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DEVELOPMENT OF VACCINES TO PREVENT WOUND INFECTIONS DUE
TO ANAEROBIC BACTERIA (U) BRIGHAM AND WOMEN'S HOSPITAL
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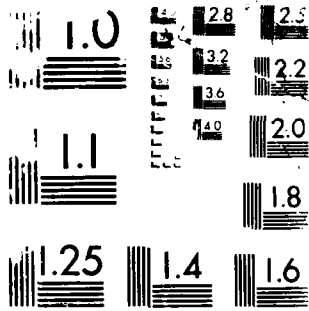
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19. ABSTRACT (Continue on reverse if necessary and identify by block number) Progress on the two major objectives of this contract were made during this year. One lot of capsular polysaccharide of <u>Bacteroides fragilis</u> vaccine was tested for protective capacity in mice and found to function to induce a protective response. One human volunteer was immunized without adverse reactions occurring and the immunogenicity will be assessed. On the second specific aim, major progress was made in producing a specific T cell factor by cloning lymphocytes in vitro to produce the protective lymphokine. A small molecular weight antigen specific factor has been produced from hybridoma clones. This factor is of T cell origin and protects mice against experimental infection with <u>Bacteroides fragilis</u> .			
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**DEVELOPMENT OF VACCINES TO PREVENT WOUND
INFECTIOUS DUE TO ANAEROBIC BACTERIA**

FINAL REPORT

Dennis L. Kasper, M.D.

April 26, 1988

**U.S. ARMY MEDICAL RESEARCH DEVELOPMENT COMMAND
Fort Detrick
Frederick, Maryland 21701-5012**

Contract No. DAMD17-83-C-3239
Contract No. DAMD17-78-C-8019

**Brigham and Women's Hospital
Boston, MA 02115**

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The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

SUMMARY

Progress on the two major objectives of this contract has been made. The first objective was to bottle and provide safety testing on a Bacteroides fragilis capsular polysaccharide preparation. The antigen was prepared in our laboratory and bottled by the Massachusetts State Public Health Laboratory. The material passed all safety and pyrogenicity studies required by the Food and Drug Administration. The second objective was to study the lymphokine produced by immunization with the polysaccharide. We have shown that a lysate of splenic T cells from animals immunized with the capsular polysaccharide, transfers protection to recipients. The protective material appears to be a lymphokine which is a protein of small molecular weight. This factor is antigen specific for the immunizing antigen.

FOREWORD

In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

For the protection of human subjects the investigator has adhered to policies of applicable Federal Law 45CFR46.

The two major objectives of this project over the course of the past four years were:

- 1) Bottling and safety testing of a B. fragilis capsular polysaccharide (CP)
- 2) Study the T cell lymphokine produced by immunization with the CP

In the past four years we have extracted, purified, characterized, bottled and sterility and safety tested capsular polysaccharide vaccine from B. fragilis. This has all been done under IND BB-1615. We have been in regular communication with the FDA. The antigen was purified on sterile columns by methods described previously¹. This involves growing organisms in a fermentor, extracting with phenol water and purification of the polysaccharide from the aqueous phase by chromatography. This antigen was a polysaccharide with the chemical characteristics described previously. It did, however, contain more glucose on a weight basis than some other lots. We believe this glucose represents a glycogen which is of bacterial origin². The glycogen is not immunologically reactive. The antigen had the same specificity as prior lots of this material. Bottling and safety testing was done in collaboration with the Massachusetts State Public Health Laboratory. The bottled material passed all safety and pyrogenicity studies required by the FDA for polysaccharide vaccines, including rabbit pyrogenicity, mouse and guinea pig toxicity and limulus lysate.

- 2) Major accomplishments have been made in characterizing the lymphokine responsible for immunity to abscesses.

We have evidence that in addition to immune T cells, a cell-free factor (ITF) prepared from an immune splenic T cell population protects experimental animals from developing intraabdominal abscesses caused by B. fragilis. Preliminary purification of this factor reveals that the active material is low in molecular weight, can bind to B. fragilis CP, and induces antigen-specific immunity to abscesses. The material is heat-labile and loses efficacy after protease, but not nuclease, digestion.

Identification of a Protective Immune T Cell Factor (ITF):

Splenic cells from mice immunized with CP and naive mice were passed over nylon wool columns to eliminate B cells and macrophages, and varying numbers of T cells were lysed by sequential freezing and thawing. The cell-free lysates, immune T cell lysate (ITF) and nonimmune T cell lysate (NITF), were transferred i.c. to naive mice. Twenty-four h later, animals were challenged with B.

¹Kasper DL, Weintraub A, Lindberg AA, Lonngren J. Capsular polysaccharides and lipopolysaccharides from two B. fragilis reference strains: Chemical and immunochemical characterization. J Bacteriol 1983; 153:991-7.

²Kasper DL, Onderdonk AB, Reinap BG, Lindberg AA. Variations of Bacteroides fragilis with in vitro passage: Presence of an outer membrane associated glycan and loss of capsular antigen. J Infect Dis 1980; 142:750-6.

GLOSSARY

BW	-	parent thymoma cell line used for producing hybridoma
IL-2	-	Interleukin 2
PEG	-	polyethylene glycol
DMSO	-	dimethylsulfoxide
DMEM	-	Dilbecco's Minimal Essential Medium
DMEM-HAT	-	above plus supplemental amino acids
FCS	-	fetal calf serum
SRBC	-	sheep erythrocytes
GBS	-	group B <u>Streptococcus</u>
CP	-	capsular polysaccharide
ITF	-	immune T cell factor

APPENDIX A

Description of Source and Preparation of Components for Lot B.f.-1R Capsular polysaccharide of Bacteroides fragilis prepared from strain 23745 (American Type Culture Collection). Other than the salt used for reconstitution and thimerosal, the polysaccharide is a pure bacterial product.

Methods, Facilities and Controls used for Manufacturing

Methods: (Materials list follows methods section)

1. Set up 12 l fermentor with pre-reduced Protease peptone-Yeast Extract media supplemented with 6% glucose and 10% fetal calf serum (FCS), and cysteine HCl.
2. Inoculate with 1500 ml broth culture of organisms, bubbling nitrogen with 3% hydrogen through the system.
3. Gram stain and subculture the inoculum aerobically and anaerobically to check for purity.
4. Incubate the 12 l for 18 h at 37°C with N₂ with 3% H₂ bubbling through continuously, maintaining pH 7.0 with 5 N NaOH using a pH titrator.
5. Collect organisms in Sharples centrifuge at 50,000 RPM to produce a 500 gm wet weight of organisms.
6. Suspend organisms in 1500 ml of sterile distilled water at 68°C.
7. Shake organisms vigorously with glass beads to suspend the organisms.
8. Mix an equal volume of 75% phenol at 68°C with the organism suspension and mix vigorously for 30 min at 68°C.
9. Mix overnight at 4°C.
10. Separate the phenol and water phases in 200 ml glass bottles at 8,000 rpm.
11. Remove the water phase on top which constitutes approximately one-half of the total volume into a vacuum flask using a vacuum aspirator and a Pasteur pipette.
12. Re-extract the residual phenol from the aqueous phase three times with equal volumes of anaesthetic grade ethyl ether.
13. Concentrate the aqueous phase and remove residual ether on a flash evaporator at 68°C.
14. Dialyze the aqueous phase for 48 h in deionized water at 4°C.
15. Concentrate the aqueous phase by partial lyophilization to 120 ml.
16. Dissolve dry ingredients of 3% sodium deoxycholate buffer in 120 ml of

sample and adjust pH to 9.

17. Chromatograph the material on a 10 x 90 cm column of S-400 (Pharmacia) in 3% deoxycholate buffer pH 9.
18. Antigen elutes just beyond void volume and is confirmed by Ouchterlony. Another peak elutes at 15,000 daltons and is the disaggregated LPS.
19. Pool Ouchterlony positive fractions and concentrate on Amicon TCF concentrator with a YM-10 membrane (Amicon).
20. Precipitate antigen from buffer with 80% ethyl alcohol at 4°C overnight. This removes the deoxycholate which is alcohol soluble.
21. Spin out precipitate at 12,000 x g for 20 min. Discard supernatant. Suspend precipitate in deionized water.
22. Dialyze for 48 h against distilled water with pH adjusted to 9.0 by adding a few drops of 0.1N NaOH. This is done to remove any trace amounts of remaining deoxycholate.
23. Dialyze for 48 h more against deionized water.
24. Lyophilize sample.
25. Suspend in 10 ml pH 7.3 0.05M Tris buffer at 37°C.
26. Add 1 mg DNase, 5 mg RNase with 3 drops of 0.1M MgCl₂ and 3 drops of 0.1 M CaCl₂. Incubate overnight at 37°C.
27. Repeat the above digestion procedure incubating for 3 h at 37°C.
28. Treat with 10 mg Pronase for 2 h at 37°C and again overnight.
29. Chromatograph on a 4.4 x 90 cm S-300 (Pharmacia) column monitoring with UV and RI monitors.
30. Identify antigen by Ouchterlony double diffusion using capsular antiserum against all fractions.
31. Pool the tubes with antigen and concentrate on PM-10 membrane (Amicon) to one-tenth of the original volume. These fractions are free of enzymes as identified by v/v absorption at 260 nm.
32. Precipitate antigen with 5 volumes of ethyl alcohol (95%) at 4°C.
33. Centrifuge at 12,000 g for 20 min.

34. Suspend pellet in deionized water and dialyze against deionized water pH 4 in 12,000 dalton exclusion size tubing for 2 days at 4°C. Dialyze against deionized water pH 7 for 2 days.
35. Lyophilize and determine dry weight (155.4 mg). Check for sterility anaerobically and aerobically.
36. Store in sterile containers until sent to Massachusetts State Biologics Laboratory for final bottling (86 mg).

Materials:

Proteose Peptone (Difco, Detroit, MI)
Yeast Extract (Difco, Detroit, MI)
Dextrose (Fisher Scientific)
Cystein-HCl (Fisher Scientific)
Fetal calf serum (Microbiological Associates, MA Bioproducts)
18 liter fermentor (LSL Biolafitte)
Nitrogen tank with 3% hydrogen
Sharples centrifuge (Penwalt Warminster, PA)
Ether, anesthesia (Baker Chemicals)
Water bath
Flash evaporator
Ethanol (Florida Distillers)
Glass beads
Sodium desoxycholate (Fisher Scientific)
Phenol (75% solution) (Fisher Scientific)
Glycine (For deoxycholate buffer) (Fisher Scientific)
EDTA (for deoxycholate buffer) (Fisher Scientific)
200 ml sterile glass centrifuge bottles
Vacuum flask 1000 ml
Tris buffer (Sigma Chemical Co.)
Deoxyribonuclease (Worthington)
Ribonuclease (Worthington)
Pronase (Calbiochem)

Labeling Information

B. fragilis capsular vaccine
IND No. 1615 Lot B.f.-1R
"Caution: New Drug - Limited by federal
law to investigational use."
Manufacture date 5-3-84
100 mcg/ml

DATE
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