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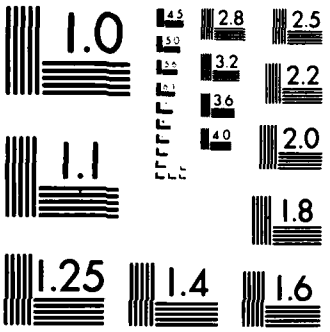

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Safety and Immunogenicity Testing of a Pilot Polysaccharide Vaccine Preparation to Pseudomonas Aeruginosa

Annual Report

Gerald B. Pier, Ph.D.

September 1980

(For period 16 August 1979 to 15 August 1980)

Supported by

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The Peter Bent Brigham Hospital
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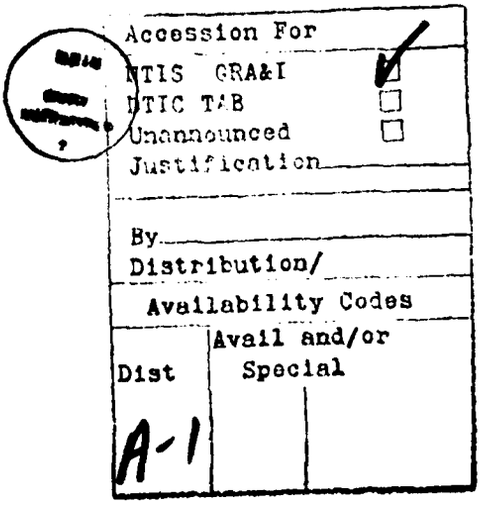
FOREWORD

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 1980. The scope of this contract included preparation and testing of a pilot vaccine to Pseudomonas aeruginosa infections up to the human trial stage, 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

Our contract proposed to prepare, by our previously described method (1), a high molecular weight PS preparation from immunotype 7 (IT-7) P. aeruginosa, test this product for chemical composition, serological activity, immunogenicity and protective efficacy in mice, determine its molecular size, and test its toxicity in rabbits, mice, and guinea pigs. IT-7 P. aeruginosa was originally chosen because the PS antigen from this molecule appeared to have the greatest degree of cross serotype protective efficacy in animal protection tests. In addition, there appeared to be a high degree of binding in both a solid phase radioimmunoassay (SPRIA) and radioactive antigen binding assay (RABA) of antisera to all 7 Fisher type strains to the IT-7 PS antigen. However, attempts to produce a vaccine from IT-7 were problematical, including low yields and serologically poorly active material. In addition, fractionation of the T-7 PS by ion exchange chromatography on DEAE Sephadex columns revealed up to 9 distinct fractions of either serologically or optically (adsorption at 206 nm) active material. These fractions were all tested for their protective efficacy in mouse immunization-challenge experiments, and the results were disappointing. None of the fractions showed very good protective efficacy. As a result, we decided to develop the vaccine from the IT-1 strain of P. aeruginosa. We have had the most experience in working with the IT-1 strain, and had consistently good yields of PS antigen from this strain. IT-1 P. aeruginosa also shows an almost equal degree of cross serotype protective efficacy as does IT-7 in mouse immunization-challenge experiments, and offers the advantage that the serologic activity of this antigen is readily destroyed by treatment with

dilute alkali at 37°C for 2 hrs. This treatment does not affect a set of serologic determinants on the LPS from IT-1 P. aeruginosa that are distinct from the PS determinants (IT-1 PS serologic determinants are all expressed on the IT-1 LPS. Their relation and chemical differences are documented in the 1st annual report on this contract).

Thus, preparation of the trial vaccine product from IT-1 P. aeruginosa has been done. We have tested the product in accordance with Food and Drug Administration (FDA) requirements for preclinical safety, toxicity and sterility standards, prepared and submitted an Investigational New Drug (IND) application to the FDA (FDA Form 1571), prepared a clinical pharmacology protocol (FDA Form 1572) to test the material in humans, notified the Surgeon General of the United States Army in accordance with our contract obligations, as well as notified the U.S. Army Human Use Review Office of our IND application. This application is appended to this annual report. In the FDA Form 1571 we have documented the following:

1) Preparation method of material
2) Chemical analyses of final product
3) Immunological and serological properties of the final product
4) Animal toxicity results of the final product
5) Sterility tests on the final product

In order to avoid repetition and for brevities sake, these data are not repeated here in the annual report.

Further progress on the development and preparation of the vaccine not documented in the appended IND application includes determination of lot to lot variation in vaccine production, and improved vaccine product isolation techniques. In order to ascertain the variability in chemical composition and immunological activity, three different lots of IT-1 PS vaccine have so far been produced. Results on the chemical composition, monosaccharide components, and serological activity of these preparations are presented in Tables I, II and III. The lot designated VL-III was the one given to the Massachusetts State Biologics Lab for bottling as our vaccine for eventual human use. Two more lots of this product are scheduled for production during the current contract year in order to establish lot to lot variation limits. However, because of the closely related results for lots VL-I, VL-II and VL-III, we felt confident enough to use lot VL-III in human trials.

We attempted to improve PS isolation techniques by use of ultrafiltered media (Trypticase Soy Broth, TSB), variations in the acetic acid strength used during the LPS hydrolyses step, use of a different molecular sieve column (a Sephacyl S-300 versus the old Sephadex G-100), and production of PS in defined media consisting only of salts with a carbon, nitrogen and sulfur source. These tests were all completed with the following results:

TABLE I

Chemical Composition of PT-1 IU
Lots VL-I, VL-II, and VL-III

COMPONENT	AMOUNT		
	VL-I	VL-II	VL-III
Carbohydrate	72.3[a]	71.9	73.5
Lipid	<0.5	<0.5	<0.5
Phosphate	<0.5	<0.5	<0.5
Nucleic Acid	1.1	1.3	0.8
Protein	0.7	1.1	0.9
Water	23.1	22.1	22.2

[a] represents weight percent

TABLE II

Monosaccharide Composition of
IT-1 PS Lots VL-I, VL-II, and VL-III

MONOSACCHARIDE	AMOUNT		
	VL-I	VL-II	VL-III
Arabinose	6.8[a]	5.1	6.1
Rhamnose	4.1	3.1	3.4
Mannose	60.0	61.1	62.2
Galactose	19.1	21.0	19.8
Glucose	7.2	7.9	8.5

[a] represents mole percent of total moles identified

TABLE III

Serologic Activity of IT-1 [illegible]
Lots VL-I, VL-II, and VL-III

ASSAY	ANTIGEN LOT		
	VL-I	VL-II	VL-III
Immunodiffusion Titration	0.125[a]	0.125	0.063
Hemagglutination Inhibition	8[b]	7	7

[a] represents lowest concentration in mg/ml giving visible precipitin line

[b] represents $\log_2$ dilution of a 1 mg/ml solution showing positive hemagglutination inhibition

1) Use of ultrafiltered TSB to eliminate possible vaccine contamination with media components. Ultrafiltration of TSB through Amicon PM-30 membranes yielded both an ultrafiltered (UF) and a retained (R) fraction. Inoculation of untreated TSB or its UF or R fractions with IT-1 P. aeruginosa produced a final optical density (OD) at 650 nm of 2.0 for untreated TSB, 1.6 for the UF fraction and 0.8 for the R fraction. Colony counts indicate about 1.3 logs less growth in the UF fraction when compared to untreated TSB, and 1.7 logs less growth in the R fraction. Since growth and PS production are directly correlated, this indicated that ultrafiltration was not a good method for improving PS yields. Attempts to use a 2X concentration of TSB gave similar results with both the UF and R fractions of 2X TSB showing poor growth of organisms. We did, however, isolate the component of TSB that is of a high enough molecular weight to possibly occur in our vaccine, in order to determine if we could assay for its presence in the final vaccine product. The material did not show any of the properties of nucleic acids or proteins, gave no peaks by gas-liquid chromatography for the presence of carbohydrate or lipids, and showed optical absorbance only in the low UV range (about 206 nm). Thus, we could not use any of our known tests to assay for its presence. However, as shown below in 2, we could assure elimination of this product from our vaccine by use of a different molecular sieve column.

2) Variations in the acetic acid strength used during the LPS hydrolyses step. In order to remove the toxic LPS component from our vaccine, we employ hydrolyses of the crude PS preparation in acetic acid in order to cleave the LPS present into its lipid A and polysaccharide ("O" side chain) components. These are then easily eliminated in subsequent purification steps. We found that when crude PS preparations are dissolved in 1% acetic acid, a concentration of acetic acid known to be able to cleave purified LPS into its components, the resultant pH is about 6.8. The pH of 1% acetic acid is 2.8. We thus tested a range of pH values from 4.0 to 5.2 for their effectiveness in cleaving the LPS while doing least harm to the PS. Our results (Table V) show that at a pH above 5.0 there remains detectable LPS in PS preparations and at a pH above 5.4 there is essentially no loss of LPS due to acetic acid hydrolyses. The presence of LPS was determined by the amount of serologically active alkali stable LPS determinant remaining after treatment. A pH of 4.8 had no effect on the amount of serologically active PS present. From these data we concluded that the pH during our hydrolyses step must be less than 4.8-5.0. This usually means an acetic acid concentration of 2.5%. Thus, we find our optimal yield of purified PS to occur when a pH of 4.8-4.9 is used. This leaves us with serologically active, immunogenic PS utilizing the least harsh treatment to eliminate LPS.

3) Use of a different molecular sieve column. We had been isolating PS by taking the void volume fractions of a Sephadex G-100 column. We found, however, that this fraction could contain molecules as small as 50,000 molecular weight. Thus the availability of a new product, Sephacyl S-300, provided us with an opportunity to improve our yield of high molecular weight PS free from smaller sized, non-immunogenic components. This column also had the added benefit of allowing a small amount of unwanted material to elute ahead of the desired PS fractions. This unwanted material may be

TABLE IV

Effect of pH on Residual Occurrence of LPS following hydrolyses of crude PS preparations

pH	HAI ACTIVITY	
	LPS	PS
Untreated	5[a]	9
6.8	6	9
6.2	6	7
5.8	5	8
5.5	5	8
5.2	3	8
5.0	1	9
4.8	-	7

[a] represents $\log_2$ dilution of treated preparation giving positive inhibition of hemagglutination assay specific for LPS or PS

residual nucleic acid, LPS, or the media components discussed above. There is a small amount of high molecular weight media component present in TSB. This material (see above, no. 1) will elute in the void volume of an S-300 column of 2.6 X 100 cm ahead of the PS fractions thus assuring its elimination from our preparation.

4) Use of defined minimal media. We attempted to prepare PS by growth of P. aeruginosa in a variety of minimal media with various carbon sources. All of these media showed comparable growth but the growth was much less than that in TSB. Also, the PS isolated from these minimal media showed no advantage in terms of greater serological activity or immunogenicity, and showed similar chemical compositions and monosaccharide components with PS isolated from TSB cultures. This media, however, was used to prepare an intrinsically labelled C^{14} PS preparation (see below).

C. Progress of Approval for Human Testing

As noted in section A above, we have prepared to submitted to the Bureau of Biologics (BOB) of the FDA Form 1571, Application for Testing an Investigational New Drug in Humans. The sponsor of this IND is the principal investigator, Gerald B. Pier. The sponsor has obtained FDA Form 1572 from the clinical investigators, Drs. Dennis L. Kasper and J. McLeod Griffiss, who will administer the vaccine in human trials. The sponsor has notified the U.S. Army Investigational Drug Review Board through the office of The Surgeon General as well as the Chief of the Human Use Review Office of the U.S. Army. Finally, Dr. Edward H. Kass, Director of the Channing Laboratory has been notified and will proceed with his review of the pre-clinical safety and toxicity studies in animals prior to commencing the human trials. Copies of the FDA forms and notifications are appended to this report.

The current contract runs through 31 December 1980. We anticipate initial human trials of the PS vaccine to commence in September, following the expiration of the 30 day waiting period required by the FDA following submission of an IND application and commencement of human testing. The completion of the first part of Phase I trials is expected by the expiration date of this contract.

D. Progress on Development of Serological Assays

We have successfully developed an easy to perform, highly reproducible radioactive antigen binding assay (RABA) to measure the immune response of immunized animals and humans to the IT-1 PS vaccine. From our studies on the growth of P. aeruginosa in a minimal salts media (see B-4 above) we decided to employ a media containing 0.7% K_2HPO_4, 0.2% KH_2PO_4, 0.01% $MgSO_4$, 0.1% $(NH_4)_2SO_4$, 0.01% and 20 MCi of C^{14} sodium acetate to produce intrinsically labelled PS. The resultant PS was serologically and chemically similar to previous PS preparations and had a specific activity of 0.52 CPM/ng. The

RABA assay employs 150 cpm of the C^{14} labelled IT-1 PS in 50 µl, and 100 µl of serum. Correlation coefficient calculations of the percent binding of immune mouse and rabbit sera with the log µg antibody concentration of these immune sera gave an r^2 value of 0.9, thus allowing us to compute the µg/ml of antibody found in rabbit and mouse sera. The test is sensitive enough to allow us to be able to predict from the results whether or not an animal is immune or not immune to challenge with the live, homologous IT-1 organism. We are currently beginning studies on human sera with this assay. A study of 50 normal human sera revealed a binding percentage range of 0-50%, with 90% of the sera showing less than 20% binding. Thus, this preliminary test indicates a low prevalence of antibody to the vaccine amongst normal human beings.

We are currently in the process of developing an enzyme linked immunoabsorbent assay (ELISA) for determination of antibody class, and an opsonophagocytosis assay for determination of antibody function.

E. Progress on the Analyses of the Immunologic Response of Mice to PS Vaccination.

We have previously shown that the C3H strain of mouse is a good antibody producer in response to immunization with 1 µg of the IT-1 PS vaccine. Another mouse strain, BALB/C, is a poor responder, showing only a minimal antibody response to a 50 µg dosage. These antibody responses have been measured by the local hemolyses in gel, or plaque forming cell (PFC) technique as well as by our RABA.

Over the past year, we have performed the following experiments:

1) Assessment of the dose-response curve to various lots of IT-1 PS. We have found that a 1 µg dose of IT-1 PS is usually the minimal dose needed to induce a good immune response in C3H mice. Although higher doses (up to 500 µg) of IT-1 PS may produce more uniform responses (Table V) in groups of individual mice, there appears to be no gain in terms of absolute amounts of antibody by using these increased doses.

2) Assessment of thymus dependency of IT-1 PS antigens. Many polysaccharide antigens are so called T independent antigens because they do not require the presence of helper T cells for induction of an immune response (2). IT-1 PS, however, appears to be a T-dependent antigen because treatment of mice with antilymphocyte serum, known to knock out T cells, reduces by 80% the PFC and RABA response of C3H/ANF mice (Table VI).

3) Genetics of the response to IT-1 PS immunization. F_1 mice of BALB/C X C3H and C3H X BALB/C crosses were all found to be good responders to immunization with IT-1 PS (Table VII). Interestingly, both male and female mice with C3H mothers showed a significantly higher response to the IT-1 PS immunization than did similar F_1 mice with BALB/C mothers. Other workers

TABLE V

Serum antibody Responses in C3H/AnF mice following immunization with PT-1 PS

DOSE (ug)	RESPONSE	
	Lot VL-I	Lot VL-III
0.5	2.0[a]	2.0
1.0	14.8	13.6
10.0	17.9	ND[b]
50.0	18.2	15.5
100	21.2	16.3
250	ND	14.8
500	ND	16.9

[a] represents average ug/ml of antibody increase over pre-immune levels for 5 mice

[b] ND - not determined

TABLE VI

Effect of administration of anti-lymphocyte serum (ALS) on C3H/ANF mouse immune response to IT-1 PS

ANTIGEN DOSE ug	TREATMENT	RESPONSE ug/ml
1.0	NMS[a]	17.2[b]
1.0	ALS	3.6
50.0	NMS	19.1
50.0	ALS	5.2

[a] NMS - normal mouse serum

[b] represents average increase over pre-immune levels of ug/ml antibody for 5 mice

TABLE VII

Response of C3H x Balb/c (CC3) and Balb/c x C3H(CC3) F_1 mice to 1 ug of LT-I PS

STRAIN	SEX	# PFC SPLEEN	SERUM ANTIBODY ug/ml
CC3	F[a]	1432[b]	14.1[c]
CC3	M	1703	13.9
C3C	F	5420	42.4
C3C	M	7195	49.8

[a] F = female, M = male

[b] represents average number of plaque forming cells/spleen from 5 mice

[c] represents average increase in ug/ml over pre-immune level for 5 mice

have shown the existence of a "maternal" effect on the presence of basal serum levels of IgA (3), and we are currently exploring a possible relation to this effect with the immune response to our vaccine. We have recently shown this effect still holds at 6 months of age for these mice, although male F_1 mice with Balb/C mothers showed less of a depressed response than did female F_1 mice with Balb/C mothers when compared to either male or female mice with C3H mothers.

A second parameter of the genetic basis of the response of mice to the IT-1 PS antigen was explored using mice with different H-2 types and mice congenic with C3H mice except at the H-2 locus. Table VIII shows no correlation of H-2 type with response, since C3H/SW mice of a different H-2 type were good responders, whereas AKR/J and CBA/J mice, both possessing the same H-2K type as C3H/ANF mice, were poor responders.

4) Determination of protective mechanism in Balb/C mice. The poor antibody response of Balb/C mice to immunization with IT-1 PS led us to investigate if the protective mechanism following immunization was cell mediated or humoral (antibody) mediated. Passive transfer of 0.5 ml of immune Balb/C serum, was, in fact, capable of conferring protection (Table IX). Adsorption of this sera with IT-1 organisms abrogated its protection. The large amount of serum needed reflected the low level of antibody present in this sera. Passive transfer of immune Balb/C spleen cells also were effective in conferring passive protection to live organism challenge (Table X). A large number of cells had to be transferred in order to confer the protection. Non-immune spleen cells were ineffective. An attempt to define the cell type involved in this protection was undertaken by treating Balb/C spleen cells with either anti- mouse Ig and complement or anti-theta serum and complement, to kill B cells and T cells respectively. The results (Table XI) suggest that a theta sensitive cell is involved in passive protection, but unfortunately the results did not reach statistical significance. These experiments are being repeated using higher cell numbers.

An interesting and potentially useful handle for getting at the problem of cell mediated immunity in response to immunization with IT-1 PS has been discovered by the principal investigator. Balb/C mice given a sub-immunogenic (1 µg) dose of IT-1 PS and 75 µg of the drug Vinblastin are protected against challenge 7 days later with the live, homologous IT-1 P. aeruginosa organisms (Table XII). This protection is specific for IT-1 organisms (Table XII), and can be passively transferred to non-immune mice with 10^7 spleen cells. This cell number is almost a log less than the number of cells needed to confer passive protection when no Vinblastin is given (Table XI). Furthermore, we have shown in over 50 individual Balb/C mice that a 1 µg dose of IT-1 PS does not induce measurable antibody in this mouse strain, even if given with Vinblastin. Current studies are underway to determine the kinetics of Vinblastin and antigen administration in inducing this phenomenon, the cell type involved, and the mechanism of enhanced immunity following Vinblastin treatment.

TABLE VIII

Response of Various Congenic Mouse Strains
to a 1 ug dose of IT-1 PS

STRAIN	H-2 TYPE	ANTIBODY RESPONSE ug/ml
C3H/ANF	k	19.4[a]
C3H/SW	b	18.2
AKR/J	k	2.1
C57/BL	b	1.9
C57/Br	b	1.5
C58/J	k	1.0

[a] represents average increase of 5 mice in ug/ml of antibody to IT-1 PS

TABLE IX

Passive Protection of Balb/c Mice with Normal Mouse Serum (NMS), IT-1 PS Immune Mouse Serum (IMS) and IT-1 PS Immune Mouse Serum adsorbed with IT-1 P. aeruginosa (IMS-ads)

SERUM	AMOUNT (ml)	NO. SURVIVORS TOTAL	%
NMS	0.5	0/5	0
IMS	0.5	5/5	100
IMS-ads	0.5	0/5	0

TABLE X

Passive Transfer of Immunity in balb/C mice using IT-1 PS Immune Spleen Cells (ISC) and Non-Immune Spleen Cells (NISC)

CELLS	NO. CELLS	NO. SURVIVORS TOTAL	PERCENT SURVIVORS
ISC	2×10^7	2/5	40
ISC	4×10^7	2/5	40
ISC	8×10^7	5/5	100
NISC	8×10^7	0/5	0

TABLE XI

Effect of Anti Ig and C' and Anti Theta and C' on Passive Transfer of Immunity in Balb/C mice using IT-1 PS Immune Spleen Cells (ISC)

CELLS	TREATMENT	NO. CELLS	NO. SURVIVORS	PERCENT SURVIVORS
ISC	None	5×10^7	3/6	50
ISC	Anti Ig + C'	5×10^7	3/6	50
ISC	Anti Theta + C'	5×10^7	0/6	0
NISC[a]	None	5×10^7	0/6	0

[a] NISC - non-immune spleen cells

TABLE XII

Active Immunization, Specificity, and Passive Transfer with Immune Spleen Cells (ISC) and Non-Immune Spleen Cells (NISC) of Balb/C Mice given 1 µg IT-1 PS and Vinblastin (Vin)

ANTIGEN AMT. (ug)	VIN. AMT. (ug)	CHALLENGE ORGANISM (IMMUNOTYPE)	NO. SURVIVORS TOTAL	PERCENT SURVIVORS
1	75	IT-1	7/9	77.8
1	-	IT-1	0/5	0
0	75	IT-1	0/5	0
1	75	IT-2	2/5	40
0	75	IT-2	0/5	0
1	75	IT-3	1/5	20
0	75	IT-3	0/5	0
1	75	IT-4	2/5	40
0	75	IT-4	0/5	0
1	75	IT-6	0/5	0
0	75	IT-6	1/5	20
ISC[a]	1×10^7	IT-1	10/10	100
NISC[a]	1×10^7	IT-1	2/10	20

[a] ISC - immune spleen cells; NISC - non-immune spleen cells

5) Basis of responsiveness of mice to LPS immunization. IT-1 PS and LPS share common serologic determinants, making it difficult to preclude the occurrence of LPS in PS preparations as being responsible for the PS' immunogenicity. This determination is further hampered by the very low amounts of P. aeruginosa LPS needed to induce an immune response in mice (1). We therefore attempted to use C3H/HeJ mice, which are genetically unresponsive to LPS from enterobacteriaceae, as a means of measuring the occurrence of LPS in PS preparations. Our results are presented in the attached manuscript, which has been submitted to the Journal of Immunology. Briefly, we found C3H/HeJ mice to be responsive to the toxic, immunogenic and mitogenic properties of P. aeruginosa LPS, thus making these mice untenable experimental tools for the assessment of P. aeruginosa LPS contamination of our PS. We therefore turned to Balb/C mice to get at this problem. Balb/C mice are excellent responders with antibody to P. aeruginosa LPS (Table XIII). We therefore made a mixture of 0.01 µg LPS with 100 µg of PS and gave this to Balb/C mice. Table XIII shows that mice given 0.01 µg of LPS had an average percentage binding of 81.4% in the RABA 5 days following immunization while mice given 100 µg of IT-1 PS only had a binding of 5.0%. Mice given the mixture of PS and LPS had a percentage binding of 37.0, lower than that of the LPS alone but significantly higher than that seen with the PS alone. These data indicate that there is at least less than 1 part in 10,000 of LPS in PS preparations. Further support comes from the data in Table XIV which describes the dose response curve of C3H/ANF mice to IT-1 PS and LPS. These mice require higher doses of LPS (about 0.05 µg) to make good antibody response to LPS. Lower doses are ineffective. These mice also show a good response to PS at 1 µg. The requirement of 5% as much LPS as PS for an immune response in these mice is well within our capabilities of detecting LPS in PS, and since chemical studies indicate less than 1 part in 1,000 of LPS in PS, we feel confident that the immune response seen in mice to PS is not due to LPS contamination.

F. Progress on in vitro Cellular Studies

In vitro studies on the ability of IT-1 PS to stimulate mitogenisis in spleen cell cultures were undertaken. Table XV shows the dose response curve of spleen cells, measuring the incorporation of tritiated thymidine, to IT-1 PS and LPS. PS was found to be mitogenic for spleen cells from C3H mice, but required about 10 times the amount of LPS needed to produce maximal mitogenesis. Treatment of these spleen cells with either anti Ig or anti-theta sera and complement (C') knocked out the PS mitogenicity, whereas treatment with anti-Ig only knocked out the LPS mitogenicity. Treatment with anti-theta sera and C' knocked out the Con A induced mitogenicity (Table XVI). The loss of PS mitogenicity by knocking out either B (anti-Ig treated) or T (anti-theta treated) cells suggests that PS is a B cell mitogen requiring T cell help for this. The B cell mitogenicity of P. aeruginosa LPS is consistent with the reports on the mitogenicity of other LPS' (4), and the data on the Con A control indicates that the T cell mitogenesis effect was indeed lost following anti-theta treatment.

TABLE XIII

Immune Response of Balb/C Mice to Immunization with IT-1 LPS, PS, and a Mixture of 0.01 µg IT-1 LPS in 100 µg IT-1 PS

ANTIGEN	AMT. (µg)	PERCENTAGE BINDING IN THE RABA
LPS	0.01	81.4
LPS	1.0	69.2
PS	50.0	5.0
LPS + PS	0.01 + 100	37.0

TABLE XIV

Dose Response Curve of C3H/ANF Mice to Immunization with IT-1 LPS and IT-1 PS

ANTIGEN	DOSE (µg)	µg/ml ANTIBODY
PS	0.5	< 2.0[a]
PS	1.0	13.6
PS	50.0	15.5
LPS	0.01	< 2.0
LPS	0.05	5.5
LPS	0.50	31.7
LPS	1.0	44.1

[a] represents µg/ml of antibody over pre-immune levels from an average of 5 mice

TABLE XV

Mitogenic Response of C3H/ANF Mouse Spleen Cells to Various Doses of IT-1 PS and IT-1 LPS

ANTIGEN	AMT. (µg/ml)	CPM OF H^3 INCORPORATED
PS	5	1,692
PS	10	1,697
PS	50	14,306
LPS	0.5	8,332
LPS	2.5	19,040
LPS	5.0	18,347
LPS	25.0	24,332
LPS	50.0	23,823
LPS	100.0	23,817

TABLE XVI

Effect of Treatment of C3H/ANF Mouse Spleen Cells with Anti Ig or Anti Theta and C' on Mitogenicity of IT-1 PS, IT-1 LPS and Concanavilin A

TREATMENT	MITOGEN	DOSE µg/ml	CPM H^3 INCORPORATED
---	IT-1 PS	50	14,000
Anti Ig + C'	IT-1 PS	50	107
Anti Theta + C'	IT-1 PS	50	39
---	IT-1 LPS	25	37,000
Anti Ig + C'	IT-1 LPS	25	600
Anti Theta + C'	IT-1 LPS	25	4,000
---	Con A	1	99,000
Anti Ig + C'	Con A	1	6,000
Anti Theta + C'	Con A	1	679

TABLE XVII

Protection of Burned Mice by IT-1 PS Vaccination

IT-1 PS AMT. (ug)	MOUSE STATUS	NO. SURVIVORS TOTAL	PERCENT SURVIVAL
0	Not burned	0/5	0
50	Not burned	5/5	100
0	Burned	0/5	0
1	Burned	0/5[a]	0
50	Burned	0/5[a]	0

[a] in these groups there was 60% survival at 48 hr. as compared to 100% mortality at this time in the group given no antigen. However, all mice were dead by 5 days in these groups.

G. Progress on PS Vaccine Efficacy in Animal Models.

We have attempted to assay the efficacy of the PS vaccine in a burned mouse model and in preventing pneumonia related death in guinea pigs. Dr. I.A. Holder of the Shriners Burn Institute in Cincinnati, Ohio, tested a PS preparation we made from his mouse strain M-2 of P. aeruginosa in burned mice. Although this preparation had good efficacy in protecting non-burned animals (Table XVII), it only slightly prolonged the time to death when tested in burned animals. Since we are not studying M-2 PS but IT-1 PS, we have sent IT-1 PS to Dr. Matt Pollock of the Uniformed Services University of the Health Sciences for testing in burned animals. Results are pending.

Dr. Jim Pennington here at Harvard Medical School has been testing the IT-1 PS in guinea pigs as a means of protecting against a P. aeruginosa induced pneumonia. The PS, however, is totally non-immunogenic in guinea pigs, as measured by humoral immunity, and therefore has not proven effective. However, IT-1 LPS, which is serologically similar to IT-1 PS, is effective in producing protection in guinea pigs. We therefore feel that the lack of immunogenicity of PS in guinea pigs is the problem here, and not the efficacy of antibody directed to the PS determinants. Guinea pigs are known poor responders to polysaccharide antigens, and their lack of response to doses as high as 1 mg of IT-1 PS attests to the lack of LPS in this vaccine. We are currently attempting to raise a high titered rabbit antisera to IT-1 PS for use in passive transfer studies of guinea pig pneumonia.

References

1. Pier, G.B., H.F. Sidberry, S. Zolyomi and J.C. Sadoff. 1978. Isolation and characterization of a high-molecular-weight polysaccharide from the slime of Pseudomonas aeruginosa. Infect. Immun. 22:908-918.

2. Amsbaugh, D.F., B. Prescott and P.J. Baker. 1978. Effect of splenectomy on the expression of regulatory T cell activity. J. Immunol. 121:1483-1485.

3. Popp, D.M. 1979. Basal serum immunoglobulin levels II. Interaction of genetic loci and maternal effect on regulation of IgA. Immunogen 9:281-292.

4. Glode, L.M., I. Scher, B. Osborne and D.L. Rosenstreich. 1976. Cellular mechanism of endotoxin unresponsiveness in C3H/HeJ mice. J. Immunol. 116:454-461.

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