(12)

AD _____

REPORT NUMBER 14

IMMUNOLOGIC INTERRELATIONSHIPS OF COLIFORM HEAT-LABILE AND HEAT-STABLE ENTEROTOXINS

Final Report

Frederick A. Klipstein, M.D.

September, 1981

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD17-77-C-7032

University of Rochester
School of Medicine and Dentistry
Rochester, New York 14642

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.

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER 14	2. GOVT ACCESSION NO. AD-A130589	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Immunological Interrelationships of Coliform Heat-labile and Heat-stable enterotoxins		5. TYPE OF REPORT & PERIOD COVERED Final Report Aug. 1, 1977 - Sept. 1, 1981 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Frederick A. Klipstein, M.D.		8. CONTRACT OR GRANT NUMBER(s) DAMD17-77-C-7032
9. PERFORMING ORGANIZATION NAME AND ADDRESS University of Rochester School of Medicine and Dentistry Rochester, New York 14642		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62770A 3M162770A871.AD.073
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research & Development Command Fort Detrick Frederick, Maryland 21701		12. REPORT DATE September, 1981 13. NUMBER OF PAGES 9
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Acute diarrhea / *Escherichia coli* / Enterotoxins / Immunization

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Investigations conducted during the four year period (1977 to 1981) of support by the USAMRDC have accomplished the following. (1) The enterotoxigenicity of certain coliform species which contaminate the small bowel in different diarrheal disorders has been established. (2) Immunization regimens have been developed which provide protection against enterotoxigenic strains of *E. coli* in experimental animals.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

SUMMARY

These investigations (a) established the fact that species of coliform bacteria other than ETEC strains of *E. coli* elaborate enterotoxins which alter gastrointestinal physiology, and (b) showed that immunization with either *E. coli* (ETEC) LT or ST toxin arouses an antitoxin response in experimental animals which protects against challenge with viable ETEC strains which produce the toxin used for immunization. Information regarding optimal immunization routes and schedules, antitoxin responses, and toxoiding of these immunogens has also been obtained. This information is being used to facilitate current work in this laboratory to develop a practical vaccine which will provide protection against diarrheal disease caused by all types of ETEC strains in humans. In view of the well-recognized incapacitating effect of acute diarrheal disease among military personnel serving in the tropics, the successful development of the vaccine should have major health implications for the military.

FOREWARD

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).

INTRODUCTION

This final report summarizes research activities conducted in this laboratory during the four year period between 1977 and 1981 that were supported by the USAMRDC and The Office of Naval Research. Since the results of all but one of the investigations conducted have been described in full detail in previously submitted annual reports, as well as in the publications listed, this report will confine itself to a brief overview of these investigations. They may be divided into two general categories as follows.

(1) Characterization of coliform enterotoxins. An assay was developed which detects, by means of in vivo marker perfusion in the rat jejunum, the presence of heat-labile (LT) or heat-stable (ST) enterotoxins produced by coliform strains other than enterotoxigenic strains of *Escherichia coli* (ETEC) (10). The toxins detected usually do not stimulate the conventional assay systems such as the Y1 adrenal cell assay for LT and the suckling mouse assay for ST. The strains examined included *Klebsiella pneumoniae*, *Enterobacter cloacae* and enteropathogenic strains of *E. coli* (EPEC) which were isolated from the gastrointestinal tract of persons with either acute or chronic diarrhea. The biologic properties of these toxins including their physical properties, their effect on gastrointestinal physiology, and their immunologic properties were delineated in a series of studies.

In summary, strains of non-ETEC coliform bacteria isolated from the jejunum of persons with tropical sprue were shown to elaborate toxins whereas those isolated from the jejunum of individuals with the blind loop syndrome or from the stool of normal persons do not (5,7). These toxins affect water and glucose transport (3,6,7). The heat-labile toxins elaborated by these strains were shown to have immunological cross-reactivity with similarly prepared toxins derived from ETEC strains (1,2). Strains of *E. coli* isolated from nursery outbreaks of infantile diarrhea, which were negative in conventional assays for ETEC toxins, were also shown to elaborate toxins by means of this assay (4) and an immunological relationship between these toxins and those elaborated by ETEC strains was established (16).

(2) Development of a program of immunological protection against diarrheal disease due to intestinal colonization by enterotoxigenic strains of *Escherichia coli*.

Preliminary studies conducted in 1978 showed that immunization of rats with an impure form (polymyxin-release) of the *E. coli* (FTEC) heat-labile (LT) toxin yields protection against challenge with either this toxin or viable ETEC LT-producing strains but not against the heat-stable toxin (ST), or viable strains which produce this toxin form (8). Subsequent investigations conducted in 1979 and 1980 delineated the influence of the immunization route, schedule and dosage of this immunogen on immediate and extended protection in this animal model (13,14,17). The validity of these observations, which were conducted using ligated ileal loops as the challenge technique to the intact gut, was established by studies which demonstrated protection in immunized gnotobiotic rats which were challenged by intestinal colonization with ETEC strains (15).

Following publication in 1980 of the methods to produce pure, homogeneous LT holotoxin and its B subunit, we made and used these materials to immunize rats. The LT holotoxin was found to be a more effective immunogen on a molar basis than the B subunit (18). Immunization with the LT holotoxin was shown to arouse an antitoxin response within the serum and/or intestinal mucosa (depending on the route of immunization), as detected by enzyme-linked immunosorbent assay (21), which provides protection against heterologous serotypes of viable LT-producing strains (20). Following publication in 1981 of methods to produce the ST toxin, we made it and immunized rats with the ST toxin coupled to a large molecular weight carrier; this immunization was shown to provide protection against this toxin and viable ST-producing strains but not against LT-producing strains (21).

PUBLICATIONS

Supported by Contract No. DAMD 17-77-C-7032

1. Klipstein FA, Engert RF: Immunological interrelationships between cholera toxin and the heat-labile and heat-stable enterotoxins of coliform bacteria. Infec Immun 13:110, 1977

2. Klipstein FA, Engert RF: Immunological relationship of different preparations of coliform enterotoxins. Infec Immun 21:771, 1978

3. Klipstein FA, Engert RF: Reversal of jejunal water secretion by glucose in rats exposed to coliform enterotoxins. Gastroenterology 75:255, 1978

4. Klipstein FA, Rowe B, Engert RF, Short HB, Gross RJ: Enterotoxigenicity of enteropathogenic serotypes of *E. coli* isolated from nursery epidemics of diarrhea. Infec Immun 21:171, 1978

5. Klipstein FA, Engert RF, Short HB: Enterotoxigenicity of colonising bacteria in tropical sprue and blind loop syndrome. Lancet 2:342, 1978

6. Klipstein FA: Enterotoxigenicity of *Klebsiella pneumoniae*. In McClung HJ (Editor), Etiology, Pathophysiology, and Treatment of Acute Gastroenteritis. Report of 74th Ross Conference on Pediatric Research. Columbus, Ohio, 1978, p. 78-82

7. Klipstein FA, Goetsch CA, Engert RF, Short HB, Schenk EA: Effect of monocontamination of germfree rats by enterotoxigenic coliform bacteria. Gastroenterology 76:341, 1979

8. Klipstein FA, Engert RF: Protective effect of active immunization with purified *Escherichia coli* heat-labile enterotoxin in the rat. Infec Immun 23:592, 1979

9. Klipstein FA: Tropical sprue. In Principles and Practice of Infectious Disease. Mandell GL, Douglas RG and Bennett JE, Editors. New York, John Wiley and Sons. 1979, p. 924-929

10. Klipstein FA, Guerrant RL, Wells JG, Short HB, Engert RF: Comparison of assay of coliform enterotoxins by conventional techniques versus in vivo intestinal perfusion. Infec Immun 25:146, 1979

11. Klipstein FA: Sprue and subclinical malabsorption. (Letter) Lancet 1:277, 1979

12. Klipstein FA: Tropical sprue in gnotobiotic rats? (Letter) Gastroenterology 76:1082, 1979

13. Klipstein FA, Engert RF: Influence of route of administration on immediate and extended protection in rats immunized with *Escherichia coli* heat-labile enterotoxin. Infec Immun 27:81, 1980

14. Klipstein FA, Short HB, Engert RF: Protective value of parenteral versus peroral immunization with *Escherichia coli* heat-labile enterotoxin. Proceedings of the Fifteenth Joint Conference on Cholera of the U.S. - Japan Cooperative Medical Science Program. National Institutes of Health, Publication No. 80-2003, 1980. p. 214

15. Klipstein FA, Engert RF, Short HB: Protective effect of immunization with heat-labile enterotoxin in gnotobiotic rats monocontaminated with enterotoxigenic *Escherichia coli*. Infec Immun 28:163, 1980

16. Klipstein FA, Engert RF, Short HB: Immunologic cross-reactivity of heat-labile enterotoxins produced by enterotoxigenic and enteropathogenic strains of *Escherichia coli*. Immunology 14:115, 1980

17. Klipstein, FA, Engert RF: Respective contributions to protection of primary and booster immunization with *Escherichia coli* heat-labile enterotoxin in rats. Infec Immun 31:252, 1981

18. Klipstein FA, Engert RF: Protective effect of immunization of rats with the holotoxin or B subunit of *Escherichia coli* heat-labile enterotoxin. Infec Immun 31:144, 1981

19. Klipstein FA: Tropical sprue in travellers and expatriates living abroad. Gastroenterology 80:590, 1981

20. Klipstein FA, Engert RF, Clements JC: Immunization of rats with heat-labile enterotoxin provides uniform protection against heterologous serotypes of enterotoxigenic *Escherichia coli*. Infec Immun 32:1100, 1981

21. Klipstein FA, Engert RF, Clements JD: Protection in rats immunized with *Escherichia coli* heat-stable enterotoxin. Infec Immun 34:637, 1981

22. Klipstein FA, Engert RF, Clements JC: Arousal of mucosal secretory immunoglobulin A antitoxin in rats immunized with *Escherichia coli* heat-labile enterotoxin. Infec Immun 37:1086, 1982.

12 copies

Director
Walter Reed Army Institute of Research
Walter Reed Army Medical Center
ATTN: SGRD-UWZ-C
Wasshington, DEC 20012

4 copies

Commander
US Army Medical Research and Development Command
ATTN: SGRD-RMS
Fort Detrick, Frederick, MD 21701

12 copies

Defense Technical Information Center (DTIC)
ATTN: DTIC-DDA
Cameron Station
Alexandria, VA 22314

1 copy

Dean
School of Medicine
Uniformed Services University of the Health Services
4301 Jones Bridge Road
Bethesda, MD 20014

1 copy

Commandant
Academy of the Health Sciences, US Army
ATTN: AHS-CDM
Fort Sam Houston, TX 78234