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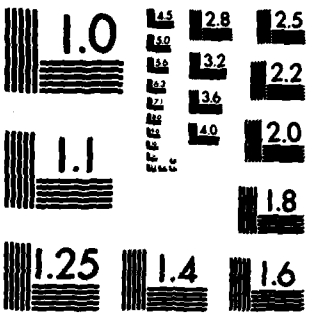

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Pseudomonas Flagella Vaccine in Burns (Trauma)

ANNUAL SUMMARY REPORT

Thomas C. Montie, Professor of Microbiology

September 1, 1984

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

Contract No. DAMD 17-82-C-2202

University of Tennessee
Department of Microbiology
Knoxville, Tennessee 37996

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188
Exp. Date: Jun 30, 1986

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approval 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 University of Tennessee 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) Knoxville, Tennessee 37996	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. Army Medical Research and Development Command 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAMD17-82-C-2202
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick, Frederick, MD 21701-5012	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 62772A; PROJECT NO. 3S162. 772A874; TASK NO. AD; WORK UNIT ACCESSION NO. 152

11 TITLE (Include Security Classification)

(U) Pseudomonas Flagella Vaccine in Burns (Trauma)

12. PERSONAL AUTHOR(S)
Thomas C. Montie

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual	FROM 15 Jul 83 TO 14 Jul 84	1 September 1984	

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		
06	11		

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

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION
22a. NAME OF RESPONSIBLE INDIVIDUAL Mrs. Virginia Miller	22b. TELEPHONE (Include Area Code) 301-663-7325 22c. OFFICE SYMBOL SGRD-RMS

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

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 USP of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW) Publication No. (NIH) 78-23, Revised 1978).

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Abstract

As indicated in our previous annual report our contract goals are directed toward: a) establishing the capacity of a Pseudomonas aeruginosa flagellar vaccine to protect burned rodents both actively and passively and b) understanding the importance of motility as a virulence factor in the burns animal models available. Progress has been made in isolating flagellar protein away from contaminating lipopolysaccharide. Experiments are underway to isolate large amounts of this material to establish the most efficient purification procedures. Carefully adsorbed antisera, anti-M-2 b type as well as a type, have been prepared. Contaminating lipopolysaccharide antibody was successfully eliminated because the antisera gave complete protection against challenge corresponding to the homologous flagellar antigenic type and not the O type or heterologous H type. Preliminary experiments indicate passive protection can be obtained against topical challenge. Experiments concerned with virulence mechanisms using four Fla^- isogenic mutants and a Mot^- genetically constructed mutant, support the hypothesis that a functionally active flagellum is required for maximum virulence. Virulence was compared using three different challenge sites, subcutaneous, topical and intraperitoneal. Depending on the mutant and the inoculation site, decreases in virulence from two to five logs were recorded. Data from sequential tissue assays following challenge with the mutants and in protection experiments indicate that motility is particularly initial in the transition from skin colonization to the circulatory system. A group of selected cystic fibrosis isolates selected for deficiencies in presumed invasive virulence factors, including motility, protease production and LPS deficiencies, show degrees of avirulence corresponding to the number of factors that are deficient.

Annual Report

A. Virulence Mechanisms

1) Assay of tissue counts in burned mice; assay modification and delineation.

Recently, we have modified the burned mouse model of Stieritz and Holder (1) to avoid use of an asbestos template to delineate the alcohol burn (BMM). We have substituted a white teflon template of 2 mm thickness. Under these conditions mice were somewhat more resistant to our standard burns organism, strain M-2. It was found that mice burned for 13 s instead of 10, survived at the 100% level, however, and were equally as susceptible, LD_{50} = 5.4 cells in CF-1 mice. Experiments with HA/ICR mice proved to be somewhat less definitive. This was particularly apparent when these mice were obtained from Cumberland View Farms. Therefore, all experiments are now performed using female CF-1 mice obtained from Charles River Co.

Detailed comparisons of the lethality of the M-2 and its isogenic ethyl methane sulfonate (EMS) mutant (M-2 Fla^-) have been made (2) and repeated in the 13 s sc. model. This strain, in keeping with previous results (2), gave an LD_{50} approximately four logs above the parent M-2.

Tissue counts of strain M-2 Fla^+ sc challenged mice, containing the 13 s burn, gave the following infection sequence following 10^2 challenge (Figure 1). Comparisons were made with the EMS Fla^- isogenic strain. Data in Fig. 1 showed that after M-2 Fla^+ 10^2 sc inoculation, the typical colonization of mouse burned skin was followed by rapid bacterial entry (within 30 h) into the blood and liver. In contrast Fla^- inoculation sc into burned skin showed relatively high skin colonization (10^7 CFU), but invasion into the liver was delayed and transient with no bacteremia. This response is consistent with the lower virulence of these strains.

Experiments were performed to test if the type of inoculation would effect the relative virulence of the two strains when challenged intraperitoneally. A difference of approximately one log was seen (Table 1). These same strains were compared following topical challenge. Suspensions (10^8 CFU) were pipetted onto the surface of the burn. The liquid was observed to be entirely absorbed into the 13 s burn area. Consistent with the above experiments the Fla^- strain showed reduced virulence of one to two logs Table 2.

The indication from these experiments is that the anatomical difference between the Fla^+ and Fla^- strain is more critical for virulence when the strains are compared by skin inoculation. This suggests that penetration and entry into the circulatory system is aided by the presence of an intact flagellum.

Characterizations of other potentially isogenic strains obtained by EMS mutation from Dr. A. Koprinski, are summarized in Table 3. These AK strains showed approximately the same proteolytic activity, and were the same O antigen type as the PAO-1 parent. Growth rates are also similar and other physiological comparisons are being made. Table 4 gives the comparative virulence data for these strains following subcutaneous challenge. The PAO-1 wild type is virulent at a level below 100 CFU. The huge loss in virulence for these strains is apparent. No killing was observed until a level of 10^7 CFU was reached, a difference of >5 logs compared to the parent.

Another isogenic comparison has been made with a transduction, isogenic motility mutant, 1200-80 (fla^+, mot^-). This strain was constructed genetically from the parent Mt 1200 (fla^+, mot^+) by Dr. Tsuda and Iino and reveals a cell that contains a straight flagellum as visualized in the EM. This flagellum is not functionally operative. This aberration has been mapped on the chromosome (3). A reduction in lethality of approximately two logs was seen with the motility mutant (Table 5). This is the first time that a genetically

defined motility mutant has been tested. Intraperitoneal challenge in the burned mouse gave a one log reduction in the mutant compared to Mt 1200.

We have been interested in examining a number of Pseudomonas strains in the BMM in an effort to determine on a broader scale whether a deletion of a number of virulence factors could result in severely reduced virulence. Further, could these factors be more quantitatively additive, or show some rough correlation between number of factors absent, and percent reduction in virulence. A first step in this direction has presented itself, since we have observed a loss of certain physiological characteristics and virulence in cystic fibrosis P. aeruginosa isolates, particularly those from patients in poor clinical conditions. These results are outlined as follows.

Twenty CF P. aeruginosa strains were serotyped using viable cells in a slide agglutination assay (Table 6). Only two of the rough strains (86f, 415 gg) were typable, a characteristic associated with intact LPS (4). Of the four rough strains which were non-typable, only one, 572b, was from a patient in good clinical condition (G strains). Forty-five per cent of the rough strains polyagglutinated with two or more antisera. One third of the classic strains tested were typable. The majority (55%) of the classical strains were polyagglutinable. However, in contrast to rough strains, only one classic strain, 541a, was non-typable. Of the twenty strains tested, 75% were either polyagglutinable or non-typable. Regardless of patient clinical condition, or colonial morphology, some type of LPS alteration is present in the majority of strains.

Thirty-two strains were screened for proteolytic activity in skim milk plates, an assay which detects both alkaline protease and elastase. Results in Table 7 show that 12 strains demonstrated proteolytic activity comparable to M-2. Nine of these 12 were G strains. In sharp contast, 13 strains demonstrated no proteolytic activity. Seventy percent of these non-proteolytic strains were from patints in poor clinical condition (P strains).

The chemotaxis of CF P. aeruginosa strains toward amino acids has recently been reported (M.A. Luzar et al., Inf. Immun., manuscript submitted). Seventy-five percent of both classic and rough G strains tested were similar to M-2 in their tactic response to amino acids. In contrast, the majority of P strains showed reduced taxis to one or more attractants.

P. aeruginosa is avirulent in normal mice, but extremely lethal in those receiving thermal trauma. In the burned mouse model developed by Stieritz and Holder (1) the LD_{50} for M-2 is 1.5×10^1 CFU following subcutaneous inoculation into the burn site of HA/ICR mice. A comparison of relative virulence revealed significant differences between M-2 and CF P. aeruginosa strains (Table 8). Injection of 310 CFU of M-2 caused 100% mortality in HA/ICR mice by day three. Of the twenty-three CF strains examined, only 35c demonstrated virulence approaching that of M-2 (3.2×10^4 CFU of 35c caused 80% mortality). Injections ranging from 3.5×10^3 CFU to 6.1×10^5 CFU caused 0-20% mortality in 97% of the strains tested.

A series of varied morphological types of CF sputum strains were then examined for virulence in the burned mouse model using CF-1 mice (Table 9). Morphological types tested were smooth and rough strains from patients in good and poor clinical conditions. One mucoid strain was also tested. Of the ten CF strains examined, 35c and 903a resembled M-2, being of classic morphology, proteolytic and O-antigen typable. They also gave the lowest LD_{50}s, 10^4 CFU or below. Strains 35c was the most virulent of the ten strains giving an LD_{50} of 8×10^2 CFU. Strains, 412a and 903a were intermediate in virulence (LD_{50} = 10^4 CFU). Avirulence of the other strains appeared to be associated with the loss of one or more important physiological characteristics reported to be associated with virulence. An LD_{50} of above 10^6 CFU resulted when two or more virulence factors were absent. For example,

strains such as 86f, 409g, 144b, 402c and 776e gave no deaths at 10^6 CFU. These strains exhibited a corresponding loss of a combination of factors such as proteolytic activity, motility-chemotaxis and appropriate O-antigen typing reflecting some alteration in lipopolysaccharide.

B. Protection Studies

Lethality data taken together with tissue colonization experiments indicate that bacterial flagellar movement (motility) is important in the infectious process by P. aeruginosa in acute burn infections. These data remain consistent with not only previous protection experiments using flagellar preparations (5, 6), but also recent data obtained using flagellar H-specific antisera in passive protection experiments to be described below. The published experiments indicated that active protection is H antigen specific (6). We have also reported results using chemical and physical treatments of flagellar antigen that demonstrate that contaminating LPS can not account for active protection. Protection by a protein component is indicated (U.S. Army Annual Report, 1983). Further experimentation is planned with flagellar antigen separated from contaminant LPS by a detergent column technique. The data to date underline the important potential of a flagellar vaccine for clinical use in high-risk patients.

To more quickly obtain utilizeable flagellar antiserum, hyperimmune rabbit antisera was prepared using partially purified flagella from strain M-2. The antisera was sequentially adsorbed with heated strain M-2 bacteria to remove only LPS antibodies. The H titre was 1:8,192. This antisera was diluted with saline 1:100 before use in the BMM.

Data tabulated in Table 10 show the protection obtained with 0.5 ml antiserum, injected ip., one day prior to challenge. Strain M-2 and a virulent shriners burn isolate (SBI-I), were used as challenge strains at a level of approximately 7 LD_{50}s. SBI-I has the same H antigen (b type) as M-2 but a

different O type. Normal rabbit antiserum was used as a control. Complete protection against both strains was obtained with the hyperimmune antiserum but none was seen in normal controls.

An important extension of this approach is summarized in Table 11. Conditions were the same as described in Table 10, except challenge was by flagellar antigen a type strains 1210 and GNB-1 (see 1983 U.S. Army Annual Report). The striking finding was that no protection was found against strains having different H antigen type. This was in complete contrast to results shown in Table 11. Strain 1210 is an $a_0a_1a_2$ flagellar type and GNB-1 is an a_0a_2 type. Both have different O antigens from M-2.

Very preliminary results are presented in Table 12 illustrating an attempt to protect against topical challenge in the model with 3×10^9 CFU. Antiserum was applied one day pre-burn and one day post-burn challenge with M-2. Delay to death and partial protection (40%) was obtained only in the M-2 antiserum immunized mice. Further adjustment of antiserum dosage and timing of application seems to be required to achieve maximum protection.

Another related passive protection experiment should be noted here. In this experiment conditions were as in Table 10 (usual conditions), but following challenge with M-2, tissue samples were assayed (Figure 2) as in previous immunization experiments (6). The pattern of protection after 24 h was similar to that found in earlier active immunization experiments, that is, liver counts were in the 10^2 - 10^3 range. However, blood counts were zero. One day controls (non-immunized) are included for comparison. An unusual feature is that we observed, for the first time in protection experiments, a two log reduction in skin counts at day one. These results suggest that passive protection may involve some action at the skin colonization site.

In summary then, in the traumatized host, we see the role of motility (and probably chemotaxis) most important at the invasion phase of entry into the

circulatory system. The presence of proteases (particularly elastase), phospholipases, and a "pot pourri" of factors (aggressins) also are of particular importance in the early infection stage. Other toxins and enzymes aid in bacterial cell survival (eg. leukocyte destruction). Once established in the liver, exotoxin A (among other unknown toxins and proteases) promotes lethality. Our approach with a flagellar vaccine therefore, is to prevent the bactermic step, and possibly with a passive treatment earlier skin colonization might be blunted.

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Appendix

List of Tables

Table 1. Virulence of two *Pseudomonas aeruginosa* strains differing in the Fla phenotype challenged intraperitoneally.

		Cumulative Mortalities (Died/Total Inoc.) Days Post-Burn						
Strain	Challenge Inocula (CFU)	1	2	3	4	5	6	7
M-2 (Fla^+-WT Parent)	2.8×10^7	10/10	-	-	-	-	-	-
	2.8×10^6	1/10	10/10	-	-	-	-	-
LD_{50} 10^4 CFU	2.8×10^5	0/10	8/10	10/10	-	-	-	-
	2.8×10^4	0/10	6/10	7/10	7/10	7/10	7/10	7/10
	2.8×10^3	0/10	1/10	1/10	1/10	1/10	1/10	1/10
	2.8×10^2	0/10	0/10	0/10	0/10	0/10	0/10	0/10
M-2 (Fla^--EMS Mutant)	3.3×10^7	10/10	-	-	-	-	-	-
	3.3×10^6	2/10	9/10	10/10	-	-	-	-
LD_{50} 10^5 CFU	3.3×10^5	0/10	5/10	6/10	6/10	6/10	6/10	6/10
	3.3×10^4	0/10	1/10	3/10	3/10	3/10	3/10	3/10
	3.3×10^3	0/10	0/10	0/10	1/10	1/10	1/10	1/10
	3.3×10^2	0/10	0/10	0/10	0/10	0/10	0/10	0/10

Table 2. Virulence of two *Pseudomonas aeruginosa* strains differing in the Fla phenotype, challenged topically.

		Cumulative Mortalities (Died/Total Inoc.) Days Post-Burn						
Strain	Challenge Inocula (CFU)	1	2	3	4	5	6	7
M-2 (Fla^+-WT Parent)	2.75×10^9	0/10	0/10	8/10	10/10	-	-	-
	2.75×10^8	0/10	1/10	7/10	7/10	8/10	8/10	8/10
LD_{50} 2×10^7 CFU	2.75×10^7	0/10	1/10	3/10	5/10	5/10	5/10	6/10
	2.75×10^6	0/10	1/10	2/10	2/10	3/10	4/10	4/10
M-2 (Fla^-, EMS Mutant)	2.13×10^9	0/10	0/10	0/10	3/10	4/10	4/10	4/10
LD_{50} 10^9 CFU	2.13×10^8	0/10	0/10	0/10	1/10	1/10	3/10	4/10
	2.13×10^7	0/9	0/9	0/9	0/9	0/9	0/9	0/9

Table 3. Characteristics of parent and corresponding motility mutants

Strain	Phenotype	Proteolytic Activity (MM)[a]	O-Antigen Type[b]
PAO-1	Fla^+ Mot^+	8.6	5
AK 1152	Fla^- Mot^-	7.7	5
AK 1153	Fla^- Mot^-	7.9	5
MT 1200	Fla^+ Mot^+	8.4	5
MT 1200-80	Fla^+ Mot^-	11.0	5

[a]Zone of clearance measured in millimeters of colonies on skim milk agar plates

[b]Slide agglutination assay using Difco-LATS Serotyping Kit

Note: Growth rates of all strains were similar in complex media

Table 4. Virulence of three *Pseudomonas aeruginosa* strains differing in the Fla phenotype challenged subcutaneously.

		Cumulative Mortalities (Died/Total Inoc.) Days Post-Burn						
Strain	Challenge Inocula (CFU)	1	2	3	4	5	6	7
PAO-1 (Fla^+ WT-Parent)	5.3×10^3	1/5	5/5	-	-	-	-	-
	5.3×10^2	0/5	4/5	4/5	4/5	4/5	4/5	4/5
LD_{50} 10^2 CFU	5.3×10^1	0/5	1/5	2/5	2/5	2/5	2/5	2/5
AK 1152 (Fla^-, EMS-mutant)	8.3×10^6	0/5	0/5	0/5	1/5	1/5	1/5	1/5
	8.3×10^5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
$LD_{50} > 10^7$ CFU	8.3×10^4	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	8.3×10^3	0/5	0/5	0/5	0/5	0/5	0/5	0/5
AK 1153 (Fla^-, EMS-Mutant)	1.1×10^7	0/5	1/5	1/5	1/5	1/5	1/5	1/5
	1.1×10^6	0/5	0/5	0/5	0/5	0/5	0/5	0/5
$LD_{50} > 10^7$ CFU	1.1×10^5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Controls	0.85% Saline	0/15	0/15	0/15	0/15	0/15	0/15	0/15

Table 5. Virulence of two Pseudomonas aeruginosa strains differing in the Mot phenotype challenged subcutaneously.

Strain	Challenge Inocula (CFU)	Cumulative Mortalities (Died/Total Inoc.) Days Post-Burn 1	2	3	4	5	6	7
MT 1200 (Fla^+ Mot^+)	6.2×10^5	0/10	6/10	10/10	-	-	-	-
	6.2×10^4	0/10	4/10	7/10	7/10	8/10	8/10	8/10
LD_{50} 6×10^3 CFU	6.2×10^3	0/10	1/10	3/10	4/10	4/10	5/10	5/10
	6.2×10^2	0/10	0/10	0/10	1/10	1/10	1/10	1/10
MT 1200-80 (Fla^+ Mot^-)	9.4×10^4	0/10	0/10	0/10	0/10	0/10	0/10	0/10
$LD_{50} > 10^5$ CFU	9.4×10^3	0/9	0/9	0/9	0/9	0/9	0/9	0/9
	9.4×10^2	0/10	0/10	0/10	0/10	0/10	0/10	0/10

Table 6. O antigen serotypes of P. aeruginosa strains from cystic fibrosis patients.[a]

Strain	Clinical condition	Colonial morphology[b]	Serotype[c]
86f	C	R	10
415gg	P	R	11
572b	C	R	NT
414mm	P	R	NT
414ii	P	R	NT
402c	P	R	NT
96e	P	R	PA
409g	P	R	PA
66g	P	R	PA
412oo	C	R	PA
320f	C	R	PA
412a	C	M	PA
35c	C	C	6
435c	C	C	PA
903a	C	C	4
902c	C	C	PA
144b	P	C	6
776e	P	C	PA
676e	P	C	PA
541a	P	C	NT
M-2	Invasive burn strain	C	5

[a]Slide agglutination assay using viable cells and O-antisera (Difco-American Scientific) diluted 1:10.
[b]C, classic; R, rough; M, mucoid.
[c]NT, non-typable, no agglutination in any typing sera; PA, polyagglutinable, agglutinated by two or more typing sera.

Table 7. Proteolytic activity of *P. aeruginosa* CF strains.

Strains[a,c]	Zone of Clearing[b]		
	+	+/-	-
Total	12/32	7/32	13/32
Good	9/32	3/32	4/32
Poor	3/32	4/32	9/32

[a]Each strain was tested at least three times.
[b]Zone of clearing (radius) on skim milk plate measured at 24 h: + $\geq$ 7 mm, +/- $\leq$ 3 mm, - = 0 mm.
[c]For each assay CF strains were compared to proteolytic burn strain M-2 (zone of clearing $\geq$ 7 mm).

Table 8. Comparison of lethality of *P. aeruginosa* CF strains in the burned mouse model (HA/ICR mice).

Strain	Dose[a]	% Lethality[b]
M-2(control)	3.1×10^2	100
437e$_2$	3.9×10^5	0
572b	4.1×10^4	20
86f	6.1×10^5	0
412oo	5.5×10^3	0
320f	3.5×10^3	20
435c	1.5×10^4	0
903e	4.6×10^3	20
35c	3.2×10^4	80
902c	5.1×10^4	0
412a	8.2×10^3	40
572d	5.6×10^4	0
402c	2.7×10^4	0
409g	3.8×10^4	0
96e	5.3×10^4	0
66g	6.2×10^4	0
415gg	7.2×10^3	0
414ii	3.8×10^4	20
414mm	4.7×10^4	20
448hh	9.3×10^3	0
341a	3.7×10^4	0
144b	3.8×10^5	20
776e	6.7×10^4	0
676e	4.6×10^4	0

[a]Subcutaneous injection of bacteria (in 0.1 ml buffer) into burn site.
[b]Lethality of a group of five mice. The number of deaths of a group of mice was recorded for seven days.

Table 9. Comparison of lethality of P. aeruginosa strains in the burned mouse model (CF-1 mice).

Strain[a]	Colonial Morphology[b]	O-antigen type[c]	Proteolytic activity[d]	% Motility[e]	% Arginine Chemotaxis[e]	LD_{50}[f]
M-2	C	5	+	100	100	1.5×10^1
35c (G)	C	6	+	58	126	8.4×10^2
903a (G)	C	4	+	106	57	1×10^4
412a (G)	M	PA	+	59	59	3.6×10^4
572b (G)	R	NT	+	127	94	7.1×10^6
320f (G)	R	PA	-	45	31	1.5×10^6
86f (G)	R	10	+/-	216	107	$> 4.0 \times 10^6$
409g (P)	R	NT	-	64	54	$> 6.8 \times 10^6$
144b (P)	C	6	-	67	17	$> 6.2 \times 10^6$
402c (P)	R	NT	-	48	13	$> 6.5 \times 10^6$
776e (P)	C	PA	+	98	46	$> 2.4 \times 10^6$

[a]All strains possess flagella as determined by electron microscopy.
[b]C, classic; M, mucoid; R, rough.
[c]NT, non-typable, PA, polyagglutinable.
[d]Zone of clearing in skim milk plates measured at 24 h (see Table 2).
[e]Compared to M-2 (100%) in capillary assay.
[f]Lethality of a group of eight mice following subcutaneous injection of bacteria (in 0.1 ml buffer) into burn site; four doses per group of mice. Strains 86f, 409g, 144b, 402c and 776e demonstrated 0% mortality after seven days at 10^6 CFU.

Table 10. Homologous protection using b type H specific antisera to challenge with flagellar b type organisms.[a]

Mice	Challenge[b] (CFU)	Cumulative Mortalities (Died/Total Inoc.) Days Post-Burn 1	2	3	4	5	6	7
Non-immunized	M-2 (1.43×10^2)	0/8	5/8	6/8	-	-	-	-
Non-immunized	SBI-I (4.0×10^4)	0/8	6/8	7/8	-	-	-	-
Immunized with NRS	M-2 (1.4×10^2)	0/8	6/8	8/8	-	-	-	-
Immunized with NRS	SBI-I (4.0×10^4)	0/8	7/8	7/8	7/8	7/8	7/8	8/8
Immunized with M-2 H antisera	M-2 (1.43×10^2)	0/8[c]	-	-	-	-	-	-
Immunized with M-2 H antisera	SBI-I (4.0×10^4)	0/8[c]	-	-	-	-	-	-

[a]Antisera (0.5 ml, ip.) injected one day prior to burning
[b]Subcutaneous
[c]Day 15 = 0/8

Table 11. Cross-challenge using M-2 b type H specific antisera and challenging with flagellar a type organisms.

		Cumulative Mortalities (Died/Total Inoc.)						
		Days Post-Burn						
Mice	Challenge[b] (CFU)	1	2	3	4	5	6	7
Non-immunized	1210 (7.9×10^3)	2/8	8/8	-	-	-	-	-
Non-immunized	GNB-1 (4.0×10^4)	0/8	6/8	8/8	-	-	-	-
Immunized with NRS	1210 (7.9×10^3)	0/8	8/8	-	-	-	-	-
Immunized with NRS	GNB-1 (4.0×10^4)	1/8	6/8	8/8	-	-	-	-
Immunized with M-2 H antisera	1210 (7.9×10^3)	0/8	7/8	8/8	-	-	-	-
Immunized with M-2 H antisera	GNB-1 (4.0×10^4)[c]	0/8	5/8	7/8[b]	-	-	-	-

[a]Antisera (0.5 ml, i.p.) injected one day prior to burning
[b]Subcutaneous
[c]Day 12 = 7/8

Table 12. Homologous challenge using b type H antisera and challenging topically with flagellar b type organisms[a]

		Cumulative Mortalities (Died/Total Inoc.)						
		Days Post-Burn						
Mice	Challenge (CFU)	1	2	3	4	5	6	7
Non-immunized	M-2 (1.11×10^3 s.c.)	0/8	6/8	8/8	-	-	-	-
Non-immunized	M-2 (2.78×10^9)[b]	0/8	0/8	6/8	6/8	7/8	-	-
Immunized with NRS	M-2 (2.78×10^9)[b]	0/8	0/8	5/8	7/8	-	-	-
Immunized with M-2 H antisera	M-2 (2.78×10^9)[b]	0/8	0/8	3/8	3/8	3/8	3/8	5/8

[a]O antisera (0.5 ml) injected i.p. one day prior to burning and one day post burn.

[b]Topical applications

FIGURES

FIGURE 1. Quantitative bacteriology of skin, liver, and blood after subcutaneous challenge with M-2 or M-2 Fla⁻.

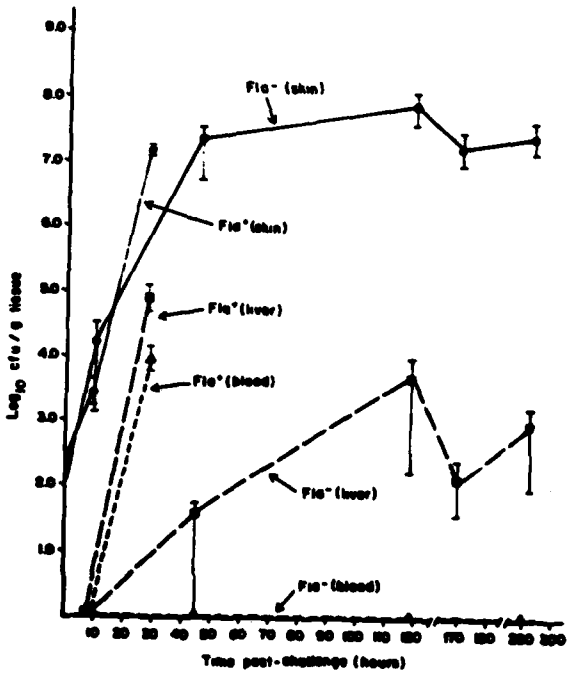
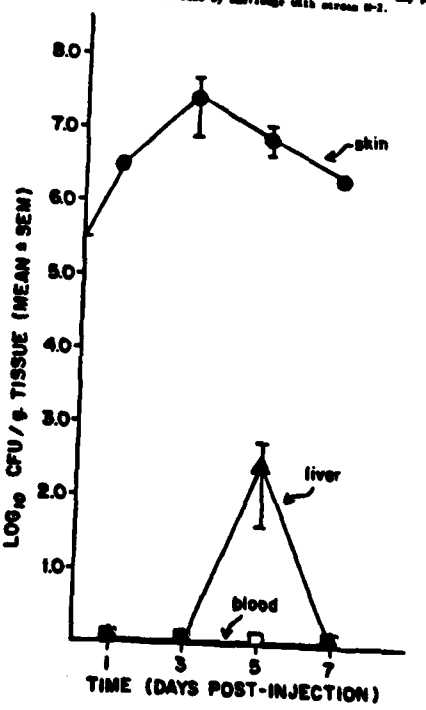
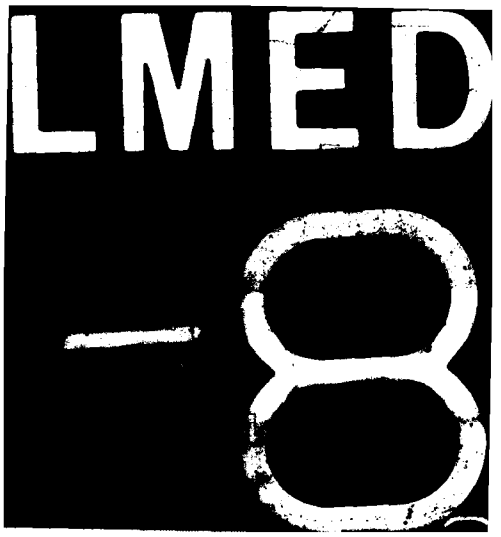


FIGURE 2. Quantitative bacteriology of skin, liver, and blood after administration of anti-M-2 antisera (1 day prior to challenge) followed by challenge with strain M-2.

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