugate vaccines by HPLC

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: C-M Tsai, Ph.D., Research Chemist, LBP, DBP, CBER
X-X Gu, M.D., Fogarty Fellow, LBP, DBP, CBER

COOPERATING UNITS *(if any)*

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INSTITUTE AND LOCATION

CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The PS content in Hib conjugate vaccines in final containers is currently determinened by measuring its ribose using colorimetric orcinol assay. However, the Merck vaccine contains a large quantity of lactose, a stabilizer, which makes the assay impossible; and an indirect rate nephelometry assay, which is based on the rate of antigen-antibody aggregation, is used to estimate the PS content of the vaccines. We have developed an HPLC method to quantitate the PS content in the vaccines. Our results show that the amounts of PS in the Merck vaccine as well as Connaught and Praxis vaccines can be measured on a Dionex carbohydrate analyzer as follows. The PS in the conjugate vaccines was first depolymerized in 0.1 N NaOH at room temperature for six hours to its single repeating unit (ribitol-ribose-phosphate). The PS repeating unit in the alkali-treated sample was then separated from the huge lactose peak by an HPLC column and quantitated. This method can detect the PS as low as 0.1 μg or at a PS concentration of 1μg/ml.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BA-02013-03 LBP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Preparation of monoclonal antibody against meningococcal A1 LPS (L8,)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: X-X Gu, M.D., Fogarty Fellow, LBP, DBP, CBER
C-M Tsai, Ph.D., Research Chemist, LBP, DBP, CBER
A. Karpas, Ph.D., LCP, DBP, CBER

COOPERATING UNITS *(if any)*

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SECTION

INSTITUTE AND LOCATION

CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Eight monoclonal antibodies (MAbs) to lipooligosaccharides (LOS) of Neisseria meningitidis were produced by immunizing mice with purified LOS from group A meningococcal strain A1. The specificities of the MAbs were examined by enzyme-linked immunosorbent assay (ELISA), immunodot assay, and ELISA inhibition using the homologous A1 LOS, 12 immunotype LOS of N. meningitidis (L1 through L12), and LOS or lipopolysaccharides from other gram-negative bacteria. Two of the MAbs, 4385G7 (IgG2b) and 4387A5 (IgG2a), had the strongest reactivity with the homologous A1 LOS, moderate reactivity with M978 (L8) LOS, but no reactivity with other LOS. The other six MAbs (4 IgM and 2 IgG3) reacted with A1 LOS but also with several or many of the 12 LOS. ELISA inhibition at 50% showed that inhibitory activity of the LOS from strains A1 and BB431 (a group B strain) to the specific MAb 4387A5 was about 10-20 times greater than that of M978 (L8) LOS. When compared with MAb 2-1-L8 (L8) by Western blot analysis and ELISA inhibition, the two specific MAbs recognized a different epitope in 3.6-kilodalton LOS of strains A1 and BB431. We propose this new epitope as L8a since the MAbs also reacted with M978 (L8) LOS. The expression of the L8a epitope in the A1 LOS requires a few monosaccharide residues in its oligosaccharide moiety and the fatty acid residues in its lipid A moiety also play a role. In whole cell ELISA using the LOS prototype strains, the two specific MAbs bound only to L8 strains A1 and M978. By comparison, MAb 2-1-L8 not only bound to L8 strains but also had a low degree of reactivity with a few other prototype strains. These results suggest that the two specific MAbs can be used for LOS typing of N. meningitidis.

This project has been completed. The manuscript has been published in J. Clin. Microbiol. 30:2047-2053, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-02015-02 LBP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular cloning of pneumolysin from *S. pneumoniae* group 19

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Susan Banks, Ph.D., Staff Fellow, LBP, DBP, CBER
C.J. Lee, Sc.D., Supervisory Research Chemist, LBP, DBP, CBER

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Bacterial Polysaccharides, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER
0.5		

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Isolation and molecular cloning of pneumolysin gene from group 19 pneumococci were studied to examine the relationship of pneumolysin to virulence. Group 19 chromosomal DNA samples were analyzed, using clonal *ply* genes from two different pneumococcal strains as probes. Genomic DNA that contained the *ply* gene from 19F, 19A, 19B and 19C strains were examined by polymerase chain reaction (PCR). The amplified gene contained a ClaI restriction enzyme site 5' and XbaI restriction enzyme site 3' encoded in the oligonucleotides utilized in the PCR mixture. The ClaI/XbaI *ply* gene was cloned into a vector, Bluescript pks(-). Recombinant clones 19A31 and 19F5 contained the *ply* gene as confirmed by restriction enzyme analysis and Southern blot.

The nucleotide structure of 19A *ply* gene was sequenced and compared with type 2 *ply* gene. Ten differences in the two genes were observed in 1420 bp of sequence, whereas a change in only two amino acids (type 2 Arg226 -->19A Asp; type 2 Asp380 -->19A Asn) was present. Deduced amino acid sequence from 19A *ply* gene showed 24.6% homology with other cytolytic toxins, e.g. listeriolysin, alveolysin and perfringolysin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-02018-02 LBP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Protective immunity of young mice to type 9V PS-protein conjugate

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Theresa Wang, B.S., Biologist, LBP, DBP, CBER
C.J. Lee, Sc.D., Supervisory Research Chemist, LBP, DBP, CBER

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Bacterial Polysaccharides, DBP

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INSTITUTE AND LOCATION

DBP, CBER, FDA, BEthesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Pneumococcal type 9V is one of the most common types causing pneumococcal disease in young children. Type 9V PS is contained in the current pneumococcal vaccine but it induces a low antibody response in young children. To increase its immune response the PS was conjugated to a protein carrier. A modified conjugation method was applied to link 9V PS covalently to inactivated pneumolysin. Type 9V PS was reacted with cystamine in the presence of carbodiimide. The amino group of the formed compound was reduced by dithiothreitol to form a thiol derivative. The carrier protein was reacted with N-succinimidyl bromoacetate to form bromoacetylated protein. The thiol derivative of PS was next reacted with bromoacetylated protein to form a PS-protein conjugate. This method has produced a 9V PS-protein conjugate with a stable covalent linkage.

Pregnant mice at 2 weeks of gestation and/or lactating mice at 1 week after delivery were injected with 9V PS-inactivated pneumolysin conjugate. Young mice at 2 weeks of age were given additional dose of conjugate. Two weeks after injection, young mice were challenged with 10 to 10 type 9V cells. Measurements of survival rate and bacterial clearance are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-02020-02 LBP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Bacterial clearance of young mice immunized with type 19F PS-PdB conjugate

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: C.J. Lee, Sc.D., Supervisory Research Chemist, LBP, DBP, CBER
J.C. Paton, Ph.D., Adelaide Children's Hospital, N. Adelaide, S. Australia

COOPERATING UNITS *(if any)*

Adelaide Children's Hospital, N. Adelaide, S. Australia

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INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have studied the effect of maternal immunization with type 19F PS-PdB conjugate during gestation and/or lactation on the antibody response and bacterial clearance in the offspring. The conjugate immunogen was injected subcutaneously to mothers during gestation, lactation or both period. Young mice were given an additional dose of conjugate IP during lactation. Serum samples were collected from young mice two weeks after injection. Young mice from mothers that were immunized with the conjugate immunogen and then received an additional dose of conjugate produced significantly higher 19F antibody and anti-pneumolysin levels.

The young mice were challenged with 10 to 10 cfu of type 19F cells. At 1, 2, and 4 hrs after bacterial challenge, blood samples were collected and distributed on the sheep blood agar plates. The number of bacteria were significantly lower in experimental groups of young mice from mothers exposed to conjugate immunogen during gestation and lactation than in non-immunized controls. These studies provide further support that maternal immunization with 19F PS conjugated with inactivated pneumolysin has conferred the protective immunity to pneumococcal infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 BA-02021-2 LBP

PERIOD COVERED
October 1, 1991 to August 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Stress proteins from group B *Neisseria meningitidis*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Gayathri Arakere, Ph.D., Staff Fellow, LBP, DBP, CBER
Carl Frasch, Ph.D., Chief, LBP, DBP, CBER

COOPERATING UNITS *(if any)*
Martin Kessel, Ph.D., Dept of Microbiology, Univ of Maryland
Nga Nguyen, Ph.D., Cytokine Biology, CBER

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INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER.
0.7	0.7	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Several stress proteins have been reported in Neisseria including proteins induced with heat chock and ones induced under iron deficient growth. We have characterized a 65-kDa stress protein enhanced under growth in stationary conditions and found that iron concentration of the medium had no influence on the enhancement of this protein. We have purified this protein using a combination of column chromatography and sucrose density gradients. Electron microscopy of the purified protein revealed structures very similar to the GroEL protein of *Escherichia coli*. The N-terminal amino acid sequence analysis reveals about 58% identity with the *E. coli* protein. We have also purified and characterized another 55-kDa protein which partitions in the same fraction as the meningococcal stress protein. This protein was found to be Glutamine synthetase by enzymatic activity and electron microscopy. Its structure was found to be identical to the *E. coli* glutamine synthetase and it cross reacted with the polyclonal antibody to *E. coli* glutamine synthetase. We are studying the immunogenicity of the 65-kDa stress protein and its role in the assembly of glutamine synthetase.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-02022-02 LBP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization and expression of group 19 pneumolysin genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Susan D. Banks, Ph.D., LBP, DBP, CBER
C.J. Lee, Sc.D., Supervisory Research Chemist, LBP, DBP, CBER

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Bacterial Polysaccharides, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5		

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

E. coli clones, 19A31 and 19F5, containing *ply* gene produced protein molecules that expressed hemolytic activity. A Western blot revealed cross-reaction to polyclonal type 1 pneumolysin antibody. Proteins from both clones showed the same molecular size of 53 kD. Large quantities (mg) of purified pneumolysin from these clones will be produced for further chemical and immunological characterization.

Molecular manipulation has been performed to induce mutation of the *ply* gene, such as a mutant gene that produces a truncated protein with elimination of hemolytic activity and retention of the antibody binding properties. We have synthesized oligonucleotides that begin at the 3' at a point 1 base (Glu19) or 21 bases (Asp19) from the cysteine codon at base 1,287. The PCR fragments generated are smaller than the *ply* gene (1,425 bp). Glu19 is 1,286 bp and Asp19 is 1,265 bp. Cloning is currently being carried out. These mutated pneumolysin molecules will be used as carrier proteins for preparation of pneumococcal PS-protein conjugate vaccines.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 BA-02025-02 LBP

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chemical synthesis of meningococcal Group A oligosaccharides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Willie F. Vann, Research Chemist, LBP, DBP, CBER
Stephen J. Freese, Ph.D., Staff Fellow, LBP, DBP, CBER

COOPERATING UNITS *(if any)*
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INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The synthesis of controlled size oligosaccharides is being pursued in order to provide material for the preparation of conjugates. These conjugates will make it possible to determine the effects of oligosaccharide size on immunogenicity of a conjugate vaccine. An appropriate solid phase support has been prepared. This support incorporates a four-carbon spacer arm which is attached to the support by a selectively cleavable disulfide bond. The support has been found to bear derivatizable groups at a concentration of 50 mmoles/gm. It was determined that the conventional b-cyanoethyl protected phosphitylating reagent, required to complete the synthesis of the activated monomer, would not be optimal for this synthesis, therefore a benzyl protected phosphitylating reagent was prepared. This reagent was preferable because it uses the same protective group as is already present in the carbohydrate moiety of the monomer. In contrast to the case of the b-cyanoethyl protected intermediate, the phosphite enantiomers of the a-anomer of the benzyl protected intermediate copurify. This makes the essential separation of anomers much less labor intensive. The required monomer, 2-acetamido-3-0-acetyl-4-0-benzyl-2-deoxy-6- fluorenylmethoxycarbonyl-a-D-mannopyranosyl-N, N-diisopropy-0-benzyl-phosphoramidite, was prepared, purified and characterized. This monomer has been used to prepare a tetrasaccharide attached to the solid phase. Measurement of coupling efficiency indicated that the synthesis can be readily extended to produce a 20-mer. Work is now progressing on the efficient removal of the saccharide from the support.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 BA-02027-01 LBP

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Serum Resistance in Neisseria meningitidis Associated with Changes in LPS.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Carl E. Frasch, Ph.D., Chief LBP, DBP, CBER
Diana Lacerda, Ph.D., Visiting Scientist, LBP, DBP, CBER

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Bacterial Polysaccharides

SECTION

INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD, 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Wildtype Neisseria meningitidis elaborate lipopolysaccharides without long O polysaccharide side chains, and are referred to as lipooligosaccharides (LOS). Others have shown that antibodies to meningococcal LOS determinants can be bactericidal. Studies have also shown that changes in the molecular size of the LOS in Haemophilus influenzae type b result in changes from serum resistance to serum sensitivity. Naturally occuring antibodies to the saccharide determinants of meningococcal LOS are present in the sera of healthy adults. Using changes in colony opacity and SDS-PAGE with silver staining, we selected a series of LOS variants from group B (BB-1 and 44/76) and group C (BB-305) N. meningitidis strains. No discernible differences were seen in the outer membrane proteins. We obtained sera from 4 healthy adults that had not worked with Neisseria. These sera were quickly frozen at -70 C to preserve complement activity. The sera were tested for bactericidal activity against each LOS variant by measuring Log decrease in viable count during 1 hour at 37 C. We found the BB-305 wildtype to be serum resistant (SR) in all 4 sera, but variant 1 (V1) was serum sensitive (SS) in all 4 sera. V2 was SR in 2 of 4 sera and SS in the other 2 sera. BB-305 wildtype LOS was 4800 daltons, V1 was 4300 daltons, and V2 was 3600 daltons. Loss of about two sugars from the wildtype resulted in a change from SR to SS, possibly due to exposure of a cross-reactive epitope. We are now using rabbit anti-LOS typing sera and monoclonals to determine epitope differences which may account for the differences in serum sensitivity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 BA-02028-01 LBP

PERIOD COVERED
March 1992 to September 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immune response to group B meningococcal polysaccharide.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Sarvamangala Devi, Ph.D., Senior Staff Fellow, LBP, DBP, CBER
Carl Frasch, Ph.D., Chief, LBP, DBP, CBER
—

COOPERATING UNITS *(if any)*
-

LAB/BRANCH
Laboratory of Bacterial Polysaccharides, DBP

SECTION

INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.35	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Group B meningococcal capsular polysaccharide (GBPS) consists of a linear polymer of alpha (2->8) linked N-acetyl neuraminic acid. Immune response to GBPS in humans has not been studied in detail. Most of the assays in use modify the structure of native antigen or its conformation to varying extent. We adopted an ELISA to measure antibodies to both GBPS and *Escherichia coli* K1, 0-acetylated polysaccharide in sera of healthy normals and group B meningococcal patients and carriers. In general, non-carriers showed higher total immunoglobulin levels to 0-acetylated K1 CPS than to GBPS, whereas patients and carriers had more antibodies to GBPS. Both IgG and IgM capsular antibodies were found. Attempts are under way to standardize ELISA to quantitate antibodies and express results in an acceptable unitage. Both the coating antigens are being used in different forms to understand finer epitope specificities. We intend to investigate possible association of various epitopes with different biologic properties. The kinetics of antibodies induced in laboratory animals by GBPS conjugates with and without adjuvants are being studied.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-02029-01 LBP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Inactivated pneumolysin as an aid to vaccine design

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: C.J. Lee, Sc.D., Supervisory Research Chemist, LBP, DBP, CBER
James C. Paton, Ph.D., Adelaide Children's Hospital, N. Adelaide,
S. Australia

COOPERATING UNITS *(if any)*

Adelaide Children's Hospital, N. Adelaide, S. Australia

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INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Genetically engineered toxoid molecules have been evaluated as potential protein carriers for pneumococcal capsular polysaccharide in a conjugate vaccine. Immunization of mice with genetically engineered toxoids protected mice from subsequent challenge with virulent pneumococci to a greater extent than wild type toxin. An inactivated pneumolysin, "pneumolysoid B (PdB)" was prepared from a mutant gene constructed by the site-directed mutagenesis of type 1 ply gene, pJCP20 plasmid in which DNA fragment that encodes for Try433 of pneumolysin was changed to Phe. Conjugation of the toxoid to pneumococcal type 19F PS enhanced the immunogenicity of both PS and pneumolysoid antigens.

The 19F PS-PdB conjugate was given to maternal mice during gestation and/or lactation and an additional immunogen was given to young after birth. Two weeks after last injection, the immunized young mice were challenged with 19F pneumococci. Almost all young mice in control group not receiving immunogen, died within first 3 days after challenge (5% survial rate), while most mice in the immunized group survived (71.4-75%). Thus, the injection of mice with 19F PS conjugated with inactivated pneumolysin has conferred protective immunity to pneumococcal infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-02030-01 LBB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effect of molecular size of PS on immune response of PS-protein conjugate

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Theresa Wang, B.S., Biologist, LBP, DBP, CBER
P.J. Baker, Ph.D., Senior Microbiologist, NIAID, NIH
C.J. Lee, Sc.D., supervisory Research Chemist, LBP, DBP, CBER

COOPERATING UNITS *(if any)*

NIAID, NIH, Bethesda, MD

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TOTAL STAFF YEARS: 0.4	PROFESSIONAL: 0.4	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Observations on the decrease in antibody response and protective immunity of bacterial polysaccharide vaccine having smaller molecular size, indicate the correlation between the molecular size of PS and immunogenicity of vaccines. To study the effect of molecular size of PS on the immunogenicity, pneumococcal type 3 PS was treated with 0.5-1 N hydrochloric acid for 0.5, 1 and 2 hrs., to prepare various size of PS with Kd values, 0.23 to 0.80. The dose-response patterns of these type 3 PS fractions were analyzed by plaque-forming cell assay. Initial results indicate that the low magnitude of immune response was induced by the PS sample having small molecular size. Their effectiveness in inducing low-dose paralysis will be examined.

The different size PS or oligosaccharide were conjugated with the inactivated 19F pneumolysin and the antibody response was determined. The results showed that the serum levels of 19F IgG1 and IgG3 were significantly higher in mice immunized with a conjugate, in which large molecular size of PS was used to prepared the conjugate, than the group injected with a conjugate, in which small molecular size of OS was used.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-02031-01 LBP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Quantitative analysis of Hib PS in DTP-Hib conjugate combined vaccine

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Theresa Wang, B.S., Biologist, LBP, DBP, CBER
Jean P. Li, M.S., Chemist, LBP, DBP, CBER
C.J. Lee, Sc.D., Supervisory Research Chemist, LBP, DBP, CBER

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Bacterial Polysaccharides, DBP

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DBP, CBER, FDA, BEthesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A "rocket" immunoelectrophoretic method was applied to quantitation of H. influenzae type b (Hib) PS in HIb conjugate alone and in a combined vaccines with DTP. Three types of HIb-protein conjugate vaccines were prepared by manufacturers by different methods, using different protein carrier, PS size, nature of linkage, and ratio of protein and PS. Furthermore, two manuracturers prepared DTP-HIb combined vaccine. With the optimum concentration of HIb antiserum (rabbit HIb antiserum, 20 ul/ml agarose gel), linear curves were obtained with HIb PS-protein conjugate at concentrations of 2 to 10 ug HIb PS/ml. For two manufacturers conjugate vaccine samples resulted in clear, straight migrating rockets. In contrast, another vaccine showed skewed rockets with open ended at the tip. Different experimental parameters will be used to find the optimum condition for IEP. Quantitative determination of Hib conjugate could be analyzed accurately combined with DTP.

A nephelometric method for quantitation of the HIb PS content of HIb conjugate and DTP-Hib conjugate combined vaccines has also been examined. More studies are required to obtain the optimum conditions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BA-02032-01 LBP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Production of oligosaccharide-protein conjugates to meningococcal LPS meningococcal

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: X-X Gu, M.D., Fogarty Fellow, LBP, DBP, CBER
C-M Tsai, Ph.D., Research Chemist, LBP, DBP, CBER

COOPERATING UNITS *(if any)*

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LAboratory of Bacterial Polysaccharides, DBP

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INSTITUTE AND LOCATION

CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The lipooligosaccharide (LOS) of N. meningitidis plays an important role in meningococcal pathogenicity. Antibodies to oligosaccharide (OS) moiety of LOS induce bactericidal activities in vitro. We are preparing an LOS derived OS-protein conjugate as a vaccine that may prevent meningococcal disease.

The OS was isolated from strain A1 LOS by acetic acid hydrolysis of the LOS and purified through P-4 chromatography. The carboxylic acid group of KDO in the OS was linked to a homobifunctional adipic acid dihydrazide (ADH) using carbodiimide (EDC) and N-hydroxysulfosuccinimide. This ADH derivatized OS was further coupled to tetanus toxoid (TT) using EDC. The molar ratio of OS to TT in three lots of conjugate preparations ranged from 10:1 to 18:1.

Antigenicity of the conjugates in vitro was similar to that of LOS as determined by ELISA in which a rabbit immune serum against whole cells was used as a binding antibody. Immunogenicity of the conjugates showed that the conjugates induced the specific IgG antibody to LOS in mice, and also induced the better antibody response in rabbits. MPL enhanced the immunogenicity of the OS-TT. The binding reactivities of 12 immunotype meningococcal LOS to the rabbit sera elicited by the OS-TT showed that M978 (B), BB431 (B), 44/76 (B), and A1 (A) LOS had the strongest reactivity which indicated that the A1 OS-TT conjugate induced the immunotype 8 specific antisera against several group B strains especially two disease isolated strains 44/76 and BB431.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-02006-10 LMDI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of the Immune Response to Polysaccharide Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:KE Stein, Ph.D., Chief, LMDI, DBP, CBER
CM Boswell, M.S., Biologist, LMDI, DBP, CBER

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular and Developmental Immunology

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.1	0.2	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The immune response to polysaccharide antigens is highly regulated and has several distinguishing features including restricted subclass, variable region gene usage and fine specificity. The basis for selective V_H and V_L gene usage in response to antigens such as bacterial levan (BL) is poorly understood. BL is a β (2→6) linked polyfructosan with β (2→1) (inulin determinant) linked branch points. We have generated a panel of 102 monoclonal antibodies (mAb) from BALB/cAnN and CBA/CAHN mice following one or two doses of 10 mcg BL. mAb from a single immunization are predominantly IgM, those from two injections are a mix of IgM and IgG3. V_H gene analysis of CBA mAb showed a biased usage of J606 and 36-60 families with an expected frequency of J558 in response to single or multiple injections. In BALB/c mAb there is a pronounced V_H restriction to the J606 family (84%) following a single injection of BL, but a more normal V_H profile in response to two injections. V_L usage is also restricted. There is a correlation in both strains (with only one exception in CBA) between VK11 usage and inulin reactivity of the mAb. Sequence data from three BALB/c J606/VK11 mAb show the use of one J606 gene, which has the same derived amino acid sequence as that of the J606 myeloma protein. 12 mAb have been generated from BL-injected CXBG/B4 mice which is a BALB/c x C57BL/6 recombinant inbred strain that expresses the BALB/c immunoglobulin heavy chain gene locus and the C57BL/6 *Sr-1* gene, a diversity gene previously shown to influence fine specificity of the response to BL. CXBG/4 mAb are a mix of IgM, IgA, and IgG3, do not cross-react with inulin, and are currently being analyzed for V region gene usage. We conclude that CBA and BALB/c mAb specific for BL differ markedly in V_H gene family usage and fine specificity, that light chain usage correlates with fine specificity, and that the BALB/c immunoglobulin heavy chain locus is, itself, not enough to explain these differences. Molecular studies are in progress to further define the basis for these differences.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-02005-07 LMDI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunity to Group C *Neisseria meningitidis* in Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:KE Stein, Ph. D., Chief, LMDI, DBP, CBER
ME Braun, Biologist, LMDI, DBP, CBER

COOPERATING UNITS *(if any)*

LJ Rubinstein, Ph.D., Merck, West Point ,PA
H. Jennings, Ph.D., NRC, Ottawa, Ontario, Canada

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DBP, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.1	PROFESSIONAL: 0.2	OTHER: 0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The capsular polysaccharide of *Neisseria meningitidis* group C (MCPS) is a homopolymer of $\alpha(2\rightarrow9)$ linked sialic acid residues. The polysaccharide is a thymus independent (TI) which is poorly immunogenic in infants and young children. Our previous studies demonstrated that the immune response to MCPS in BALB\c mice provides a model system which closely parallels the response in man. In order to compare the response to MCPS with that to MCPS-TT, a thymus dependent form of the antigen, we have generated two panels of monoclonal antibodies (mAb) from mice immunized with MCPS-TT and boosted with MCPS-TT (C2 mAb) or formalin fixed bacteria (CP mAb). The anti-MCPS mAb are primarily of the IgG3 (53%) and IgM (27%) isotypes, whereas the anti-C2 and anti-CP mAbs are mainly IgG1 (87% and 88% respectively). The data indicate a change in fine specificity as assessed on native MCPS and a naturally occurring non-O-acetylated form, OAc-. As reported earlier, the anti-MCPS mAb are predominantly of two specificities, MCPS specific (47%) or OAc- >> MCPS (20%). The anti-C2 mAb are more diverse, none are MCPS only specific, 27% are MCPS ≈ OAc-, 20% are MCPS > OAc- and 20% are OAc- >> MCPS. The anti-CP mAb were more similar to the anti-C2 mAbs than the anti-MCPS mAbs suggesting that the secondary antibody repertoire is determined by the primary immunization. Moreover the data also suggest the anti-C2 and anti-CP mAbs are of higher avidity than the anti-MCPS mAbs. The [OD 2] (concentration of mAb that results in fluorescence unit = 2 on a scale of 0-4 in a direct binding ELISA) for the majority of the anti-C2 and anti-CP mAbs are 10-100 fold lower than the [OD 2] for most of the anti-MCPS mAbs. To examine differences in affinity maturation between the TI and TD form of MCPS, we are currently establishing methods to measure intrinsic affinity constants. We will also test the IgG1 anti-C2 and anti-CP mAbs for bactericidal activity using mouse complement.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 BA-02008-05 LMDI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Ontogeny of Immunoglobulin Variable Region Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:SH Feng, Ph.D., Staff Fellow, LMDI, DBP, CBER
KE Stein, Ph.D., Chief, LMDI, DBP, CBER
MI Toledo, M.S., Guest Worker, LMDI, DBP,CBER

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular and Developmental Immunology

SECTION

INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.7	1.1	0.6

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies of murine antibody gene organization have shown that the heavy chain variable region (VH) is comprised of several gene families. Expression of these gene families appears to be random in adult mice, by Northern analysis of B cell hybridomas and transformed cell lines. Mice that carry the *xid* gene defect have been shown to be unresponsive to polysaccharide antigens. In earlier studies we asked whether the lack of responsiveness to polysaccharide antigens in *xid* mice could be accounted for by an inability to express certain VH gene families. We showed that *xid* mice could express all of the 9 VH gene families examined and with normal frequencies except for the VHJ558 family in CBA/N (*xid*) female mice. Moreover, female CBA/N mice showed individual variation in which VH gene family was predominantly expressed. VH expression was normal in CBA/N male mice, however, suggesting that two defective X chromosomes might result in greater expression of the defect than one defective X. To further explore the relationship of the abnormal VH gene expression to the *xid* defect, we examined mice with the *xid* defect on a BALB/c background. We found that in this strain, C.CBA/N, both males and females showed individual variation in which VH gene family was predominantly expressed. Furthermore, some of the VH families that were over-expressed showed strain variation, related to the background strain and not the *xid* gene. For example, the S107 family was over-expressed in some CBA/N mice, but not in C.CBA/N mice. Conversley, VGam3-8 was overexpressed in C.CBA/N but not in CBA/N mice. These results indicate that the *xid* gene does result in abnormal VH gene family expression. Studies are in progress to determine the molecular basis for this abnormality.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-02009-03 LMDI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mitogenic capacity of various components from gram negative bacteria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:KE Stein, Ph. D., Chief LMDI, DBP, CBER
ME Braun, Biologist, LMDI, DBP, CBER

COOPERATING UNITS *(if any)*

CM Tsai, Ph. D., Research Chemist, LBP, DBP, CBER

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DBP, CBER, FDA, Bethesda, MD, 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has three parts involving the capacity of three different components of gram negative bacteria to stimulate mitogenesis of murine B cells. The first involved the question of whether the *Haemophilus influenzae* type b capsular polysaccharide is a murine B cell mitogen as reported in 1986. We found that vaccine quality polysaccharide is not, itself, a B cell mitogen and only research grade polysaccharide containing significant contamination with lipopolysaccharide was mitogenic.The second part of this project was to determine if an *H. influenzae* type b polysaccharide-meningococcal outer membrane protein conjugate vaccine (Hib-OMP) was a murine B cell mitogen like the outer membrane protein alone which was reported by two labs to be mitogenic. We found that Hib-OMP was, indeed, a mitogen for murine B cells. The above two parts were completed previously. Studies have been completed for part 3, to determine if lipooligosaccharides from human pathogens such as *H. influenzae*, *Neisseria meningitidis* and *Bordetella pertussis* containing short O-side chains, are more potent B cell mitogens than lipopolysaccharides. The results indicate that if the various preparations are compared on an equal molar basis they have similar mitogenic activities, although, the lipooligosaccharide from *Neisseria meningitidis* was consistently found to be somewhat more stimulatory and exhibited a broader dose response curve. We found there was no correlation between mitogenic capacity and O-side chain length, comparing these lipooligosaccharides to a smooth *Escherichia coli* lipopolysaccharide. We completed these studies by examining the mitogenic capacity of several *Salmonella minnesota* mutants having different O-side chain lengths to wild type smooth LPS. Again, no correlation was found between O-side chain length and mitogenic capacity. Manuscripts of these studies are in preparation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-02002-04 LBP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic and Structural Analysis of _Neisseria meningitidis_ Outer Membrane Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Gerardo Zapata, Ph.D., Staff Fellow, LBP, DBP, CBER
Carl E. Frasch, Ph.D., Chief LBP, DBP, CBER

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Bacterial Polysaccharides, DBP

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DBP, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Neisseria meningitidis is a major cause of bacterial meningitis. Strains of _N. meningitidis_ express one of two porin proteins. These proteins have been identified as the class 2 and class 3 proteins, and express serotype specific epitopes. They have been evaluated as vaccine constituents. We obtained the gene for the class 3 protein by PCR from a serotype 4 strain as a 1,025 bp fragment. The sequence of this gene was obtained and compared to two recently published sequences. Based upon this comparison we identified two possible variable regions that may be associated with serotype specificity. Primers were prepared to obtain sequences in the VR1 and VR2 regions from 5 additional group B _N. meningitidis_ strains of serotypes 1, 4, 8, 12 and 15, all expressing class 3 proteins. The VR1 and VR2 regions were hypervariable and flanked by highly conserved regions among eight different class 3 sequences. These two hypervariable regions of 15 and 9 amino acids are predicted to be in surface exposed loops.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-02003-4 LBP

PERIOD COVERED

October 1, 1991 to August 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Lipid terminal of Bacterial Polysaccharide

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Gayathri Arakere, Ph.D., Staff Fellow, LBP, DBP, CBER
Carl Frasch, Ph.D., Chief, LBP, DBP, CBER
—

COOPERATING UNITS *(if any)*

-

LAB/BRANCH

Laboratory of Bacterial Polysaccharides, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS 0.3	PROFESSIONAL: 0.3	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The lipid terminal of several bacterial polysaccharides have been hypothesized to have a role in anchoring the polysaccharides to the membrane. We have studied the association of isolated membranes from a noncapsular variant of group B *Neisseria meningitidis* using two polysaccharides possessing very different structures and whose lipid terminals are well characterized, from group C *N. meningitidis* and *Haemophilus influenzae* type b. The polysaccharides were treated with Phospholipase A_2 or D to remove the fatty acid at the 2 position and the entire lipid terminal respectively. With native group C polysaccharide (both O-acetyl positive and negative), there was about 15% association with isolated membranes which was completely eliminated when the polysaccharide was treated with Phospholipase A_2. The native polysaccharide from *H. influenzae* type b also associated with the isolated membranes from *N. meningitidis* and the Phospholipase D treated polysaccharide did not. The association of the polysaccharide with the membrane seems to be eliminated whether only a fatty acid is removed or the entire lipid terminal is removed. The binding seems to be due to hydrophobic interactions rather than receptor mediated. We are looking into whether there are more quantitative differences in binding with Phospholipase A_2 and D treatments and whether the structures of the polysaccharides are altered in any way.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 BA 03001-03 LBT

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Identification of the ganglioside binding domain of tetanus toxin.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Jane L. Halpern, Ph.D., Pharmacologist, LBT, DBP, CBER
Anne Loftus, M.S, Microbiologist, LBT, DBP, CBER
Lura Williamson, Ph.D., LDN, NICHD, NIH
Elaine Neale, Ph.D, LDN, NICHD, NIH

COOPERATING UNITS *(if any)*
Laboratory of Developmental Neurobiology, National Institute of Child Health and Human Development, NIH

LAB/BRANCH
Laboratory of Bacterial Toxins, DBP

SECTION

INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.75	0.25	0.50

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Tetanus toxin is a protein neurotoxin of 1315 amino acids. The mechanisms by which tetanus toxin binds to and enters cells, and inhibits neurosecretion are not well understood. Hc (previously referred to as Fragment C) is a ~50,000 M.W. peptide derived by proteolysis from the carboxyl terminus of tetanus toxin that retains the ability to bind to gangliosides present in neuronal cell membranes. We have previously prepared a number of deletion mutants of Hc and identified the carboxyl terminal 5 amino acids as a region critical for binding to purified ganglioside. To determine if the requirements of Hc binding to purified ganglioside are similar to those for binding to target cells, these mutant forms of Hc have been examined for the ability to bind to spinal cord neuronal cultures. The binding of these proteins to neuronal cells paralleled the binding to purified ganglioside. Mutants with up to 263 amino acids deleted from the amino terminus retained ganglioside binding activity. The detection of 5 amino acids from the carboxyl terminus did not effect binding while the deletion of 10 or more carboxyl terminal amino resulted in a 75-80% loss of binding relative to Hc. The deletion mutants were more sensitive than Hc to proteolysis with trypsin, suggesting alterations in conformation. The receptor binding activity of Hc was not retained in a peptide corresponding to the carboxyl terminal 20 amino acids. These data suggest the carboxyl terminal of Hc is important for maintaining a conformation necessary for binding to receptor.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 BA 03003-05 LBT

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Global Regulation of Virulence Factors in *Bordetella pertussis*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: E. Scott Stibitz, Ph.D., Senior Staff Fellow, Microbiologist, LBT, DBP, CBER
M-S. Yang, M.S., Microbiologist, LBT, DBP, CBER
T.L. Garletts, COSTEP, LBT, DBP, CBER

COOPERATING UNITS *(if any)*
-

LAB/BRANCH
Laboratory of Bacterial Toxins, DBP

SECTION

INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS: 1.6	PROFESSIONAL: 1.6	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The primary focus of work in our laboratory has been the *vir* locus of *Bordetella pertussis*. This locus is responsible for the regulation of *B. pertussis* virulence determinants (pertussis toxin, filamentous hemagglutinin, etc.) in response to environmental stimuli. This locus is also the site of a reversible frameshift mutation which results in the phenomenon of phase variation. In the past we had observed that two closely related strains of *B. pertussis* underwent phase variation at dramatically different rates. We have recently extended this observation by constructing a defined frameshift mutation in a kanamycin resistance gene and showing that the reversion of this mutation also occurs at dramatically different rates in these two strains. We are currently engaged in the genetic mapping of this hypermutable phenotype, with an eye to the isolation of the gene(s) responsible. This mapping is made possible because we have recently completed a physical map of the *B. pertussis* chromosome. Physical mapping of previously isolated genes is a simple and straightforward task, and we have several collaborations with other workers in the field to map specific genes. However, the derivation of this map also allows the use of genetic tools for mapping. Specifically, we have designed and constructed new strains and new vectors which allow us to do genetic mapping using Hfr-like strains of *B. pertussis*. Such an approach will allow the isolation of some interesting genes which has not been possible using standard cloning techniques. The primary scientific finding of the chromosomal map was that the genes for *B. pertussis* virulence determinants are unlinked, i.e. are scattered around the chromosome. The implications of this finding are currently being extended by constructing maps of other strains of *B. pertussis* and other species of *Bordetella* with the hopes of further defining their evolutionary relationships.

U.S. GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 BA 03004-03 LBT

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Models for evaluating the toxicity of cytokines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Sherry Ansher, Ph.D., Senior Staff Fellow, LBT, DBP, CBER
Walter Thompson, B.S., Biologist, LBT, DBP, CBER
Raj Puri, M.D., Ph.D., LCI, DCB, CBER

COOPERATING UNITS *(if any)*
Division of Cytokine Biology, CBER

LAB/BRANCH
Laboratory of Bacterial Toxins, DBP

SECTION

INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD 2089

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER:
0.7	0.7	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The administration of cytokines has been associated with a variety of effects on many organ systems. One consequence of administration of cytokines is the inhibition of hepatic drug metabolism. The mechanism for this effect remains unknown. We have administered IL2 and IFNα to mice. The coadministration of these two cytokines causes inhibition of cytochrome P-450 levels, decreased microsomal drug metabolizing enzymes and increased hexobarbital-induced sleep times. All of these effects are more pronounced when both agents are given together than with either cytokine alone at a similar dose. Similar effects have been observed when IL-1 or IFN-gamma has been administered. As with vaccine administration, we are isolating the livers of mice treated with cytokines and are using cDNA probes to meausre specific isozymes of cytochrome P-450. In addition, probes to IL2 and IL1 have been used to see if these cytokines are elevated in the livers of mice treated with DTP vaccine. These results will be compared to the effects of direct cytokine administration to elucidate the cytokines involved in the inhibition of drug metabolizing enzymes.

Mice infected with murine AIDS show a number of alterations in hepatic drug metabolism. Studies are in progress to determine the extent of the alterations and the possible mechanism. Spleen cell cultures are being examined for cytokine induction to see if altered cytokine levels could account for some of the observed effects. In addition, studies at the level of the RNA are planned to see if there are increased cytokine mRNAs which might explain the changes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 BA 03005-03 LBT

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
The Effects of DTP Vaccine on Hepatic Drug Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Sherry Ansher, Ph.D., Senior Staff Fellow, LBT, DBP, CBER
Walter Thompson, B.S., Biologist, LBT, DBP, CBER
Philip J. Snoy, DVM, DPQC, CBER

COOPERATING UNITS *(if any)*
Division of Product Quality Control, CBER

LAB/BRANCH
Laboratory of Bacterial Toxins, DBP

SECTION

INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD 2089

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have demonstrated that administration of DTP vaccine to mice causes alterations in hepatic drug metabolism in a dose and time dependent manner. The mechanism for the observed changes is unknown, as are the components responsible for the alterations. Endotoxin (LPS) is a common component of many bacterial vaccines and causes a variety of effects in vivo. In order to understand the role of LPS in vaccine-induced hepatotoxicity, endotoxin sensitive and endotoxin resistant mice have been treated with vaccines and endotoxin. There is a marked difference in hexobarbital-induced sleep times and cytochrome P-450 levels between LPS-sensitive and LPS-resistant mice treated with 50 ug of B.pertussis LPS. In contrast, DTP vaccine pretreatment causes similar responses in both strains of mice. These differences between LPS and DTP vaccine persist for at least 1 week. Polymyxin B sulfate is able to completely neutralize the effects of soluble LPS, but there is still residual rabbit and limulus endotoxic activity when it is mixed with DTP vaccine. This may be due to incomplete accessibilty of the endotoxin in the vaccine. An additional approach has been to use DT vaccine mixed with LPS to simulate bound endotoxin. This mixture does not alter drug metabolism in the same manner as DTP vaccine. The acellular version of DTP does not produce the same effects as the whole cell vaccine in mice. Serum cytokine levels (IL6 and TNF) are elevated in a number of disease states or following injury or trauma. We have found that IL6 and TNF are elevated 2-4 hours after a single dose of vaccine or endotoxin. The serum profiles of these cytokines are similar in responsive mice treated with either LPS or DTP vaccine; in nonresponsive mice neither agent produces a marked increase. Studies are continuing to identify other factors which may be involved.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA 03006-02 LBT

PERIOD COVERED

March 25, 1991 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure-function relationships of diphtheria toxin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Virginia G. Johnson, Ph.D., LBT, DBP, CBER
Peter J. Nicholls, Ph.D., SNB, NINDS
Richard J. Youle, Ph.D., SNB, NINDS

COOPERATING UNITS *(if any)*

Surgical Neurology Branch, National Institute of Neurological Disorders and Stroke, NIH.

LAB/BRANCH

Laboratory of Bacterial Toxins, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS: 0.5	PROFESSIONAL: 0.5	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Diphtheria toxin is a 58,000 dalton protein. The crystal structure of the toxin reveals three distinct domains corresponding to the three functional activities of the toxin: receptor binding, membrane translocation and enzymatic activity. Our studies are directed at a better understanding of the structure-function relationships of the translocation and enzymatic domains. Residues within the enzymatic cleft have been mutagenized to better define to toxin's ability to transfer ADP-ribose from NAD to elongation factor 2.

The translocation domain of the toxin reveals a high concentration of α-helical regions. Two the these α-helices are hidden in the center for the domain at neutral pH. It is believed that when the toxin encounters acidic conditions in the endosome, the translocation domain undergoes a conformational change, exposing these two α-helices and allowing them to insert into the lipid bilayer as the initial step in membrane translocation. A proline residue at position 345 is one of the amino acids separating these two α-helices. We have changed this Pro to Glu or Gly and found that while the mutant toxins demonstrated comparable binding activity and enzymatic activity to the native protein, they were 100-fold less toxic to cells reflecting a 100-fold reduction in translocation activity. These results demonstrate the critical role of Pro 345 in membrane translocation.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-BA-05001-0 LMCB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Characterization of *Mycobacterium intracellulare* λgt11 recombinant clones.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Sheldon L. Morris, Ph.D., Commissioned Corps, LMCB, DBP, CBER
David A. Rouse, B.A., Biologist, LMCB, DBP, CBER
Jay Nair, M. Phil., Biologist, LMCB, DBP, CBER

COOPERATING UNITS *(if any)*
Arthur Karpas, Ph.D., Hybridoma Coordinator, LMCB, DBP, CBER

LAB/BRANCH
Laboratory of Mycobacteria, DBP (formerly: Immunology)

SECTION

INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.4	0.8	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

During the past year, the Laboratory of Mycobacteria has continued the evaluation of the genetic, biochemical, and immunologic characterization of two immunoreactive *Mycobacterium intracellulare* antigens.

MI43 - Nucleotide sequence analyses have previously indicated that the MI43 gene encodes a 27 kDa lipoprotein. Recently, we have confirmed biochemically, using detergent phase separation experiments and metabolic labeling with ^{3}H palmitic acid that MI43 is a lipoprotein. We have also demonstrated that purified MI43 is recognized by 70% of the sera from patients with tuberculosis, 80% of sera from HIV-seronegative patients with MAC disease, and 50% of sera from AIDS patients with MAC disease.

MI85 - Our nucleotide sequence analysis and expression in *Escherichia coli* suggest that the MI85 gene encodes a catalase-peroxidase, a bi-functional enzyme that may be critical for the intraphagosomal survival of mycobacteria. Because of the importance of MI85, we have generated a number of monoclonal antibodies which recognize epitopes on this antigen. Induction experiments using one of these antibodies have demonstrated that MI85 is induced three-fold by peroxide *in vitro*. In addition, we have identified the nucleotide sequences containing the MI85 catalase promoter and are currently characterizing the structure and activity of the promoter. Finally, we have cloned and are characterizing the catalase-peroxidase genes of *M. tuberculosis* and *M. avium*.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BA-05003-04 LMCB

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of *Mycobacterium kansasii* antigens in a λgt11 gene LMCBbrary

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Sheldon L. Morris, Ph.D., Commissioned Corps, LMCB, DBP, CBER
Geraldo Armoa, M.S., Graduate Student/Guest Scientist
David A. Rouse, B.A., Biologist, LMCB, DBP, CBER

COOPERATING UNITS *(if any)*

Lewis F. Affronti, Ph.D., The George Washington University

LAB/BRANCH

Laboratory of Mycobacteria, DBP (formerly: Immunology)

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER:
0.5	0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previously, we had identified a recombinant bacteriophage from a λgt11 gene library which expressed an immunogenic *Mycobacterium kansasii* antigen (MK35). We had also demonstrated that MK35 is reactive against sera from patients with mycobacterial disease and evokes a large skin test response in sensitized guinea pigs. In FY92, we have completed the DNA sequence of the gene encoding MK35. Nucleotide sequence analysis has indicated that MK35 is a 26 kDa lipoprotein. Using polymerase chain reaction methodologies, we have identified homologous genes from *M. tuberculosis* and *M. avium*. These genes are currently being characterized.

U.S. GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-BA-05004-04 LMCB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
AIDS-related Mycobacterial Disease--Antibodies to *M. avium* in AIDS sera

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Sheldon L. Morris, Ph.D., Commissioned Corps, LMCB, DBP, CBER
David Rouse, B.A., Biologist, LMCB, DBP, CBER
Francis Chuidian, M.D. (Guest Worker), LMCB, DBP, CBER

COOPERATING UNITS *(if any)*
Kuzzel Institute, San Francisco, CA (Dr. Luis Bermudez)
Georgetown University (Drs. Henry Yeager and Rod Schwartz)

LAB/BRANCH
Laboratory of Mycobacteria, DBP (formerly: Immunology)

SECTION

INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
0.4	0.4	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To evaluate the feasibility of developing serodiagnostic tests for detecting *Mycobacterium avium* complex (MAC) infections, antibodies to MAC antigens were measured by ELISA and immunoblot assays in 20 patients with AIDS and disseminated MAC disease, 5 HIV-seronegative patients with pulmonary MAC infections, and 20 healthy controls. Wheras ELISA titers for healthy controls and patients with AIDS and MAC disease were comparable, HIV-seronegative patients with MAC disease had higher anti-MAC antibody titers ($P<0.01$). Immunoblot analyses with the same *M. avium* sonic extracts indicated that each of the three groups had a limited heterogeneous response to *M. avium* antigens. No significant differences in immunoblot reactivities were detected. However, immunoblot studies with recombinant nontuberculous mycobacterial antigens revealed that over 90% of the patients with MAC disease and only 25% of controls recognized a recombinant protein derived from a 35 kDa mycobacterial protein. Although sonic extracts do not permit adequate discrimination of antibody reactivity in patients with MAC disease, recombinant antigens may be useful indicators of disease.

We have also been utilizing MAC-specific monoclonal antibodies to detect *M. avium* antigens in the sera of AIDS patients with MAC disease. We are currently testing a protocol that permits the detection of MAC antigen in AIDS patient sera using MAb 4004 F1, an antibody that reacts specifically with a 20 kDa MAC protein.

U.S. GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BA-05005-03 LMCB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Analysis of natural and synthetic antigens and epitopes of mycobacteria.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Sheldon L. Morris, Ph.D., Commissioned Corps, LMCB, DBP, CBER
David A. Rouse, B.A., Biologist, LMCB, DBP, CBER
Jay Nair, M.Phil., Biologist, LMCB, DBP, CBER
Gill Han Bai, DVM, Ph.D., Fogarty Fellow, LMCB, DBP, CBER

COOPERATING UNITS *(if any)*

-

LAB/BRANCH

Laboratory of Mycobacteria, DBP (formerly: Immunology)

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS. 1.6	PROFESSIONAL: 1.6	OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

In an effort to define immunodominant epitopes and to develop monospecific skin test antigens, T cell epitopes from the homologous 19 kDa proteins from *Mycobacterium intracellulare* and *M. tuberculosis* were defined with overlapping synthetic peptides. These two antigens, exhibiting 78% sequence homology, present an excellent opportunity for defining species-specific epitopes. The T cell activity of these mycobacterial peptides were first tested in vitro by challenging anti-*M. tuberculosis*, anti-*M. avium*, and anti-*M. kansasii* T cell lines and assaying for T cell proliferation. Six peptides derived from the 19 kDa *M. tuberculosis* antigen stimulated T cells. Two of these peptides were specifically active against only the *M. tuberculosis* T cell line. Four the peptides derived from the *M. intracellulare* 19 kDa antigen induced T cell proliferation, and two of these peptides had specific reactivity. The peptides were also assayed for their capacity to elicit delayed-type hypersensitivity (DTH) reactions in guinea pigs. All six of the tuberculosis peptides that were active in skin test assays elicited non-specific responses. However, one of nine active *M. intracellulare* peptides did elicit a monospecific DTH response. These experiments suggest that specific skin test reagents may be developed from synthetic peptides.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-839

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BA-05006-02 LMCB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Rapid Identification of Mycobacterial Infections Using Polymerase Chain Reaction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Sheldon L. Morris, Ph.D., Commissioned Corps, LMCB, DBP, CBER
Gill Han Bai, DVM, Ph.D., Fogarty Fellow, LMCB, DBP, CBER
~~Z.M~~. Li, LP, DBP, CBER

COOPERATING UNITS *(if any)*

-

LAB/BRANCH

Laboratory of Mycobacteria, DBP (formerly: Immunology)

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS 0.7	PROFESSIONAL: 0.7	OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Polymerase chain reaction (PCR) protocols designed to rapidly and specifically detect *Mycobacterium avium*, *M. intracellulare*, and *M. kansasii* infections were developed. These protocols utilize PCR primers derived from unique sequences within the mycobacterial 16s rRNA genes. The *M. avium*, *M. intracellulare*, and *M. kansasii* PCR procedures specifically detect 1-10 fentograms of homologous genomic DNA. No PCR reactivity was demonstrated against DNA from 18 other strains of mycobacteria or from 7 closely related bacterial strains. Furthermore, bacterial titration experiments indicated that 1-10 nontuberculous mycobacterial bacilli could be directly detected using these PCR protocols. Currently, these PCR methodologies are being adapted for direct assays of clinical samples.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-BA-05007-01 LMCB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Molecular Characterization of Drug Resistance in *Mycobacterium tuberculosis*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Sheldon L. Morris, Ph.D., Commissioned Corps, LMCB, DBP, CBER
David A. Rouse, B.A., Biologist, LMCB, DBP, CBER
Jay Nair, M.Phil., Biologist, LMCB, DBP, CBER

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Mycobacteria, DBP (formerly: Immunology)

SECTION

INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.2	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Because of the emergence of multiply drug resistant *Mycobacterium tuberculosis* strains in clinical isolates, our laboratory has begun a molecular characterization of drug resistance in *M. tuberculosis*. Resistance to isoniazid (INH), the primary drug for treatment of tuberculosis, is frequently correlated with a loss of catalase-peroxidase activity. Recently, we have demonstrated by using peroxidase assays and an affinity purified polyclonal antibody that an INH resistant mutant of the catalase-peroxidase positive *M. tuberculosis* H37Rv strain has no detectable catalase protein. Furthermore, Southern blot hybridizations with a catalase-peroxidase gene probe have demonstrated that the gene encoding this protein is deleted from the INH resistant strain. We are currently examining other INH resistant strains to elucidate the role of catalase in INH resistance.

In prokayotes and eukaryotes, resistance to streptomyocin (SM), another primary tuberculosis drug, often results from mutations in the ribosomal S12 protein gene. We have recently demonstrated with PCR and nucleotide sequence analyses that 2 different SM resistant *M. tuberculosis* strains have identical mutations in the S12 protein gene. The location of these mutations within the *M. tuberculosis* S12 gene is identical to the site of S12 mutations previously described in other SM resistant cells.

PHS 6040 (Rev 1/84)

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-BA-06001-08 LM

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Mycoplasma pneumoniae-induced Pneumonia in Chimpanzees

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Michael F. Barile, Ph.D., Chief, LM, DBP, CBER
Marion W. Grabowski, B.S., Microbiologist, LM, DBP, CBER
Bobby Brown, D.V.M., Primate Research Institute, Univ. of New Mexico
Donna K.F. Chandler, Ph.D., LM, DBP, CBER
Kaity Kapatais-Zoumbos, M.D., Medical School of Patrus, Patrus, Greece

COOPERATING UNITS *(if any)*
Primate Research Institute, Univ. of New Mexico, Holloman, NM
Medical School of Patrus, Patrus, Greece

LAB/BRANCH
Laboratory of Mycoplasma, DBP

SECTION

INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Six sero-negative, culture-negative young adult chimpanzees were experimentally-infected with *Mycoplasma pneumoniae*. Each chimpanzee became colonized with peak titers of 10^6 to 10^8 ccu/ml. The oropharyngeal and tracheal tissues remained colonized for up to 70 days and lungs for 26 days. Seroconversion developed within two weeks post-challenge; the titers peaked between 1:256 and 1:1024, and antibody titers persisted throughout the study period. Positive cold agglutinin titers were detected at day 12 to 15 post-inoculation, and the response persisted for up to 40 days with peak titers ranging from 1:160 to 1:640. Animals developed overt signs of disease that corresponded with peak lung colonization, the development of positive x-ray findings and cold agglutinin titers. The infected chimpanzees developed a persistent cough and low grade fever, and some developed rhinitis, oropharyngitis, diarrhea, and lot of appetite. The specific IgA immunoglobulin values in the lung lavage fluids were 2 to 3 times greater than the IgG values. The two non-inoculated chimpanzees that served as negative controls became infected during the course of study, but we were unable to determine the mode of transmission. The experimentally-induced pneumonia in chimpanzees were remarkably similar to naturally occurring primary atypical pneumonia in humans, and this animal best reflects and parallels the pneumonia in humans, and this animal best reflects and parallels the pneumonia in humans. Manuscript is being prepared for publication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BA-06003-03 LM

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Evaluation of *Mycoplasma pneumoniae* vaccines in chimpanzees

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Michael F. Barile, Ph.D., Chief, LM, DBP, CBER
Marion W. Grabowski, B.S., Microbiologist, LM, DBP, CBER
Donna K.F. Chandler, DBIND, CBER
Bobby Brown, D.V.M., Primate Research Institute, Univ. of New Mexico
Kaity Kapatais-Zoumbos, M.D., Medical School of Patrus, Patrus, Greece

COOPERATING UNITS *(if any)*

Primate Research Institute, Univ. of New Mexico, Holloman, NM
Medical School of Patrus, Patrus, Greece

LAB/BRANCH

Laboratory of Mycoplasma, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.05	0.05	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Chimpanzees were immunized with a formalin inactivated or an acellular extract vaccine by the intraperitoneal-priming, intratracheal-booster immunization schedule shown previously to protect hamsters on challenge (Barile et al., 1988b). Although immunization with either vaccine induced some seroconversion, there was only a slight increase in the serum specific IgM values, and no increase in IgG values. Following immunization, there was little or no increase in the specific IgA or IgG values in lung lavage fluids. Thus, these two vaccines did not induce a meaningful immunological response. Two weeks after immunization, animals were challenge with 1 x 107 CCU of virulent strain PI-1428. Mean antibody titers for the acellular vaccine-immunized animals peaked within four weeks at 1:256, and then declined, whereas the formalin-inactivated vaccine animals' titers peaked at 1:85 within six weeks and persisted throughout the 10 week study period. The oropharygeal, tracheal and lung tissues of immunized and non-immunized animals became colonized, and peak titers ranged from 106 to 108 CCU/ ml. The animals immunized with either vaccine developed less colonization of the oropharynx and a lesser amount of overt disease than the non-immunized controls. The formalin inactivated vaccine that was used in a field trial study protected 67% of the vaccinees (Wenzel et al., 1977), and provided partial protection to these chimpanzees on challenge. Prior to challenge, the serum antibody titers of two chimpanzees experimentally infected six months earlier with M. pneumoniae were both 1:64. Following challenge, the mean antibody titer peaked at 144 within two weeks, and then declined. The oropharygeal, tracheal and lung tissues of one chimpanzee did not become colonized and the other animal had only one positive culture, a lung specimen with a titer of only 102 CCU/ml. These findings indicate that a prior mycoplasmal infection protects the animal host against subsequent challenge or exposure. Manuscript is being prepared.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	**PROJECT NUMBER** Z01-BA-06005-05 LM

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular analysis of *Mycoplasma hominis*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: L.D. Olson, Ph.D., Staff Fellow, LM, DBP, CBER
A.A. Gilbert, M.A., Microbiologist, LM, DBP, CBER
L.A. Adler, B.S., Howard Hughes Preceptor Program Guest Worker

COOPERATING UNITS *(if any)*
-

LAB/BRANCH
Laboratory of Mycoplasma, DBP

SECTION

INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	0.3	1.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mycoplasma hominis strain 1620 was isolated from the synovial fluid of a chronically infected septic arthritis patient. We have been studying the variable expression of surface exposed, acylated, integral membrane proteins that may play a role in the persistance of this pathogen in its human host.

Three monoclonal antibodies (Mabs) that bind specifically to the variable *M. hominis* surface antigens have been previously constructed and characterized. The Mabs are being used as probes of *M. hominis* gene banks in our effort to isolate, clone and characterize a gene or genes that encode the protein portion(s) of the membrane lipoproteins.

Additionally, we are analyzing the expression of the Mab-specific surface antigens in generations of clones. Individual colonies vary in relative size from small to medium to large. We have determined that the large colonies express high molecular weight proteins that are absent from the smaller colonies. The antigenic switching that occurs is under study.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 BA 06008-02 LM

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Sera Immunoblots of Chimpanzee Infected with *Mycoplasma pneumoniae*.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: M. F. Barile, Ph.D., Chief, LM, DBP, CBER
G. Franzoso, Fogarty Fellow, LM, DBP, CBER
G.A. Meloni, Universita di Padova, Padova, Italy
P-C. Hu, Dept. of Pediatrics, Univ. of North Carolina Medical School

COOPERATING UNITS *(if any)*
Universita di Padova, Padova, Italy
Dept. of Pediatrics, Univ. of North Carolina Medical School, Chapel Hill, NC

LAB/BRANCH
Laboratory of Mycoplasma, DBP

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INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.75	0.75	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The immunoblot analyses of convalescent sera from groups of chimpanzees that were either experimentally-infected, immunized and challenged or rechallenged following a previous infection were examined and compared in an attempt to identify the protective immunogens. The sera of the infected chimpanzee reacted with 17 to 20 protein bands and the immunoblot patterns produced were remarkably similar to the convalescent serum of a patient with natural-occurring *Mycoplasma pneumoniae* disease. Whereas the sera of chimpanzees immunized with the acellular vaccine recognized three proteins (61, 42 and 30 kDa), the sera of the formalin-inactivated vaccine were sero-negative prior to challenge. All immunized animals seroconverted after challenge. Although the immunoblot patterns among the three groups were similar following challenge, a moderate degree of variations was noted among individuals and between groups. The previously infected chimpanzees, who were fully protected, showed the most impressive immunologic responses on challenge. The most immunodominant component on challenge in each group was the 169 kDa protein band, that co-migrated with the well-established P1-adhesin. The other immunodominant protein bands were the 117, 86, 35 and 30 kDa. Of these, the 86 and 35 kDa polypeptides were most noteworthy because these two surface exposed, immunodominant proteins were present in the cytadsorbing, pathogenic strains PI-1428, M129 and FH, but were absent in the non-cytadsorbing, non-pathogenic strain B176. These components were also the most immunodominant components in the convalescent sera of patients with naturally-occurring disease. For these reasons, the 169, 86 and 35 kDa proteins probably represent the most promising immunogens for inclusion in the development of a protective acellular vaccine. The manuscript is being prepared for publication.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA 06009-02 LM

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mycoplasma-induced septic arthritis in chimpanzees.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M. F. Barile, Ph.D., Chief, LM, DBP, CBER
Philip J Snoy , D.V.M., Pathobiol. and Primatol. Lab, DPQC, CBER
Marion W Grabowski, LM, DBP, CBER
Donna K.F. Chandler, Ph.D., LM, DBP, CBER

COOPERATING UNITS *(if any)*

Division of Product Quality Control, CBER

LAB/BRANCH

Laboratory of Mycoplasma, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS: 0.1	**PROFESSIONAL:** 0.1	**OTHER:**

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study involved the induction of septic arthritis in chimpanzees with strains of *M. hominis* and *U. urealyticum* and with a pathogenic strain of *M. pneumoniae*. An acute septic arthritis was experimentaly induced in chimpanzees inoculated intraarticularly with strains of *Mycoplasma hominis* and *Ureaplasma urealyticum*, each isolated from a patient with septic arthritis. The disease induced was similar to that observed in patients with regard to clinical signs of disease, degree of colonization, and the extent of the inflammatory and antibody responses produced. A very large inoculum of the arthritogenic *Mycoplasma hominis* strain 1620 given intravenously or of the non-cytadsorbing laboratory reference type strain PG21 given intraarticularly produced no overt clinical disease.

U. urealyticum serovar SVII strain 2010B isolated from the septic joint of a patient with hypogammaglobulinema and in low broth passage produce severe disease, whereas the laboratory reference Serovar SVII type strain OC in high passage produced mild disease. Whereas the arthritogenic *Ureaplasma* strain produced an intense inflammatory response and a weak metabolism inhibition antiboey response, the high passage laboratory type strain produced a mild arthritis and minimal inflammation but a greater metabolism inhibition antibody response. Thus the source of the original isolate, attentuation of virulence by continuous broth passage and the ability to attach may all be important factors in determining the ability of *Mycoplasmas* to experimentally induce arthritic disease. The manuscript is being prepared for publication.

U.S. GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA 06011-01 LM

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Attachment Specificity of *Mycoplasma hominis*.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: L.D. Olson, Ph.D., Staff Fellow, LM, DBP, CBER
A.A. Gilbert, B.S., Microbiologist, LM, DBP, CBER

COOPERATING UNITS *(if any)*

-

LAB/BRANCH

Laboratory of Mycoplasma, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.45	0.20	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Mycoplasma hominis strain 1620, isolated fro the synovial fluid of a chronically infected spetic arthritis patient, has been the primary strain under study to determine the types of receptors used for colonization of the host. We have found that *M. hominis* binds only to sulfatide or sulfated glycolipids and to no other glycolipid or glycoprotein tested.

M. hominis colonizes the human urogenital tract. Both male and female tracts possess a preponderance of sulfated glycolipids on the surface of the tissues. It is therefore reasonable that the mycoplasma make use of this prevalent compound as its receptor for attachment.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA 06012-01 LM

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of the Pathogenicity of Strains of *M. fermentans*.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: L.D. Olson, Ph.D., Staff Fellow, LM, DBP, CBER
A.A. Gilbert, M.A., Microbiologist, LM, DBP, CBER
Robin N. Shepard, Biological Laboratory Aid, LM, DBP, CBER
P. Snoy, D.V.M., Pathobiol. and Primatol. Lab, DPQC, CBER

COOPERATING UNITS *(if any)*

Division of Product Quality Control, CBER

LAB/BRANCH

Laboratory of Mycoplasma, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.11	0.10	0.01

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mycoplasma fermentans, a mycoplasma species that colonizes humans, was first described in 1953. It has been detected in and isolated from tissues of AIDS patients and appears to be associated with a fatal necrotic disease. In order to satisfy Koch's postulates for demonstration of pathogenicity, we have intraperitoneally inoculated six groups of 4 rhesus macaque monkeys each with one of the following 3T3 tissue cell culture-grown preparations: the original *M. fermentans* isolate from an AIDS patient, strain *incognitum;* reference *fermentans* strain, PG18; a synovial *fermentans* isolate, strain KL-4; a tissue-culture dependent strain, IM-1; a heterologous mycoplasmal synovial isolate, *M. hominis* strain 1620; or just 3T3 cells alone.

The monkeys were inoculated November 1, 1991. The four monkeys from each group have had their temperatures taken and been weighed weekly. Blood is drawn every month. At this date, at least one monkey from each *fermentans* group has evinced either a weight loss or an inability to maintain weight. Nevertheless, no serum antibodies have been detected in any of the 16 monkeys that received *M. fermentans*. In contrast, the 4 monkeys inoculated with *M. hominis* have demonstrated substantial rises in serum antibodies against *hominis* proteins. Cytokine responses are currently being measured. These results are similar to those observed during the initial *M. fermentans* study in animals performed by Dr. Shyh-Ching Lo (WRAIR) as well as an on-going SIV study in rhesus monkeys at the University of Wisconsin.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA 06014-01 LM

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Mycoplasma fermentans metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: L.D. Olson, Ph.D., Staff Fellow, LM, DBP, CBER
C.A. Renshaw, B.S., Biologist, LM, DBP, CBER
S. Rottem, Visiting Scientist, LM, DBP, CBER
J. Boal, Ph.D., Staff Fellow, BL, DBB, CBER

COOPERATING UNITS *(if any)*

-

LAB/BRANCH

Laboratory of Mycoplasma, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.75	0.75	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mycoplasma fermentans, a species that colonizes humans, is unique among mycoplasmas. Most mycoplasmas are divided metabolically into species that ferment glucose or species that are nonfermenters but hydrolyze arginine. *M. fermentans*, however, possesses both metabolic pathways. Furthermore, *M. fermentans*, apparently unlike other human mycoplasmas which are obligate extracellular parasites, is an intracellular parasite that has been implicated as the agent of a fatal necrotic disease.

The arginine dihydrolase pathway includes arginine deiminase (AD), which converts L-arginine to L-citrulline; ornithine transcarbamylase (OTC) which converts citrulline to ornithine and carbamyl phosphate; and carbamate kinase, which yields carbamyl phosphate to CO_2, ATP and NH_3.

AD is an immunosuppressive substance which has been shown to exert a lymphocyte blastogenesis inhibitory activity. If *M. fermentans* is capable of causing an immunosuppressive disease, better understanding of its metabolism is warranted. Since both fermentation and arginine hydrolysis are occurring within the cell, we investigated whether repression by glucose of arginine hydrolysis is a regulatory mechanism used by *M. fermentans*.

Whole cell perfusion NMR was used to demonstrate that *M. fermentans* is ferments glucose concomittantly with arginine hydrolysis. Enzyme assays of the AD from *M. fermentans* grown with or without glucose did not show any repression of the activity. In contrast, the specific acitivities of *M. fermentans* OTC from cells grown with or without glucose demonstrated vividly that this enzyme was subject to glucose repression. The OTC from *M. hominis* was, as expected, unaffected by growth in glucose. Therefore regulation of the arginine dihydrolase pathway in *M. fermentans* occurs in large part at the level of OTC repression by glucose, while arginine deiminase is unaffected by glucose metabolism.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA 06015-01 LM

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Immunodominant components of *M. pneumoniae* involved in attachment.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M. F. Barile, Ph.D., Chief, LM, DBP, CBER
G._Franzoso, M.D., Fogarty Fellow, LM, DBP, CBER
G. A. Meloni, M.D., University di Padova, Padova, Italy
P.-C. Hu, Ph.D., University of North Carolina Medical School

COOPERATING UNITS *(if any)*

University di Padova, Padova, Italy; Department of Pediatrics, University of North Carolina Medical School, Chapel Hill, NC

LAB/BRANCH

Laboratory of Mycoplasma, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.75	0.75	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Convalescent sera of experimentally infected chimpanzees or monoclonal antibodies (MAbs) specific to the 90 kDa and 40 kDa proteins indicated that both proteins were present in cytadsorging, pathogenic *M. pneumoniae* strains PI-1428, M129, and FH, but were not present in the non-cytadsorbing, non-pathogenic strain M129-B176. Adsorption of convalescent chimpanzee sera with the avirulent strain M129-B176 did not remove or inhibit reactivity of these two proteins. Using proteolysis and hte specific MAbs, we demonstrated that the 90 and 40 kDA proteins were surface exposed. Western blot analyses using surface-bound antibodies supported these findings. Electron microscopy studies with gold-labeled MAbs showed that the 90 kDa protein is localized on the terminal tip attachment apparatus. Thus, in addition to the well established 169 kDa P1-adhesin, the immunodominant, surface exposed 90 and 40 kDa proteins might also be involved in mycoplasma attachment. These proteins represent important immunogens and should be considered as potential candidates for inclusion in an acellular *M. pneumoniae* vaccine. The manuscript is being prepared for publication.

PHS 6040 (Rev 1/84)

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 BA 06016-01 LM

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cloning and characterization of the 52 kilodalton protein.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Hilda Marcus, Ph.D., Visiting Scientist, LM, DBP, CBER
Elizabeth Leininger, Ph.D., Staff Fellow, LAIC, DBP, CBER

COOPERATING UNITS *(if any)*
Laboratory of Allergy and Immunochemistry, DBP, CBER

LAB/BRANCH
Laboratory of Mycoplasma, DBP

SECTION

INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Krivan H. C. et al (1989 J. Biol. Chem) reported that *M. pneumoniae* binds avidly to sulphatides. A membrane preparation *M. pneumoniae* was applied to a dextran sulphate affinity column, and two proteins of 52 kDa and 32 kDa molecular weight bound to the column (Geary S. et al. 1990, IOM Abst.). The 52 kDa protein can be detected in western blots of membrane preparations from *M. pneumonie* using the monoclonal antibody (Ab) CP3-46F5 (F5) (Chandler et al. 1989. Infect. Immun. 57:1131-1136). Chromosomal DNA from *M. pneumoniae* M129 was restricted and ligated to the phagemid vector Bluscript (Stratagene). *Escherichia coli* XL1-Blue colonies expressing epitopes of the 52 kDa protein were detected immunologically by reaction with monoclonal antibody CPs-46F5. Three positive phagemid constructs were identified. They code for peptides that are smaller than the size expected for fusion protein on western blots. The sequenc of the cloned *Mycoplasma* DNA inserts is being analyzed to determine the presence of UGA condons. UGA condon code for tryptophan in *Mycoplasma* spp., but shen cloned into *E. coli* result in production of truncated proteins. Surface proteolysis of strain M129 indicated the surface exposed nature of the protein . Monoclonal antibody F5 was purified from ascites fluid in order to affinity purify the protein on an Ab affinity column. Location of the 52 kDa protein on *M. pneumoniae* will be studied by immune electron microscopy. Localization of the protein at the adherence tip structure would indicated the importance of the protein in adherence. The role this protein plays in the adherence of *M. pneumoniae* will be evaluated in adherence inhibition studies.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA 06017-01 LM

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Cloning and characterization of the protease produced by *M. pneumoniae*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Hilda Marcus, Ph.D., Visiting Scientist, LM, DBP, CBER
Dan C. Stein, Ph.D., University of Maryland
--

COOPERATING UNITS *(if any)*

University of Maryland

LAB/BRANCH

Laboratory of Mycoplasma, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS.	PROFESSIONAL	OTHER.
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Infection of the respiratory tract by *M. pneumoniae* is initiated by attachment of the organism to ciliated cells. Attachment of the organism to the respiratory epithelium is a prerequisite to colonization. Attachment is followed by ciliostasis, alteration and loss of cilia, and destruction of the mucosal epithelium. The nature of events leading to the destruction of the respiratory epithelium is not clear. Chandler et al (1980. I & I. 29:1111-1116) demonstrated that a cell free extract of a virulent *M. pneumoniae* caused ciliostatis of hamsters ciliated epithelium, hemagglutination of red blood cells, and proteolysis of a synthetic tetrapeptide substrate S-2222. An open reading frame 6 (ORF6) coding for a 130 kDa protein, is part of the "P1 adherence operon" of *M. pneumoniae* (Colman et al. 1990. Gene. 87:91-96). The 40 kDa and 90 kDa protein coded for by the ORF are important in positioning P1 adhesin at the tip structure of the organism. Processing signals for production of the two proteins can not be identified on a nucleic acid level. Our current working hypothesis is that the protease is required to cleave the 130 kDa scaffolding protein. OFR6 will be recloned into phagemid Bluescript and the 130 kDa protein will be labeled radioactively under the T7 polymerase system. The labeled protein will serve as a substrate for digestion by the protease. Two approaches have been taken to study the protease. First, the protease will be isolated from a cell free extract, and its specificity will be studied using protease inhibitors and substrates. Second, a gene coding for a UGA suppressor $tRNA^{trp}$ was obtained from Michael Yarus (Univ. of Colorado). The gene was recloned into plasmid pRK415 that is compatible with the ColEI replicon plasmids used to construct a chromosomal DNA library from *M. pneumoniae* strain M129. The library, constructed in a IacIq strain under the Iac promoter, will be induced and screened for production of the protease.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA 06018-01 LM

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of genes in the PI operon of *Mycoplasma pneumonia*.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Dan Stein, Ph.D., IPA Appointment, LM, DBP, CBER
Mike Barile, Ph.D., Chief, LM, DBP, CBER
P.-C. Hu., Department of Pediatrics, Univ. of North Carolina Medical School

COOPERATING UNITS *(if any)*

Department of Pediatrics, University of North Carolina Medical School, Chapel Hill, North Carolina

LAB/BRANCH

Laboratory of Mycoplasma, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Inamine et al. (Gene 73:175-183) identified an operon in *M. pneumoniae* that encodes a gene responsible for attachment of *M. pneumoniae* to the human host. The PI gene product appears to be essential for attachment, although the role that the other genes in this operon play in attachment remain unknown. In this project we will focus on Orf 4, and its possible role in attachment. This project has two primary goals: 1) The DNA encoding this Orf will be subjected to site-specific mutagenesis to allow for the expression of this gene in *E. coli* and 2) the expressed protein will be purified from *E. coli* for use in various biological experiments. *M. pneumoniae* does not read the genetic code in the same manner as most bacteria, it uses the TGA codon to encode tryptophan, rather than using it as a translation termination signal. In order to express this gene, the TGA codons found in this Orf need Id to TGG. A PCR-based site-directed mutagenesis procedure will be used. Individual regions will be amplified using the PCR, and various restriction sites will be incorporated into the Orf. All of the fragments will be cloned into pMal-p-2. This cloning vector is specially designed to facilitate the purification of cloned proteins. The purified protein will be studied in a variety of ways. One hypothesis that we have is that this protein is involved in the proteolytic processing of the protein encoded by Orf4 of the PI operon. We will use the purified protein to determine if the Orf1 protein can cleave the Orf4 protein. This project will interrelate with another project from this lab "Cloning and characterization of the protease..". We will use substrates purified in that project to determine if the Orf1 protein encodes a protease. We will use a variety of animal seras to determine if the Orf1 protein is immunogenic in animals. We will generate antibodies against the purified protein and determine where this protein is localized in the mycoplasma cell.

U.S. GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 BA-07001-24 LP

PERIOD COVERED
October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Serologic Methods for Pertussis Research: Development and Standardization.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Bruce D. Meade, Ph.D., Microbiologist, LP, DBP, CBER
Theresa A. Romani, B.S., Biologist, LP, DBP, CBER
Freyja V.C. Lynn, B.S., Biologist, LP, DBP, CBER
Charles R. Manclark, Ph.D., Microbiologist, Chief, LP, DBP, CBER

COOPERATING UNITS *(if any)*
National Bacteriological Laboratory, Stockholm, Sweden (SBL)--Hans Hallander, M.D.
Connaught Laboratories, Ltd, Toronto Canada; Biocine Sclavo spa, Siena, Italy;
SmithKline Biologics, Rixensart Belgium; Vanderbilt University, Nashville, TN

LAB/BRANCH
Laboratory of Pertussis, DBP

SECTION

INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS: 0.9	**PROFESSIONAL:** 0.9	**OTHER:**

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Objectives: (a) To develop, standardize, and evaluate sensitive and specific assays for antibodies to *Bordetella pertussis* antigens. (b) To serve as a reference laboratory for pertussis serology by writing reference methods, procuring reference reagents, training visiting personnel, and performing comparative testing with outside laboratories. For all assays listed below, protocols have been distributed, visitors were trained, and comparative testing with outside laboratories has been performed. (1) Assays for which standardized protocols and reference reagents are available are the microagglutination assay, the CHO--cell pertussis toxin neutralization assay, and ELISA assays for IgG antibodies to pertussis toxin and filamentous hemagglutinin. Protocols and reagents have been distributed to more than 20 laboratories. The Laboratory continued to evaluate capsulation programs for ELISA. (2) *Quality Control:* Laboratory continues its ongoing program to assess the intra- and inter-assay reproducibility of serologic assays and to receive and qualify new reagents. Assisted Stockholm laboratory in testing and validating the anti-human IgG conjugate that will be used in the acellular pertussis vaccine efficacy trial in Sweden. (3) *Assessment of inter-laboratory reproducibility:* Laboratory has demonstrated inter-laboratory reproducibility of testing methods by performing collaborative studies with testing laboratories at Universities, manufacturers and national control agencies. Problems with demonstrating inter-laboratory reproducibility of the anti-pertacti ELISA has led to a multi-laboratory collaborations. (4) *Development of new assays:* (a) continued work to develop new ELISA-based assays using capture systems and polyclonal and monoclonal antibodies. (b) Methods are being established to measure the opsonic potential of specific antibodies and to assess the effect of opsmins on phagocyte bactercidal activity.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-BA-07002-03 LP

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Serological response to *Bordetella pertussis* antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Drusilla L. Burns, Ph.D. Research Chemist, LP, DPB, CBER
Juan L. Arciniega, Sc.D., Visiting Scientist, LP, DPB, CBER
Charles R. Manclark, Ph.D., Chief, LP, DPB, CBER;
Erik L. Hewlett, M.D., Univ. of Virginia; Adamadia Deforest, Ph.D., Temple Univ.;
Kathryn M. Edwards, M.D., Vanderbilt Univ. School of Medicine, Nashville, TN.

COOPERATING UNITS *(if any)*
University of Virginia School of Medicine, Charlottesville, VA; Temple University School of Medicine, Philadelphia, PA; Centers for Disease Control, Atlanta, GA; Vanderbilt University School of Medicine, Nashville, TN

LAB/BRANCH
Laboratory of Pertussis, DBP

SECTION

INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.4	0.4	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The human serological response to several envelope-associated proteins and adenylate cyclase toxin of *B. pertussis* was examined using immunoblot techniques. Antigens recognized by sera from individuals with culture-confirmed pertussis as well as by sera from infants immunized with three doses of conventional whole-cell pertussis vaccine included a 63,000 Da protein that was shown to be antigenically related to a mycobacterial heat-shock protein and structurally related to the chaperonin family of proteins involved in protein folding and assembly. *B. pertussis* lipooligosaccharide was also recognized by antibodies in certain of these sera. A 29,000 Da species reacted with sera from convalescent individuals whereas a 91,000 Da species reacted with sera from vaccinated individuals. Antibodies to adenylate cyclase toxin were common in sera from individuals diagnosed with pertussis, however these antibodies were rarely found in children who had received three doses of DTP vaccine. Sera from neonates (cord blood) and corresponding maternal sera were examined for antibodies to *Bordetella* adenylate cyclase. Similar levels of antigen specific antibodies (IgG isotype) were found in each serum sample of a given neonate-maternal pair suggesting that antibodies to adenylate cyclase are passively transferred through the placenta. These antibodies may have been elicited by previous exposure of the mothers to *B. pertussis* or by vaccination of the mother with pertussis vaccine since all four pertussis vaccines currently in use in the US were capable of eliciting in mice antibodies reactive with adenylate cyclase. Alternatively, these antibodies may have been elicited by exposure of mothers to cross-reactive antigens such as *E. coli* α-hemolysin since all of the neonatal sera contained antibodies reactive with this antigen. Antibodies to adenylate cyclase have been previously shown to passively protect mice against challenge with *B. pertussis*. Therefore the possibility exists that these antibodies may help protect the newborn child against pertussis. This project has been completed.

U.S. GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BA-07003-04 LP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Evaluation of Serodiagnostic Methods for Pertussis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Bruce D. Meade, Ph.D., LP, DBP, CBER
ChrisAnna M. Mink, M.D., LP, DBP, CBER; Theresa A. Romani, B.S., LP, DBP, CBER
C.R. Manclark, Ph.D., Microbiologist, Chief, LP, DBP, CBER
Steven Wassilak, M.D., CDC
Adamadia Deforest, Temple University; David Addiss, CDC
Tatsuo Aoyama, M.D., Municipal Hospital, Kawasaki, Japan

COOPERATING UNITS *(if any)*

Temple University School of Medicine, Philadelphia, PA
Center for Disease Control, Atlanta, GA; Kawasaki Municipal Hospital, Kawasaki, Japan

LAB/BRANCH

Laboratory of Pertussis, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☑ (a1) Minors
  ☑ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Objectives: (a) To evaluate the serologic response to infection with *B. pertussis* and immunization with pertussis vaccine (b) To determine which serologic assays have the highest diagnostic sensitivity and specificity for pertussis and (c) To understand pertussis epidemiology in the community through seroprevalence and outbreak studies.

(1) *CDC Multicenter Surveillance Study*: Evaluation of data collected for this study has continued. A manuscript has been published in which these data were used in the analysis of the efficacy of whole cell pertussis vaccines in U.S. children four years of age and younger.

(2) *Serodiagnosis*: (a) Assays are in place to measure IgG, IgA, and IgM antibodies to PT, FHA, LOS, pertactin, fimbriae, and whole bacteria. These 18 assays plus the whole-cell microagglutination assay have been performed on paired sera from immunized and infected individuals. No single assay has the required characteristics and analyses are underway to select the optimal combination of assays. (b) Historical data have shown that for children immunized with whole-cell vaccine, pertussis agglutinating antibodies correlated with clinical protection. Analysis of the serologic data indicates that IgG anti-fimbriae antibodies correlate best with agglutinins in immunized children while IgG and IgA anti-fimbrial antibodies correlate well with agglutinins in convalescent individuals.

(3) IgG and IgA antibodies to Pt, FHA, pertaction and fimbriae have been measured in serial serum samples collected from 72 Japanese individuals with culture-confirmed pertussis. The data are being analysed and prepared for publication.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BA-07004-04 LP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Epidemiology and diagnosis of *Bordetella pertussis* infection in Washington DC

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: ChrisAnna Morgan Mink, M.D., LP, DBP, CBER
Bruce D. Meade, Ph.D., LP, DBP, CBER
Theresa A. Romani, B.S., LP, DBP, CBER
C.R. Manclark, Ph.D., Microbiologist, Chief, LP, DBP, CBER
Roberta Shahin, Ph.D., Staff Fellow, LP, DBP, CBER
Bernhard L. Wiederman, M.D., and Joseph Campos, Ph.D., Children's Hospital, DC

COOPERATING UNITS *(if any)*

Children's Hospital, Washington, DC.

LAB/BRANCH

Laboratory of Pertussis, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.9	0.9	

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☑ (a1) Minors
☑ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Objectives:

1) To evaluate diagnostic techniques for *B. pertussis* in relation to the stage of illness and to discern which techniques provide optimal recognition of *B. pertussis* infection.

2) To determine the reservoir of *B. pertussis* infection and to assess the significance of adult transmission to infants.

3) To better understand the serologic response to contact with *B. pertussis*, and to discern which antibodies are important for immunoprotection.

To date, six index cases and 19 household contacts have been enrolled and evaluated. Nasopharyngeal specimens have been processed with routine culture medium and direct fluorescent assay. In addition, newly developed techniques have been used with the clinical specimens. These include mouse monoclonal antibodies, developed in the Laboratory of Pertussis, for the immunofluorescent assay, and serologic methods to detect pertussis-specific antibodies.

No additional recruitment occurred in FY92. IgG and IgA antibodies to PT, FHA, pertactin and fimbriae as well as total IgA and total protein were measured in serum and saline of the study population. Pertussis-specific IgA antibodies were routinely detected in saliva of both infected individuals, as well as symptomatic and asymptomatic household contacts, indicating that *B. pertussis* infection elicits a mucosal immune reponse in humans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BA-07005-09 LP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Immune Response to Three versus Four Doses of DTP Vaccine

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Bruce D. Meade, Ph.D., Microbiologist, LP, DBP, CBER
Theresa A. Romani, B.S., Biologist, LP, DBP, CBER
Christina B. Swartz, B.S.
Charles R. Manclark, Ph.D., Microbiologist, Chief, LP, DBP, CBER
Roger Bernier, Ph.D., (CDC)
Stevan Wassilak, M.D. (CDC)
Adamadia DeForest, Ph.D., St. Christopher's Hospital, Philadelphia, PA

COOPERATING UNITS *(if any)*

Centers for Disease Control, Atlanta, GA
St. Christopher's Hospital, Philadelphia, PA

LAB/BRANCH

Laboratory of Pertussis, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
0.4	0.4	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

CDC is funding studies with the following objectives: a) To compare the safety and antigenicity of three doses of DTP administered at 2, 4, and 18 months of age to four doses of DTP administered at 2, 4, 6, and 18 months. b) To evaluate the safety and antigenicity of DTP administered to premature infants compared to full-term infants. The serologic evaluations will be done at CBER.

Study design called for a total of 500 children to be enrolled and 2500 blood specimens to be collected. After approximately 80 children had completed the study, preliminary serological analyses were performed by Dr. DeForest using agglutination and CHO-cell neutralization assays. These revealed that the 3-dose group had antibody titers significantly lower than the 4-dose group. The study was stopped at that time. The Laboratory of Pertussis performed ELISA assays on these samples to determine if ELISA assays would also demonstrate reduced antibody levels in the 3-dose group. Approximately 400 sera were tested and 1600 test results were reported. Analysis of these data has been completed and a manuscript is being prepared for publication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BA-07006-03 LP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Evaluation of the Human Antibody Response to Acellular Pertussis Vaccines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Bruce D. Meade, Ph.D., Microbiologist, LP, DBP, CBER
Theresa A. Romani, B.S., Biologist, LP, DBP, CBER
Charles R. Manclark, Ph.D., Microbiologist, Chief, LP, DBP, CBER
Hans Hallander, SBL; George Curlin, NIAID, NIH
Ghanshyam Gupta, OEB, CDER
Tatsuo Aoyama, M.D., Kawasaki Municipal Hospital, Kawasaki, Japan

COOPERATING UNITS *(if any)*

(SBL) National Bacteriological Laboratory, Stockholm, Sweden
National Institute of Allergy and Infectious Diseases, NIH
Kawasaki Municipal Hospital, Kawasaki, Japan

LAB/BRANCH

Laboratory of Pertussis, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
1.6	1.6	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Purpose: To utilize standardized procedures to measure the immune response in clinical trials of acellular pertussis vaccine (ACPV). 1. *NIAID - Multicenter Phase II Trial*: The Laboratory of Pertussis served as the serologic reference laboratory for the multicenter trial of 13 candidate ACPVs. Four ELISA assays (IgG-PT, IgG-FHA, IgG-69k, IgG fimbriae) and whole-cell agglutinins were performed on over 4000 sera for this study (more than 25,000 individual assay results were reported; of these, approximately 1500 were performed in FY92). As part of the study, FDA methodology and reagents were transferred to the NIAID-supported lab which performed confirmatory testing on a 10 percent sub-sample of sera from the study and to the lab which performed the CHO-cell testing. (a) FDA scientists advised NIAID statisticians on the procedures for analysing and presenting the serologic data from the NIAID multicenter trial. (b) Quality assurance analyses of the FDA serologic data demonstrated a high reproducibility and consistency of the data generated in the FDA lab and demonstrated a high degree of correlation among the results obtained in the 3 testing laboratories. (c) FDA scientists analysed and presented the serologic data from the trial. (d) Analysis of the data demonstrated a good correlation between IgG anti-PT and CHO-cell titers for children immunized with any of the whole-cell or acellular vaccines. (e) Analysis of the data demonstrated a good correltation between IgA anti-fimbriae antibodies and agglutinin titers for children immunized with any whole-cell or acellular vaccine containing fimbriae. 2. The 4 ELISA assays were performed on sera from approximately 80 Japanese children immunized with either the Biken or Takeda acellular vaccines. These results will be compared to responses in U.S. children immunized with vaccines from the same manufacturer. 3. *Development of new assays.* Procedures are being developed to measure epitope specific responses in individuals immunized with acellular vaccines.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BA-07007-13 LP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Aerosol Infection of Mice as a Model for Pertussis Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: R.D. Shahin, Ph.D., Senior Staff Fellow, LP, DBP, CBER
M. Leef, Biologist, LP, DBP, CBER
Charles R. Manclark, Ph.D., Microbiologist, Chief, LP, DBP, CBER
T. Finn , Ph.D., Visiting Scientist, LP, DBP, CBER
J. Mekalanos, Ph.D., Professor, Harvard Medical School
D. Beatty, Ph.D., Harvard Medical School

COOPERATING UNITS *(if any)*

Dept. Microbiology and Molcular Biology, Harvard Medical School, Cambridge, MA

LAB/BRANCH

Laboratory of Pertussis, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Respiratory infection of neonatal mice with an aerosol of *Bordetella pertussis* provides a reproducible system for the study of the host-pathogen interactions involved in respiratory infection and subsequent disease. Analysis of genetic mutants of *Bordetella pertussis* in this model is a powerful tool that allows us to identify new virulence determinants. Following infection with wild-type *B. pertussis* 18323, *B. pertussis* grows in the lungs of infected mice, concomitant with an increase in peripheral leukocyte count and decrease in weight, followed by death. We have previously identified a new virulence associated factor, activated by the *vir* locus in this model. A second class of transposon insertion mutants, whose expression is repressed by the *vir* locus, was analyzed in the mouse aerosol model. One of these mutants was determined to be defective in respiratory tract persistence, lymphocytosis, and the ability to kill, while a second *vir* repressed mutant remained as virulent as wild-type *B. pertussis* 18323. The role of these *vir* repressed genes in pathogenesis, remains to be elucidated.

U.S. GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BA-07008-05 LP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Evaluation of antigens protective in the mouse aerosol model

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: R.D. Shahin, Ph.D., Senior Staff Fellow, LP, DBP, CBER
D.F. Amsbaugh, Biologist, LP, DBP, CBER;
M~~.F~~. Leef, Biologist, LP, DBP, CBER;
C.R. Manclark, Ph.D., Microbiologist, Chief, LP, DBP, CBER;
D.L. Burns, Ph.D., Research Chemist, LP, DBP, CBER;
J. Arciniega, D.Sc., Visiting Fellow, LP, DBP, CBER;
W. Neal Burnette, Ph.D, Amgen, Inc.; Josee Hamel, Ph.D. and Bernard Brodeur, Ph.D, Canadian Lab. Centre for Disease Control; Rino Rappuoli, Ph.D., Sclavo Res. Cen.

COOPERATING UNITS *(if any)*

Amgen, Inc., Thousand Oaks, CA
Canadian Laboratory Centre for Disease Control, Ottawa, Canada
Sclavo Research Center, Siena, Italy

LAB/BRANCH

Laboratory of Pertussis, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS 1.4	**PROFESSIONAL** 1.4	**OTHER:**

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Animal models are used for the evaluation of acellular pertussis vaccine components before use in clinical trials and administration to humans. The aerosol challenge model provides a reproducible system for the study of virulence factors in immunity involved in respiratory infection and subsequent disease. We are evaluating purified antigens of *B. pertussis* for their ability to protect neonatal mice against lethal respiratory infection. Active immunization with the B oligomer of pertussis toxin, as well as with a genetically engineered non-toxic mutant of pertussis toxin elicits specific antibody in the serum and lungs of mice and protects neonatal mice against lethal respiratory infection. Monoclonal antibodies directed against the lipooligosaccharide of *B. pertussis* outer membrane protein protects against leukocytosis and death, as well as decreased bacterial infection when passively administered. Current studies are in progress to evaluate additional soluble and membrane-associated proteins protective ability and antigenicity.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BA-07009-03 LP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Mucosal immunity to pertussis antigens in mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: R.D. Shahin, Ph.D., Senior Staff Fellow, LP, DBP, CBER
D.F. Amsbaugh, B.S., Biologist, LP, DBP, CBER
M.F. Leef, Biologist, LP, DBP, CBER
C.R. Manclark, Ph.D., Microbiologist, Chief, LP, DBP, CBER
T. Finn, Ph.D., Visiting Scientist, LP, DBP, CBER

COOPERATING UNITS *(if any)*

-

LAB/BRANCH

Laboratory of Pertussis, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Pertussis is a respiratory disease caused by bacteria that grow only on the ciliated epithelium of the respiratory tract. We are currently studying the dissemination of a protective mucosal immune response from the gut associated lymphoid tissue to the respiratory tract in a mouse model of respiratory infection. Intraduodenal as well as intranasal immunization of adult mice with endotoxin-free filamentous hemagglutinin prior to aerosol challenge decreases the number of *B. pertussis* recovered from the lungs and tracheas in comparison to unimmunized infected animals. Antibodies to FHA can be detected in the lungs and serum of intraduodenally or intranasally immunized mice at the time of respiratory challenge. We have developed an *in vitro* assay designed to enumerate antigen specific memory B lymphocytes recovered from the respiratory mucosa following oral immunization. Using this assay, we have detected a significant increase in the number of FHA specific memory B lymphocytes in the lungs of mice following infection as well as after intranasal immunization. Current efforts are also directed toward the cloning of protective epitopes of *B. pertussis* antigens into bacterial vectors that will colonize the murine gut mucosa in order to evaluate the protective capacity of a live oral vaccine against *B. pertussis*.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BA-07010-05 LP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Mucosal Immunity to Pertussis in Humans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: C.M. Mink, M.D., Staff Fellow, LP, DBP, CBER
B.D. Meade, Ph.D., Microbiologist, LP, DBP, CBER
R.D. Shahin, Ph.D., Staff Fellow, LP, DBP, CBER
C.R. Manclark, Ph.D., Microbiologist, Chief, LP, DBP, CBER
M.A. Sprauer, M.D., CDC
T.A. Romani, B.A., LP, DBP, CBER

COOPERATING UNITS *(if any)*

Centers for Disease Control, Atlanta, GA

LAB/BRANCH

Laboratory of Pertussis, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In humans, serum and secretory IgA antibodies to antigens of *B. pertussis* are detected in convalescent sera and secretions, but not in sera or secretions from vaccinees. Parenterally administered vaccines, currently in use, elicit systemic but not mucosal immunity. Induction of a vigorous local immune response at the mucosal epithelium of the respiratory tract may provide superior protection against pertussis, in contrast to parenteral vaccines. Procedures have been developed to measure the following in saline and nasopharyngeal aspirants: total protein, total IgA and IgA to specific pertussis antigens (PT, FHA, pertactin and fimbriae). (1) The procedures were used to evaluate the mucosal response in individuals from Children's Hospital National Medical Center (project Z01 BA-07004-03 LP). (2) These assays were performed on 50 individuals in a pilot study in Sweden designed to determine the optimal diagnostic assays to be used to evaluate cases in upcoming efficacy trials. Results will be compared with PCR and culture to define assay sensitivity and specificity.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-BA-07011-08 LP

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Studies on the Structure and Function of Pertussis Toxin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Drusilla L. Burns, Ph.D., Research Chemist, LP, DBP, CBER
S.Z. Hausman, M.A., Microbiologist, LP, DBP, CBER
Juan L. Arciniega, Sc.D., Visiting Fellow, LP, DBP, CBER
Charles R. Manclark, Ph.D., Microbiologist, Chief, LP, DBP, CBER
W. Neal Burnette, Ph.D. Amgen, Inc; Vernon Mar, Amgen, Inc.;
Timothy D. Bartley, Ph.D., Amgen, Inc..David Whiteley, Amgen, Inc.

COOPERATING UNITS *(if any)*
Amgen, Inc., Thousand Oaks, CA

LAB/BRANCH
Laboratory of Pertussis, DBP

SECTION

INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.8	1.8	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Studies have ben initiated to examine the mechanism of entry of pertussis toxin (PT) into the eukaryotic cell. The interaction of pertussis toxin with cells and model membranes was investigated by examining PT-induced intoxication of Chinese hamster ovary (CHO) cells and by studying the binding of PT and its subunits to phospholipid vesicles. Since certain bacterial toxins require an acidic environment for efficient interaction with membranes and subsequent entry into the cell, the requirement for an acidic environment for PT action was examined. PT, unlike bacterial toxins such as diphtheria toxin, did not require an acidic environment for efficient intoxication of Chinese hamster ovary cells. Potential modes by which PT might interact with biological membranes were studied by examining the binding of PT to model membrane system. PT was found to be capable of interacting with phospholipid vesicles, however efficient binding of the toxin to the vesicles, however, efficient binding of the toxin to the vesicles occurred only in the presence of both ATP and reducing agent. The A subunit portion of the toxin bound preferentially to the vesicles while little binding of the B oligomer portion of PT to the model membranes was observed. Isolated A subunit, in the absence of the B oligomer, also bound to the vesicles with optimal binding occurring in the presence of reducing agent. After cleavage of the A subunit by trypsin, probably at Arg181, Arg182 and/or Arg193, large fragments which lacked the C-terminal portion of PTA no longer associated with the lipid vesicles. These results suggest that the A subunit of PT can interact directly with a lipid matrix and, if freed from the constraints imposed by the B oligomer, may be capable of interacting with cellular membranes.

The binding of PT and its B oligomer to lipid vesicles containing glycosphingolipids was also also studied.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA 07015-04 LP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Adhesins of *Bordetella pertussis*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.J. Brennan, Ph.D., Biologist, LP, DBP, CBER
J.H. Hannah, M.P.H., Microbiologist, LP, DBP, CBER
E. Leininger, Ph.D., Staff Fellow, LCP, DBP, CBER
J.G. Kenimer, Ph.D., LCP, DBP, CBER
Z.M. Li, M.D., Visiting Associate, LP, DBP, CBER
C.R. Manclark, Ph.D., Microbiologist, Chief, LP, DBP, CBER
S. Stibitz, Ph.D., Staff Fellow, LBT, DBP, CBER; A. Bhargava, M.D., Visiting Scientist, LP, DBP, CBER; I. Charles, Ph.D., Wellcome Biotech, Kent, U.K.

COOPERATING UNITS *(if any)*

Laboratory of Cellular Physiology, DBP, CBER
Laboratory of Bacterial Toxins, DBP, CBER
Wellcome Biotech, Kent, U.K.

LAB/BRANCH

Laboratory of Pertussis, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER.
1.1	1.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

B. pertussis expresses a number of proteins including the 69K OMP (pertactin), filamentous hemagglutinin, and pertussis toxin that may promote adherence of *B. pertussis* to mammalian cells. In order to study the potential role of these proteins in bacterial adherence mutants specifically lacking pertactin, FHA or pertussis toxin are being constructed using the pertussis suicide vector pRTP1. Through the use of antibiotic resistance markers and double recombination events mutants deficient in the expression of pertactin, FHA or both proteins have been constructed and characterized. A decrease in adherence to mammalian cell lines is seen with all the mutants, while the mutant lacking both proteins demonstrates a synergistic effect. Mutants lacking pertussis toxin or all virulent associated proteins are presently being constructed in order to assess the contribution of other bacterial proteins to adhesion.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 BA 07016-03 LP

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Pertactin and FHA as Adhesive Proteins of *Bordetella pertussis*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: M.J. Brennan, Ph.D., Biologist, LP, DBP, CBER
E. Leininger, Ph.D., Staff Fellow, LCP, DBP, CBER
J.H. Hannah, M.P.H., Microbiologist, LP, DBP, CBER
J.G. Kenimer, Ph.D., LCP, DBP, CBER
M.S. Peppler, Ph.D., University of Alberta
C.A. Ewanowich, University of Alberta
C. Locht, Ph.D., Institut Pasteur
G. Renauld, Ph.D., Institut Pasteur

COOPERATING UNITS *(if any)*
Laboratory of Cellular Physiology, DBP, CBER
University of Alberta, Edmonton, Alberta, Canada
Institut Pasteur, Lille, France

LAB/BRANCH
Laboratory of Pertussis, DBP

SECTION

INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS: 0.2	**PROFESSIONAL** 0.2	**OTHER**

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Attachment studies using Chinese Hamster (CHO) cells suggest that the 69K OMP (pertactin) might play an important role in the adherence of bacteria to eukaryotic cells since *B. pertussis* mutants which are deficient in the production of pertactin adhere less well to CHO cells than wild-type bacteria. CHO cells were shown to attach to plastic wells coated with either purified pertactin or with the mammalian cell attachment protein fibronectin. Monoclonal antibodies reactive with pertactin inhibit CHO cell attachment to pertactin but not to fibronectin. Recent genetic analysis has shown that the sequence RGD, which is the cell binding site on fibronectin, occurs twice in the pertactin sequence. RGD-containing peptides inhibited CHO cell attachment to pertactin as well as to fibronectin, suggesting that CHO cell attachment to pertactin occurs through an RGD-containing site.

Filamentous hemagglutinin (FHA) is another surface protein of *B. pertussis* which functions as an adhesin. Like pertactin it also contains an RGD sequence. However, an FHA-derived peptide containing the RGD sequence did not block attachment of CHO cells to FHA or other cell attachment proteins. Our studies suggest that FHA may contain a lectin site that interacts with carbohydrates present on the surface of mammalian cells.

Recently, it has been shown that *B. pertussis* can invade and survive within mammalian cells. The RGD peptide from pertactin inhibits invasion but the RGD peptide from FHA does not. A monoclonal antibody (Mab) directed against the RGD region of pertactin also blocks invasion. These results suggest that pertactin may play a role in bacterial invasion.

These studies are directed toward the identification of important functional and immunological epitopes on pertactin and FHA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA 07017-05 LP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Characterization of the Porin Protein of *Bordetella pertussis*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.J. Brennan, Ph.D., Biologist, LP, DBP, CBER
A.C. Steven, Ph.D., NIDDK, NIH; M. Kessel, Ph.D., Visiting Scientist, NIDDK, NIH;
J.H. Hannah, M.P.H., Microbiologist, LP, DBP, CBER;
Z.M. Li, M.D., Visiting Associate, LP, DBP, CBER;
S. Stibitz, Ph.D., Staff Fellow, LBT, DBP, CBER;
N.Y. Nguyen and J.B. Ewell, DBB, CBER;

COOPERATING UNITS *(if any)*

National Institute of Diabetes and Digestive and Kidney Diseases, NIH
Division of Biochemistry and Biophysics, CBER
Laboratory of Bacterial Toxins, DBP, CBER

LAB/BRANCH

Laboratory of Pertussis, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previously ultrastructural studies have shown that *B. pertussis* has a crystalline surface lattice consisting of a 40 kDa porin protein. We have developed large scale methods for the purification of this protein in order to study the structural and functional characteristics of this protein. Porin protein was purified by sequential extractions of cell envelopes with Triton X-100 and Zwittergent 3-14 followed by DEAE ion-exchange chromatography. NH2-terminal amino acid analysis of the purified porin was performed and an oligonucleotide was constructed from this sequence. This oligo was used to probe a λgt11 library of *B. pertussis* DNA and a clone was identified that consists of a truncated porin gene containing the N-terminal portion of the protein. This gene sequence was ued to design an additional oligonucleotide that was used to identify a second clone which overlapped with the first clone and contained a termination condon. The structural gene deduced from this sequence would encode a 365 amino-acid polypeptide with a predicted mass of 39103 daltons. The predicted *B. pertussis* porin protein sequence contains regions that are homologous to regions found in porins expressed by *Neisseria* species and *Escherichia coli*, including the presence of phenylalaine as the carboxy-terminal amino acid. DNA hybridization studies indicated that both virulent and avirulent strains of *B. pertussis* contain only one copy of this gene and that *Bordetella bronchiseptica* and *Bordetella parapertussis* contain a similar gene. Identification of the porin gene will allow further molecular studies on the structure and function of this outer membrane protein.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 BA 07018-03 LP

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Mammalian Cell Receptors for *Bordetella pertussis*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: M.J. Brennan, Ph.D., Biologist, LP, DBP, CBER
J.H. Hannah, M.P.H., Microbiologist, LP, DBP, CBER;
E. Leininger, Ph.D. Staff Fellow, LCP, DBP, CBER
J.G. Kenimer; A. Karpas; P. Probst; LCP, DBP, CBER
C.R. Manclark, Ph.D. Microbiologist, Chief, LP, DBP, CBER

COOPERATING UNITS *(if any)*
Laboratory of Cellular Physiology, DBP, CBER

LAB/BRANCH
Laboratory of Pertussis, DBP

SECTION

INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.4	0.4	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The nature of receptors on mammalian cells for *B. pertussis* and its adhesins is currently being investigated. Results indicate that virulent strains of *B. pertussis* can bind to certain glycolipids present in lung tissues. The structure of these glycolipids is being investigated as is the nature of the bacterial adhesins mediating this interaction. The role of integrins as receptors for the RGD-containing *B. pertussis* proteins, pertactin and FHA, is also under investigation. Our studies suggest that FHA may contain a lectin site that interacts with sulfated glycolipids, cell surface receptors which are present in large quantitites in human trachea and lungs. We are currently mapping this lectin binding site on the FHA molecule.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA 07019-03 LP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Development of Tests for Acellular Pertussis Vaccines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Drusilla L. Burns, Ph.D., Research Chemist, LP, DBP, CBER
Charles R. Manclark, Ph.D., Microbiologist, Chief, LP, DBP, CBER
Juan L. Arciniega, D.Sc., Visiting Fellow, LP, DBP, CBER
Bruce D. Meade, Ph.D., Microbiologist, LP, DBP, CBER
Theresa A. Romani, B.S., Biologist, LP, DBP, CBER

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pertussis, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Because of adverse effects associated with the administration of whole cell vaccine, development of improved pertussis vaccines is of high priority. The Food and Drug Administration is responsible for ensuring that these vaccines are both safe and effective. We have therefore developed a battery of assays to test the safety and potency of these vaccines. These assays test the vaccines for purity, lack of toxicity, lack of the ability to revert to a toxic form, and immunogenicity. These tests have been applied to four lots of acellular pertussis vaccines being evaluated in large scall efficacy trials in Sweden and Italy, as well as other vaccines currently under IND and PLA status.

Efforts are underway to develop and standardize potency and toxicity tests which can be used as reliable measures of the reproducibility of manufacturers of these vaccines. A standardized ELISA is currently being developed which will measure the immune response in mice to the four antigens found in acellular pertussis vaccines. These antigens are filamentous hemagglultinin, pertussis toxoid, 69 kDa protein, and fimbriae. The preparation of suitable control reagents for the tests are underway. A pilot study to define optimal immunization schedules in order to prepare mouse hyperimmune reference serum is complete and the immunization of mice is in progress.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA 07020-40 LP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Control Testing of Whole Cell and Acellular Pertussis Vaccines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Bruce D. Meade, Ph.D., Microbiologist, LP, DBP, CBER
Juan L. Arciniega, D.Sc., Visiting Scientist, LP, DBP, CBER
Deborah L. Jansen, B.A., Biologist, LP, DBP, CBER
Theresa A. Romani, B.S., Biologist, LP, DBP, CBER
Freyja V.C. Lynn, B.S. Biologist, LP, DBP, CBER
M.M. Belcher, Biological Technician, PPL, DPQC, CBER
C.R. Manclark, Ph.D., Microbiologist, Chief, LP, DBP, CBER

COOPERATING UNITS *(if any)*

Division of Product Quality Control, CBER

LAB/BRANCH

Laboratory of Pertussis, DBP

SECTION

INSTITUTE AND LOCATION

DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS: 0.6	PROFESSIONAL: 0.6	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues X☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The Laboratory of Pertussis is responsible for routine control testing of pertussis vaccine potency and toxicity. The toxicity and potency of the pertussis vaccine component of licensed vaccines are tested before the product can be released.

Control testing in the Laboratory of Pertussis is used to determine that the pertussis component of vaccine issued by U.S. licensed establishments have adequate and stable potency through the dating period and are free from untoward reactivity due to toxicity.

Satisfactory estimates of potency, stability and protective units per total human immunizing dose are determined using mice as animal models. In addition, mice are used to determine vaccine toxicity by observing early and late weight changes.

On December 17, 1991, the first acellular pertussis vaccine was licensed in the U.S. The consistency of manuyfacturing for lots of acellular pertussis vaccines is tested by a potency test evaluating immunigencity in mice by ELISA and a toxicity test assessing residual PT bioactivity by histamine sensitization in mice.

In addition to laboratory examination of each vaccine submitted for release, control testing involves reviewing the manufacturer's protocol for satisfactory production methods and control tests performed by the manufacturer.

During the period described by this report, no significant changes in potency and toxicity of pertussis vaccines submitted for release were noted.

When materials allow, the Laboratory of Pertussis also performs control testing on vaccines from foreign manufacturers and serves as a reference testing laboratory for the World Health Organization.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 BA 07021-25 LP

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Selective Breeding to Establish a "Standard" Mouse

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Charles R. Manclark, Ph.D., Microbiologist, Chief, LP, DBP, CBER
Carl T. Hansen, Ph.D, Geneticist, DRS, NIH
D.L. Jansen, B.A., LP, DBP, CBER

COOPERATING UNITS *(if any)*
Division of Research Services, NIH

LAB/BRANCH
Laboratory of Pertussis, DBP

SECTION

INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Two strains of mice, HSFS/N(susceptible) and HSFR/N(resistant), have been developed by selectivity breeding for their ability to be sensitized to histamine by pertussis vaccine. The responses after more than 30 generations of selection indicate that susceptibility to sensitization to histamine is a heritable trait. The HSFS/N and HSFR/N strains have been shown to differ widely in their susceptibility to the lethal effects of histamine as measured by the median histamine-sensitizing dose. Assays for 12 biochemical markers in the HSFS/N and HSFR/N strains indicate that they are isogenic lines. Both strains were homozygous for all loci tested, as would be expected after 26 generations of sib-matings. The two strains carry the same alleles at all loci except for hemoglobin beta-chain (Hbb) and malic enzyme (Mod-1). HSFS/N carries the *d*-allele of Hbb and the *a*-allele for Mod-1; HSFR/N carries the *s*- and *b*-alleles for Hbb and Mod-1, respectively. Once a genetic profile is established it is possible to distinguish one strain from others. Since genetic contamination is a constant threat in an animal facility that houses more than one strain of a particular species, the genetic integrity of an animal colony can be monitored.

In addition to their possible use to evaluate the potency and toxicity of pertussis vaccines, the histamine susceptible and histamine resistant strains will be used in pharmacologic studies to examine the mechanism of action of LPF (HSF) in lowering the seizure thresholds to various intermediates. Now that purified pertussis toxin (PT), the agent believed to be responsible for the HSF (LPF) response, is available we are comparing the strains' response to PT and whole cell vaccine. Final characterization of the HSFS/N and HSFR/N strains awaits the breeding of sufficient animals to complete the necessary laboratory tests. This project will be placed on inactive status until space and personnel are available.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-BA-07022-03 LP

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Microencapsulation of Pertussis Antigens.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: R.D. Shahin, Ph.D, Senior Staff Fellow, LP, DBP, CBER
M.F. Leef, Biologist, LP, DBP, CBER
D.F. Amsbaugh, Biologist, LP, DBP, CBER
C.R. Manclark, Ph.D, Chief, LP, DBP, CBER

COOPERATING UNITS *(if any)*
-

LAB/BRANCH
Laboratory of Pertussis, DBP

SECTION

INSTITUTE AND LOCATION
DBP, CBER, FDA, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The objective of this project is to encapsulate purified antigens of *B. pertussis* into biodegradable, biocompatible microspheres in an immunogenic form for use as an experimental oral pertussis vaccine in an animal model of *B. pertussis* infection. Preliminary studies have shown that *B. pertussis* filamentoushemagglutinin (FHA) can be successfully encapsulated in biocompatible, biodegradable microspheres. FHA is one of several pertussis antigens that may allow *B. pertussis* bacteria to adhere and cause infection in the respiratory tract. FHA microspheres elicited a profound serum antibody response in mice that remained at high levels for at least 70 days after immunization. This work has established that the preparation of antigen for encapsulation and the encapsulation process itself does not destroy the antigenic integrity of FHA, a prerequisite to the evaluation of these microspheres for potential use as an oral pertussis vaccine. Experiments to determine the mucosal immunogenicity of these microcapsules are currently in progress. The significance of this project lies in its application to the development of an oral vaccine for pertussis.

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 BA-07023-02 LP

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Molecular Chaperones

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Drusilla L. Burns, Ph.D., Research Chemist, LP, DBP, CBER
Jeanine Gould-Kostka, Microbiologist, LP, DBP, CBER
Juan L. Arciniega, Ph.D. Visiting Scientist, LP, DBP, CBER
Martin Kessel, Ph.D., University of Maryland

COOPERATING UNITS *(if any)*
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TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.2	0.2	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The GroEL-like protein of *B. pertussis*, the major molecular chaperone produced by this organism, was purified. This protein was found to have the tetradecameric structure typical of the GroEL family of proteins and to contain epitopes similar to other members of this family, including a human GroEL-like protein. Studies were initiated to examine the structure/function relationships of GroEL in the hope of better understanding the role that this protein plays in protein folding. An IgG1 monoclonal antibody (mAb 54G8) which binds to both *B. pertussis* GroEL and *E. coli* GroEl was produced. MAb 54G8 was found to abolish the ability of GroES to inhibit the ATPase activity of both *B. pertussis* GroEl and *E. coli* GroEL. Electron microscopy was used to map the binding site of the monoclonal antibody on the *B. pertussis* GroEL molecule. In the absence of the antibody, *B. pertussis* exhibited the tetradecameric structure typical of GroEL. Both end views (showing seven-fold symmetry of the face of the molecule) and side views were evident. When mAb 54G8 was bound, *B. pertussis* GroEL molecules appeared to be cross-linked together so that they formed long chains. Only side views of the molecules were seen in these long chains. When *B. pertussis* GroEL complexed with Fab fragments of mAb 54G8 was examined, chains were no longer observed. Instead, side views of *B. pertussis* GroEL were often seen with Fab fragments extending from the ends of the molecule. These data indicate that mAb 54G8 appears to bind at or near the end of the *B. pertussis* GroEL molecule and are consistent with a model for the GroEL-GroES complex [Creighton, T.E> (1991) Nature: 352:17-18] in which it is proposed that GroES binds to the end of the GroEL molecule.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BA-07024-02 LP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Characterization of a new *vir*-regulated *B. pertussis* surface antigen

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: T. Finn, Ph.D., Visiting Scientist, LP, DBP, CBER
M.J. Brennan, Ph.D., Biologist, LP, DBP, CBER
J. Hannah, M.P.H., Microbiologist, LP, DBP, CBER
Z.M. Li, M.D., Visiting Associate, LP, DBP, CBER
R. Shahin, Ph.D., Immunologist, LP, DBP, CBER

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TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 1.0	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The *bvg* or vir locus in *B. pertussis* is responsible for coordinate regulation of the virulence associated factors. Using Tn*phoA* mutagenesis several *vir* activated genes (*vags*) have been identified. Some of these fusions were deficient in previously characterized factors such as pertussis toxin, or filamentous hemagglutinin. However one of these Tn*phoA* fusion strains was defective in a 95kDa outer membrane protein. This strain has been shown to be unable to cause lymphocytosis or to persist in a mouse aerosol model of pertussis. The Tn*phoA* insertion mutant has been used to isolate the wild type gene, which is being sequenced. The 95 kDa protein has been purified from the triton insoluble outer membrane fraction of the wild type strain 18323 using a carboxy methyl cellulose column. Purified material has been used to make monoclonal antibodies and ascites fluid is currently being made. A further *vag* product has been characterized. Identified by TnphoA mutagenesis this *vag* product appears to be a 30 kDa major outer membrane protein. The gene has been cloned and sequenced and encodes a 60 kDa protein. However, there is a potential processing site at 30 kDa similar to that in pertactin. In addition, the C terminal portion of this protein is very homologous to that of pertactin. The mature protein contains an RGD sequence and we are currently investigating whether this protein may have a role in adhesion. The role of the C terminal portion of this protein in secretion, targeting or anchoring to the membrane is also being investigated.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BA-07025-02 LP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Diagnosis of pertussis by molecular and immunological techniques.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.J. Brennan, Ph.D., Biologist, LP, DBP, CBER
Z.M. Li, M.D., Visiting Associate, LP, DBP, CBER
D. Jansen, B.A., Biologist, LP, DBP, CBER; T. Finn, Ph.D., LP, DBP, CBER
S. Halperin, M.D., Dalhousie Univ., Halifax, Nova Scotia
E. Eastman, M.D., Lark Sequencing Inc., Houston, TX
K. Bromberg, M.D., Downstate Medical Center, Brooklyn, NY
S. O'Connor, Ph.D., CDC, Atlanta, GA
Charles R. Manclark, Ph.D., Microbiologist, Chief, LP, DBP, CBER

COOPERATING UNITS *(if any)*

Dalhousie Univ., Halifax, Nova Scotia; Downstate Medical Center, Brooklyn, NY; Lark Sequencing Inc., Houston, TX; CDC, Atlanta, GA

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.6	0.6	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

There are several new methods for the detection of *B. pertussis* under investigation. Some of these involve the use of DNA probe technology and PCR. We propose a dual approach to the development of a DNA-based diagnostic test(s) specific for *B. pertussis*. One approach involves the further characterization of sequences associated with the porin gene to identify a short sequence which is unique to *B. pertussis*. The porin gene of *B. pertussis* has been cloned and sequenced by LP/CBER. Sequence differences were found when regions adjacent to the 5' end of the porin genes of *B. pertussis* and *B. parapertussis* were analyzed. Two pairs of primers were designed that amplified a 159 bp fragment from *B. pertussis* DNA and a 121 bp fragment from *B. parapertussis* DNA respectively. PCR was performed on 195 aspirates. It detected 88% of the samples which were both culture and CHO positive with a specificity of 97%. "Shared-primer" PCR was designed for detecting *B. pertussis* and *B. parapertussis* simultaneously. A non-radioactive Digoxigenin detection system which could tremendously increase the sensitivity of the PCR is currently under investigation. Hybridization of *Bordetella* specific DNA probes to colony lifts from culture plates of suspected pertussis patients is also being developed. The objective of these studies is to develop rapid, sensitive and species-specific clinical assays for the detection of *B. pertussis*

Hybridomas producing monoclonal antibodies that specifically detect *B. pertussis* in clinical samples have been developed for the detection, by immunofluorescence, of infection in children suspected of having whooping cough. These monoclonal antibodies are being evaluated in the clinical environment.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-07026-02 LP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Immunologically important epitopes on pertactin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.J. Brennan, Ph.D., Biologist, LP, DBP, CBER
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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Previously, we have identified and purified a 69kDa surface protein (pertactin) found on virulent strains of *B pertussis*. We found that this protein protects mice against infection following a respiratory challenge with *B. pertussis*. Three hybridomas were established that produce monoclonal antibodies (Mabs) directed against this protein. One of these Mabs was found to passively protect mice in the aerosol disease model. The epitopes for the Mabs were identified using fusion proteins of various lengths constructed from the P. 69 gene. An immunodominant and "protective" epitope was found near the C-terminus of the protein. This project has been completed.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 BA-07027-01 LP

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Secretion of Proteins from Gram-negative Organisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Drusilla L. Burns, Ph.D., Research Chemist, LP, DBP, CBER
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies have been initiated to examine the pathway of secretion of pertussis toxin (PT) from *B. pertussis*. Several mutants which lack the ability to secret pertussis toxin have been isolated. These mutations have been mapped to a locus which extends from about 1 kilobases (kb) to 9 kb downstream of the PT structural genes. One of these mutations was characterized in some detail and was found to produce the same amount of PT as the parent strain, yet the PT produced remained cell associated and was not secreted into the medium. Secretion of filamentous hemagglutinin and export of the outer membrane protein pertactin were unaffected in this mutant suggesting that the mutation was specific for PT secretion.

The region of the *B. pertussis* chromosome which appeared to be important for secretion of PT as indicated by mutational analysis was sequenced. Sequence analysis revealed seven open reading frames (ORFs). A search of the Swiss protein data base for proteins homologous to the protein predicted by ORF A did not reveal any good match. In contrast, the proteins predicted by ORF B, C, D, E, F and G were found to be homologous to the VirB4, VirB6, VirB8, VirB9, VirB10, and VirB11 protein, respectively, from the plant pathogen, *Agrobacterium tumefaciens*. The VirB proteins have been implicated in the transfer of a piece of DNA, T-DNA, across bacterial membranes. After release from the bacterial cell, the T-DNA ultimately crosses plant cell membranes, integrates into the plant cell genome, and codes for biosynthesis of plant growth hormones. Overexpression of these hormones leads to a loss of division control and tumors.

Our data suggest that several accessary proteins may be involved in secretion of PT from *B. pertussis* and that this transport system may be a member of a family of transport systems found in other types of bacteria.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BA-07028-01 LP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regional System for Vaccines (SIREVA)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Charles R. Manclark, Ph.D., Microbiologist, Chief, LP, DBP, CBER
Juan L. Arciniega, D.Sc., Visiting Fellow, LP,. DBP, CBER
Bruce D. Meade, Ph.D., Microbiologist, LP, DBP, CBER
Deborah L. Jansen, B.A., Biologist, LP, DBP, CBER

COOPERATING UNITS *(if any)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is to provide assistance to the Organization of a Network of Quality Control Laboratories for Vaccines in Latin America as part of the program for a Regional System of Vaccines (SIREVA). During FY92, the following items and support were provided to SIREVA.

- Master cultures of *B. pertussis* 18323 for use in establishing a seed lot system of cultures to be used in the intracerebral challenge of pertussis vaccine mouse potency assays.
- Methods for propagating, freeze-drying and maintaining a seed lot system provided in English, Spanish and Portuguese.
- Methods for the intracerebral challenge mouse potency assay provided in English, Spanish and Portuguese.
- 4000 vials of freeze dried vaccine to be used as the Working Standard Pertussis Vaccine for the Western Hemisphere.
- Details manufacture, freeze drying and standardization of the Working Standard Pertussis Vaccine.
- Results of preliminary pertussis vaccine potency assays to determine the unitage contained in the Working Standard Pertussis Vaccine.
- Proposed protocol for collaborative assays of the Working Standard Pertussis Vaccine to validate and confirm the number of protective units contained therein.
- 50 vials of the International Standard Pertussis Vaccine, Lot No. 2.
- Computer programs specifically designed to calculate vaccine potency and related laboratory assays, supplied under two operating systems (DOS and Mac) and in three languages with appropriate documentation on applications and instructions for use.
- Methods for the freedom from toxicity for pertussis vaccine.
- Methods and computer programs for the randomization of bioassays in general and pertussis vaccine provided in English, Spanish and Portuguese.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0—864-939

PUBLICATIONS LISTED BY INTRAMURAL PROJECT NUMBERS

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Gu, X-X., Tsai, C-M., Karpas, A. B. 1991. Production and Characterization of Monoclonal Antibodies to Type 8 Lopooligosaccharide of Neiseria meningitidis. Accepted, J. Clin Micro. 30(8)

Klutch, M., Karpas, A., Woerner, A., Zhang, P-f., Marcus-Sekura, C.J. 1991. Production of Type-Specific Anti-Human Immunodificiency Virus Type I Monoclonal Antibodies Reactiec with Epitopes in the C-Termini ofIntegrase and Reverse Transcriptase. Submitted to J.Vir.

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Klutch, M., Karpas, A., Woerner, A., Marcus-Sekura, C. 1991. Characterization of Anti-HIV-1 Integrese Hybridomas. NIH Research Festival. Sept 23-24. Poster L8.

Z01-BA-04004-04-LAIC

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Z01 BA-01011-03 LAIC

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Z01 BA-01008-03 LAIC

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Project Z01 BA-02005-07 LMDI

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Z01-BA-5001-05 LMCB

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Z01-BA-05004-04 LMCB

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Z01-BA-05005-03 LMCB

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Z01 BA 06011-02 LM

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Z01 BA 06012-02 LM

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Z01 BA 07002-03 LP

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Z01 BA 07003-04 LP

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Z01 BA 07006-03-LP

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Englund JA, Reed GF, Edwards KM, Decker MD, Pichichero ME, Rennels MB, Steinhoff MC, Anderson EL, Meade DB, Deloria MA, and the NIAID Acellular Pertussis Vaccine Study Group. 1992. Effect of maternal antibody on development of pertussis toxin and filamentous hemagglutinin antibody after acellular and whole-cell pertussis vaccines in infants. Annual Meeting of the Society for Pediatric Research, Baltimore, Maryland (Abstract).

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