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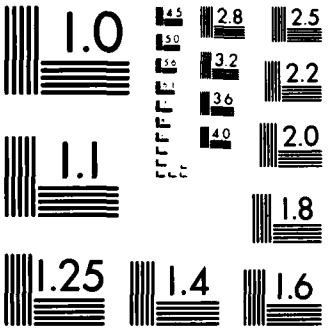

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Safety and Immunogenicity Testing of a Pilot Polysaccharide Vaccine Preparation to Pseudomonas Aeruginosa

Annual Report

Gerald B. Pier, Ph.D.

September 1, 1981

(For period 16 August 1980 to 1 August 1981)

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FOREWORD

In conducting the research described in this report, the investigator(s) 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(s) have adhered to policies of applicable Federal Law 45CFR46.

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A. Contract Background

The principal investigator was awarded a contract from the USAMRDC to run from 1 March 1979 to 31 December 1981. The scope of this contract included preparation and testing of a pilot vaccine to Pseudomonas aeruginosa infections including human phase 1 trials, development of assays for measuring the antibody response of immunized and infected individuals, testing of the immunogenicity of the vaccine product in animals, assessment of the material in compromised animal host models as a protective agent, assessment of cell mediated protective mechanisms induced by the vaccine, including in vitro cellular studies, determinations of the genetic basis of response to the vaccine in inbred mouse strains, and assessment of the role of lipopolysaccharide (LPS) as an immunogenic or biologically active component of the vaccine. The vaccine is a high molecular weight polysaccharide (PS) material isolated from the outer cell surface or cultural supernates of P. aeruginosa (1). Similar types of polysaccharides have been shown to be effective vaccines for a number of bacterial infections, such as meningitis caused by Neisseria meningitidis and pneumonia caused by Streptococcus pneumoniae. Since P. aeruginosa infections are common complications of wound and burn injuries that occur in military combat, this high molecular weight PS product is being developed as a potential preventative measure for these infections.

B. Progress of Vaccine Development and Preparation

This contract has centered around the production of a high molecular weight (MW) polysaccharide (PS) fraction from cultural supernates of the organism P. aeruginosa. Our method of preparation has been described in both publications (1,2) and previous annual reports submitted to the USAMRDC. The contract for the current year involved testing of alternative preparation procedures to purify the high MW PS. Our current procedure employs the use of hydrolyses of crude preparations of PS in acetic acid at 90-95^{0} for 18 hr to split contaminating lipopolysaccharide (LPS) into its lipid and polysaccharide components for subsequent removal. We proposed to test the feasibility of three alternative procedures to purify high MW PS: use of preparative isoelectric focusing, ion-exchange chromatography, and a combination of ultracentrifugation and column chromatography in the presence of a disaggregating buffer, sodium deoxycholate. In order to do this we first had to assess the affect of the conditions utilized during the performance of these procedures on the immunogenicity and serological reactivity (antigenicity) of high MW PS. In particular, we were concerned that the pH conditions that the PS would be exposed to during preparative isoelectric focusing or ion-exchange chromatography would be deleterious to the PS immunologic properties. Table 1 summarizes the results of exposure of purified PS to various pH conditions, ultracentrifugation and column chromatography in the presence of sodium deoxycholate. Exposure of PS to pH between 2.8 and 11.5 did not affect antigenicity. However, immunogenicity was lost at a pH below 4.8 or above 8.8, thus precluding the use of isoelectric focusing or ion-exchange chromatography as preparative procedures. Isoelectric focusing would expose the PS to a pH of 3.5 and in order to get PS to bind to anion exchange resins a pH of 9.5 is needed. Ultracentrifugation alone did not affect PS antigenicity and immunogenicity. However, chromatography in the presence of sodium deoxycholate

TABLE 1

Effect of Exposure of High MW PS to various conditions on serological activity (SA) and immunogenicity (IG)

CONDITION:	PARAMETER	
	SA_a	IG
pH 5.0	+	+
pH 4.8	+	±
pH 4.5	+	-
pH 3.7	+	-
pH 2.8	+	-
pH 8.0	+	+
pH 8.8	+	-
pH 10.2	+	-
pH 11.5	+	-
pH 12.0	-	-
NaD Chromatography[b]	+	-
Ultracentrifugation	+	+

[a] represents no change in gel precipitin or immunogenicity in mice when compared to untreated PS; - represents loss of activity

[b] NaD: sodium deoxycholate
exposure conditions were room temperature for 48 hours

caused a loss of immunogenicity but not antigenicity. Thus combination of these later two procedures was not deemed feasible because it yielded a non-immunogenic PS.

We therefore have stuck with our original use of hydrolyses in the presence of acetic acid as a means of purifying PS. Recent data we have obtained indicates that this procedure may actually be crucial to the isolation of an immunogenic high MW PS product. Analyses of PS from IT-1 P. aeruginosa by Dr. Harold Jennings of the National Research Council, Ottowa, Canada, indicates a relatively high percentage of mannose in the IT-1 PS. This mannose composes a highly branched mannan molecule, analogous to that produced by yeast cells. Complexed to this mannan is the serologically active component of PS. This serologically active component is identical to the "O" specific side chain determinant on the LPS. We have previously documented (1,2,3) the serological cross reactivity of PS and "O" side chains, and have shown that PS differs from "O" side chain by chemical constituents, monosaccharide components, and immunogenicity in animals. Since "O" side chains are released from the LPS during the acetic acid hydrolyses step, it would be available to complex with the mannan upon cooling of the heated solution. This complexing might therefore provide the necessary molecular size and complexity to confer immunogenicity on the "O" side chain determinant. Precedence for the complexing of two distinct polysaccharides present together in heated solutions upon cooling have been reported (4, 5). Experiments to isolate the mannan entity itself are currently underway, after which we will attempt to complex the "O" side chain with the mannan. Since Dr. Jennings has been unable to dissociate the serologically active component of PS (identical to "O" side chain) from the mannan using 6M urea or 8M guanidine, this is good evidence that the 2 entitites, mannan and "O" side chain, are complexed in our final product. Finally, as documented below, the PS from IT-1 P. aeruginosa has been found to be immunogenic and non toxic in humans, so the need to explore alternative isolation procedures is voided.

C. Progress on Vaccine Testing in Humans.

1) Phase I trials in adult volunteers

The Bureau of Biologics, Food and Drug administration has assigned number 1495 to our Investigational New Drug (IND) application for phase I testing of the high MW PS vaccine from IT-1 P. aeruginosa in humans. We have also been informed by LTC Roger A. Bennett of the MSC, Chief, Human Case Review office, that this proposal complies with the Army and Federal Regulations regarding human use and investigational drugs. Therefore, we have immunized 42 persons with the vaccine, interviewed them for reactions to the shot, and drawn blood samples at 14, 28, and 180 days for analyses in a radioactive antigen binding assay (RABA) and an opsonophagocytosis assay. The results of this work are documented in "Safety and Immunogenicity of High Molecular Weight Polysaccharide Vaccine"(6). Briefly, the vaccine was found to produce a statistically significant rise of binding antibody, as measured in the RABA, in persons given 150 and 250 ug (Table 2). These doses of vaccine were associated with minimal toxicity, producing no greater reactivity then a slightly sore and tender arm at the site of vaccination. The ability to opsonize live P. aeruginosa organisms for killing in a phagocytic assay was increased 4 fold or greater in 9 of 12 persons given 150 ug and 16 of 18 persons given 250 ug (Table 3). Thus, the antibody induced was functional. These results are highly encouraging

TABLE 2

Immunogenicity of PS vaccine from
Immunotype 1 *P. aeruginosa*

Antibody Concentration in ug/ml
geometric mean ± standard deviation (range)

dose (ug)	Wk after immunization 0	2	4
50	13.2 ± 12.8 (3.9-40.0)	30.5 ± 29.2 (4.0-88.4)	31.2 ± 30.3 (4.0-86.8)
75	22.8 ± 41.9 (4.1-97.8)	42.8 ± 73.9 (3.8-174.3)	44.5 ± 76.8 (3.8-175.2)
150	5.9 ± 3.6 (1.7-14.9)	63.8 ± 55.8 (6.1-147.4)	66.7 ± 54.3 (6.1-155.8)
250	4.9 ± 1.9 (3.6-10.2)	55.9 ± 61.8 (3.7-250)	56.3 ± 59.3 (3.7-232)

TABLE 3

Serum Titers in the Opsonophagocytosis Assay Following Immunization With P. aeruginosa immunotype 1 PS vaccine

Dose	Pre-Immunization Titer			Post Immunization Titer			
(ug)	≤2	4-8	>8	≤2	4-9	>8	No. persons with 4 fold or greater rise
50	5*	2	0	3	2	2	3
75	4	1	0	3	1	1	2
150	9	1	2	1	5	6	9
250	14	4	0	0	5	13	16

*represents number of vaccinees with this titer

because the antibody levels achieved were high, the antibody induced was relevant to human immunity, and the vaccine had a minimal toxicity. Furthermore, the antibody titers remained elevated for at least 6 months, indicating maintenance of the response (Table 4).

2) Initiation of vaccine studies in military recruits.

The current contract calls for initiating vaccination studies in military recruits upon completion of the phase I trials noted above. However, initiation of these studies has not been undertaken because Col Philip Russell, MD, Director of the Walter Reed Army Institute of Research has deemed it unnecessary to perform these studies. Although we initially thought there could be useful data gained from recruit studies, we concur with Col Russell's judgment in this matter. A copy of his letter of decision is attached.

D. Progress of the development of the immunologic response of mice to PS vaccination.

1) Specificity of the immune response of mice for strains of P. aeruginosa.

We have investigated the cross protective efficacy to live organism challenge following immunization with PS preparations from the 7 Fisher types of P. aeruginosa. Table 5 shows a degree of cross protective efficacy exerted by each PS to serotypes other than the one from which the PS was isolated. This indicates that contaminating LPS is not responsible for the induction of cross protective immunity since LPS only induces a serotype specific protection (7,8). Current studies are under way, to be completed before the end of the contract year, that would correlate the serological reactivity of PS immune sera with the protective efficacy observed. This correlation is to be performed utilizing the RABA and passive transfer studies of immune sera. A preliminary report of this data for PS from types 1 and 2 that has been submitted for publication is appended(7).

2) Assessment of cellular immunity in mice.

This work was to be performed under a sub-contract with Dr. Richard Markham of Washington University Medical School. The review committee eliminated this subcontract section from the contract and Dr. Markham has applied for his own funding for this work.

E. Progress of PS Vaccine Efficacy in Animal Models.

We have been collaborating with Dr. Matt Pollock of the USUHS on evaluating the protective efficacy of IT-1 PS vaccine in burned mice. These studies have shown that mice immunized with 3 50ug doses on days 0,2 and 5 were significantly more resistant to challenge of an alcohol flame burn site inoculated with P. aeruginosa (Table 6). Passive transfer of rabbit antiserum to IT-1 PS also afforded protection to challenge (Table 7). Mice that were burned them immunized with the IT-1 PS made an equivalent immune response as did non-burned animals (Table 8). Thus, these results have been very encouraging. We are currently involved in the assessment of cross protective efficacy following PS immunization utilizing the burned mouse model.

TABLE 4

Duration of Antibody Levels
in persons receiving 150 ug of immunotype 1
P. aeruginosa PS vaccine

Antibody Concentration in ug/ml- geometric mean ± standard deviation (range)		
Time after immunization		
0	6 months	difference (post-pre)
5.9 ± 3.6	32.7 ± 29.3	27.3 ± 28.8
(1.7 -14.9	(4.2 - 94.2)	(1.1 - 87.4)

TABLE 5

Cross Protective Efficacy of High MS PS
from immunotypes 1-7 in a mouse intraperitoneal infection.

Immunizing PS from Immunotype	Challenge Strain-Immunotype 1	2	3	4	5	6	7
1	90[a]	50	50	30	50	90	10
2	80	100	30	50	50	80	60
3	70	90	70	0	70	30	10
4	60	80	50	60	50	100	0
5	50	50	100	10	90	90	50
6	20	50	100	30	40	90	10
7	10	10	50	10	20	60	60
Saline	0	10	10	0	0	10	10

[a]represents precent survival (out of 10) of mice challenged with 1-2 LD_{100} of live P. aeruginosa 7 days after immunization with a 50 ug dose of the indicated PS in 0.5 ml saline.

TABLE 6

Protective Efficacy of High MW PS vaccine against murine burn wound infection and death from *P. aeruginosa*

GROUP	Challenge Dose (no. organisms)						
	10^9	10^8	10^7	10^6	10^5	10^4	10^3
Non-immune	0[a]	0	0	0	60	100	100
Immune[c]	0	60	80	80	100	60[b]	100

[a] represents percent survival

[b] two mice probably died from causes other than infection based on clinical observations.

[c] LD50 for non immune animals = 1.6×10^5, for immune animals 6.5×10^7. Difference is highly significant ($p < .05$).

TABLE 7

Passive Protective Efficacy of Immune Sera to IT-1 PS in Protecting Mice from Burn Wound P. aeruginosa Infection and Death

GROUP	Challenge Dose (no. organisms)						
	$5x10^8$	$5x10^7$	$5x10^6$	$5x10^5$	$5x10^4$	$5x10^3$	$5x10^2$
NRS[a]	0[b]	0	0	0	40	60	100
IRS[c]	0	0	20	100	100	100	100

[a]NRS, normal rabbit serum
IRS immune rabbit serum

[b]represents percent survival

[c]LD_{50} for NRS treated group was 1×10^4; for IRS treated group 6.3×10^5. Highly significant ($p<.05$) difference

TABLE 8

Antibody concentration (ug/ml, ± standard deviation)
in mice either burned or not burned then immunized with
IT-1 P. aeruginosa

Status	ug/ml ± SD	
Burned	10.3 ± 5	p = .6
Not Burned	11.7 ± 6.9	

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