

AD-A194 645

STUDIES OF THE BIOLOGICAL AND MOLECULAR BASIS OF THE
INHIBITION OF ACTIVITY OF PHAGOCYtic CELLS
BY ANTHRAX TOXIN

Final Report

George G. Wright

January 1988

Supported by

U.S. Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701-5012

Grant No. DAMD17-83-G-9565

The University of Virginia
School of Medicine
Charlottesville, Virginia 22908

DTIC
SELECTED
MAY 19 1988
S E D

Approved for public release; distribution unlimited

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.

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION Unclassified			1b. RESTRICTIVE MARKINGS		
2a. SECURITY CLASSIFICATION AUTHORITY			3. DISTRIBUTION / AVAILABILITY OF REPORT Approved for public release; distribution unlimited		
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE					
4. PERFORMING ORGANIZATION REPORT NUMBER(S)			5. MONITORING ORGANIZATION REPORT NUMBER(S)		
6a. NAME OF PERFORMING ORGANIZATION The University of Virginia		6b. OFFICE SYMBOL (If applicable)		7a. NAME OF MONITORING ORGANIZATION	
6c. ADDRESS (City, State, and ZIP Code) The University of Virginia School of Medicine Charlottesville, Virginia 22908		7b. ADDRESS (City, State, and ZIP Code)			
8a. NAME OF FUNDING / SPONSORING ORGANIZATION US Army Medical Research & Development Command		8b. OFFICE SYMBOL (If applicable)		9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAMD17-83-G-9565	
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, MD 21701-5012		10. SOURCE OF FUNDING NUMBERS			
		PROGRAM ELEMENT NO. 61102A		PROJECT NO. 61102A	TASK NO. AA
				WORK UNIT ACCESSION NO. 110	
11. TITLE (Include Security Classification) (u)Studies of the Biological and Molecular Basis of Inhibition of Activity of Phagocytic Cells by Anthrax Toxin.					
12. PERSONAL AUTHOR(S) Wright, G.G.					
13a. TYPE OF REPORT Final		13b. TIME COVERED FROM 09/30/83 TO 03/31/87		14. DATE OF REPORT (Year, Month, Day) 1988, January	
15. PAGE COUNT 6					
16. SUPPLEMENTARY NOTATION					
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB-GROUP			
06	13		Anthrax Toxin, Polymorphonuclear Neutrophils, Platelets		
06	03		Priming, Lipopolysaccharide, Chemotaxis, Superoxide Anion		
19. ABSTRACT (Continue on reverse if necessary and identify by block number) The primary effect of anthrax toxin on polymorphonuclear neutrophils (PMN) is the inhibition of priming by bacterial products, as represented by lipopolysaccharide (LPS) and by muramyl dipeptide, a synthetic mitogen analogous to bacterial peptidoglycans. Priming increases superoxide release and enzyme exocytosis of PMN after stimulation with chemotactic peptide; inhibition of priming by prior treatment with toxin reduces these critical antimicrobial functions. These observations could explain the major contribution of the toxin to virulence of <u>Bacillus anthracis</u> . Priming inhibits chemotaxis of PMN; inhibition of priming by LPS present as a contaminant evidently is responsible for the apparent stimulation of chemotaxis by prior treatment of PMN with toxin. Priming is mediated by factors released from platelets and probably other cells by bacterial products.					
20. DISTRIBUTION / AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS			21. ABSTRACT SECURITY CLASSIFICATION Unclassified		
22a. NAME OF RESPONSIBLE INDIVIDUAL Mary Frances Bostian			22b. TELEPHONE (Include Area Code) (301) 663-7325		22c. OFFICE SYMBOL SGRD-RM1-S



Accession For	
NTIS GRA&I	☒
DTIC TAB	☐
Unannounced	☐
Justification	
By _____	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	

Foreword

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

(For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR4)

Summary of Research Carried Out under the Grant

First annual report

Investigations are presented on the effects of the toxin of Bacillus anthracis on phagocytic cells, and certain alterations in biochemical reactivity associated with these effects. Combinations of protective antigen (PA) plus edema factor (EF), and PA plus lethal factor (LF) markedly stimulated chemotaxis of human polymorphonuclear neutrophils (PMN), but had little effect on random migration (1). These effects were accompanied by a marked inhibition of the oxidative activity of these cells, as measured by a decrease in the formation of chloramines. PA + EF, but not PA + LF, produced a small but consistent increase in 3'5' - adenosine monophosphate (cAMP) in PMN; the levels of cAMP were markedly lower than those produced by PA + EF in certain tissue culture cells, and those produced by the adenylate cyclase of Bordetella pertussis in PMN.

Second annual report

The anthrax toxin was known to exert antiphagocytic and antibactericidal effects on PMNs, which are believed to contribute to the essential role of the toxin in virulence. Toward elucidation of these effects, we studied pretreatment of human PMN with purified preparations of the toxin components - PA, EF, and LF - and its effect on their release of superoxide anion (O_2^-) after stimulation with the chemotactic peptide N-formyl-methionyl-leucyl-

phenylalanine (FMLP). PMN isolated with minimal exposure to lipopolysaccharide (LPS) released only small amounts of O_2^- after FMLP stimulation; O_2^- release was increased 5.2-fold by treatment with 3 ng per ml of LPS for 1 hour at 37 C prior to FMLP stimulations, an effect referred to as priming. PMN were primed to an equivalent extent by treatment with N-acetylmuramyl-L-alanyld-isoglutamine (muramyl dipeptide-MDP) 100 ng per ml. Pretreatment of PMN with anthrax toxin components PA + EF or PA + LF inhibited priming by LPS or MDP as shown by inhibition of release of O_2^- up to 90% relative to controls not treated with toxin; single toxin components were inactive. The inhibition was reduced markedly if priming with LPS or MDP were carried out prior to exposure to toxin. O_2^- release after stimulation by phorbol myristate acetate was not increased by priming, and pretreatment with toxin did not inhibit O_2^- release after this stimulus. Evidently anthrax toxin inhibits the priming normally induced in PMN by bacterial products and necessary for full expression of antibacterial effects by these cells; inhibition of priming represents a new mechanism by which a bacterial toxin increases the virulence of the bacterium that produces it. (2).

Third annual report

The foregoing experiments were carried out with human PMN partially purified without exposure to LPS. Further purification of the PMN on Percoll gradients removed most remaining mononuclear cells and platelets, yielding PMN preparations approximately 98%

pure. We found that these PMN suspensions were not susceptible to priming by LPS; susceptibility was restored to a major degree by reintroduction of platelets, approximately 5 per PMN.

Incubation of platelets, which had been isolated without LPS exposure, with LPS at concentrations of the order of 10 ng per ml released a soluble factor that produced priming responses in PMN of at least five-fold. The priming factor had properties of a labile protein, and did not resemble previously described mediators derived from platelets. It was non-dialyzable, did not pass an ultrafilter with 30,000 Dalton cut-off, and was precipitated by 40% saturation with ammonium sulfate. Activity of the crude filtrate was destroyed immediately at pH 5 or below; moderate activity was retained after brief exposure to pH 10. Efforts to extract priming activity in lipid solvents gave negative results. Anthrax toxin, previously shown to inhibit priming of PMN by LPS, also inhibited priming of PMN by platelet-derived priming factor, but had no evident effect on release of priming factor from platelets.

Evidently platelet-derived priming factor mediates a portion of the overall priming effect of LPS described previously, thereby modulating the level of O_2^- generation by PMN.(3).

Publications Supported by the Grant

Complete papers:

1. Wade, BH, GG Wright, EL Hewlett, SH Leppla, and GL Mandell. 1985. Anthrax toxin components stimulate chemotaxis of human polymorphonuclear neutrophils. Proc. Soc. Exp. Biol. Med. 179: 159-162.
2. Wright, GG and GL Mandell. 1986. Anthrax toxin blocks priming of neutrophils by lipopolysaccharide and by muramyl dipeptide. J. Exper. Med. 164: 1700-1709.
3. Wright, GG, PW Read, and GL Mandell. 1988. Lipopolysaccharide releases a priming substance from platelets that augments the oxidative response of polymorphonuclear neutrophils to chemotactic peptide. J. Infect. Dis. in press.

Abstracts of presentations at national scientific meetings:

4. Hewlett, EL, HJ Anderson, AA Weiss, GA Meyers, RD Pearson, GG Wright, and SH. Leppla, 1985. Adenylate cyclase toxins of Bordetella pertussis and Bacillus anthracis. Clinical Research, 33: 405A.
5. Wright, GG and GL Mandell. 1986. Inhibition by anthrax toxin of superoxide anion formation in stimulated neutrophils. Abstracts of the Annual Meeting of the American Society for Microbiology, pg. 52.
6. Wright, GG, PW Read, and GL Mandell. 1987. Platelets release a potent neutrophil priming factor upon exposure to lipopolysaccharide. Clinical Research, 35: 619.

Personnel receiving financial support from the grant:

**George G. Wright
Agbor Egbewatt
Craig Lombard
Paul W. Read**

None received graduate degrees as a direct result of their appointments under the grant. Egbewatt, Lombard, and Read have initiated graduate or medical studies subsequently.

DISTRIBUTION LIST

5 copies **Commander**
US Army Medical Research Institute of Infectious
Diseases
ATTN: SGRD-UIZ-M
Fort Detrick, Frederick, MD 21701-5011

1 copy **Commander**
US Army Medical Research and Development
Command
ATTN: SGRD-RMI-S
Fort Detrick, Frederick, MD 21701-5012

12 copies **Defense Technical Information Center (DTIC)**
ATTN: DTIC-DDAC
Cameron Station
Alexandria, VA 22304-6145

1 copy **Dean**
School of Medicine
Uniformed Services University of the Health
Sciences
4301 Jones Bridge Road
Bethesda, MD 20814-4799

1 copy **Commandant**
Academy of Health Sciences, US Army
ATTN: AGS-CDM
Fort Sam Houston, TX 78234-6100