APPLIED MICROBIOLOGY, July 1971, p. 84-88

Vol. 22, No. 1
Printed in U.S.A.

Immunogenicity of Plague Vaccines in Mice and Guinea Pigs

E. VON METZ, D. M. EISLER, AND G. A. HOTTLE

University of California, School of Public Health, Naval Biomedical Research Laboratory, Berkeley, California 94720

Received for publication 25 January 1971

The median effective doses (ED_{50}) of 28 lots of killed *Pasteurella pestis* strain 195/P vaccine were determined in mice and guinea pigs. Mice were injected with vaccine alone, whereas guinea pigs received vaccine suspended in incomplete Freund's adjuvant. Potency ratios of vaccines were obtained by comparing the ED_{50} of the test with that of a reference vaccine. Mean potency ratios of 1.82 ± 0.50 in mice and 3.22 ± 0.56 in guinea pigs were obtained, and the difference between these means was significant, $P = <0.01$. The number of organisms in the challenge dose did not significantly affect the ED_{50} of a vaccine in guinea pigs. However, irrespective of vaccinating route, nearly 1,000 times as much vaccine was required in the absence of adjuvant as in its presence to produce comparable protective indexes in the guinea pig. The response of guinea pigs did not offer any improvement over mice in evaluating the efficacy of plague vaccines.

This laboratory has evaluated commercially prepared vaccines for several years. In our efforts to improve the test, a comparison of immunity in guinea pigs and mice was conducted. The results from this study form the basis of this report.

Although it was thought (2) that killed plague bacilli were relatively nonimmunogenic in guinea pigs, Keppie, Cocking, and Smith (3) and subsequently Chen, Foster, and Meyer (1) showed that in fact guinea pigs could be immunized with various antigens of *Pasteurella pestis* as well as the whole organism. We compared the potencies of formaldehyde-killed whole organism plague vaccines in mice and guinea pigs by methods outlined by Chen, Foster, and Meyer (1).

MATERIALS AND METHODS

Vaccines. The plague vaccines were prepared by Cutter Laboratories as described by Chen et al. (1). They consisted of different lots of formaldehyde-killed suspensions of *P. pestis* strain 195/P containing 2×10^9 particles per ml, with phenol as preservative.

A lyophilized reference standard vaccine was supplied by the Division of Biologics Standards, National Institutes of Health, Bethesda, Md., and was stored at 4 C. Samples were resuspended in 0.85% sodium chloride (saline) solution for testing. Each experimental vaccine was evaluated by comparison with the reference standard assayed concurrently.

Immunogenicity tests in guinea pigs. Hartley strain guinea pigs weighing 350 to 500 g were used in the trials. Groups of 16 animals each were injected intramuscularly with 0.5 ml of a mixture of saline-diluted vaccine emulsified in an equal volume of incomplete Freund's adjuvant (1). The adjuvant consisted of 9 parts of Drakeol 6 VR (light mineral oil, Pennsylvania Refining Co., Butler, Pa.) and 1 part of Arlacel A (mannide monoleate) obtained from Hill Top Laboratories, Inc., Cincinnati, Ohio. Three weeks later each animal was challenged subcutaneously with *P. pestis* strain 195/P [0.85×10^6 to 3.2×10^6 colony-forming units (CFU)]. In some experiments, dilutions of vaccine without adjuvant were injected intramuscularly or intraperitoneally. Control animals receiving adjuvant or saline solution without vaccine were included with each experiment. The animals were observed for 3 weeks after challenge.

Immunogenicity tests in mice. The potency test was performed as described by the U.S. Public Health Service (7). Vaccines were diluted in saline solution and two doses (0.2 ml each, 7 days apart) were injected intraperitoneally into mice (NAMRU strain) at 20 per test dilution. Seven days after the second injection, each animal was challenged subcutaneously with 288 to 504 CFU of *P. pestis* strain 195/P and observed for 2 weeks.

Challenge culture. Stock cultures of *P. pestis* strain 195/P were grown on blood-agar slants (Difco blood-agar base plus 3% sheep blood) and stored at 4 C. Organisms were injected into guinea pigs and reisolated at regular intervals to maintain their virulence. For use in challenge, a 100-ml volume of heart infusion broth (Difco), containing 0.003 M calcium chloride, 0.02 M magnesium chloride, and 0.2% xylose, was heavily inoculated from a slant and incubated at 37 C for 24 hr on a rotary shaker. A second passage was made by subculturing 2 ml of suspension into a second flask of broth. After growth at 37 C for 25 hr, the cell number was determined on spread plates

(Difco blood-agar base medium) by using 1% peptone water as diluent. The plates were counted after incubation for 48 hr at 28 C. Meanwhile, the broth culture was stored at 4 C for the 48 hr before use in the test. This practice made it possible to obtain a fairly uniform challenge dose. Storage at 4 C did not affect the viability or virulence of the organism.

Median effective dose (ED_{50}) and potency ratio. The ED_{50} of the test or reference vaccine, assayed concomitantly, is the reciprocal of the dilution protecting half of the animals as calculated by the method of Reed and Muench (4). The potency of each test vaccine was expressed as a ratio of its ED_{50} to that of the reference.

Determination of the median lethal dose (LD_{50}) of P. pestis strain 195/P. The LD_{50} (number of organisms estimated to kill 50% of control animals) of the challenge culture was determined anew for each test, or group of tests, by injecting control animals with suitable dilutions of it. The LD_{50} value was calculated by the method of Reed and Muench (4) and ranged, in mice, from <0.4 to 1.8 CFU. In guinea pigs, it ranged from 0.9 to 10 CFU.

RESULTS

Comparison of immunogenicity of vaccines in mice and guinea pigs. Table 1 shows the responses of mice and guinea pigs immunized with 28 lots of vaccines and challenged with *P. pestis*. The mouse potency ratios are listed in descending order of value. The corresponding potency ratios from guinea pig tests do not fall in the same order.

A comparison (Table 2) of the results between mice and guinea pigs shows a probability of <0.01. The 28 vaccines were not tested in mice and guinea pigs at the same time. As will be shown, the use of adjuvant is responsible for the generally higher ED_{50} in guinea pigs.

Reproducibility. Inasmuch as vaccine lots were tested on different occasions, reproducibility was gauged by testing two vaccines three times, concomitantly in mice and guinea pigs. Table 3 shows potency ratios obtained in both species. The variation in mouse potency ratios was 2.5-fold for

TABLE 1. *Immunogencity of plague vaccines in NAMRU strain mice and Hartley strain guinea pigs*

Vaccine no.	Mice				Guinea pigs			
	Challenge no. LD_{50}[a]	ED_{50}[b]		Potency ratio	Challenge no. LD_{50}[a]	ED_{50}[b]		Potency ratio
		Test	Reference			Test	Reference	
28	531	220	38	5.8	10^6	1,000	843	1.2
14	265	180	43	4.2	10^6	1,980	860	2.3
25	337	118	31	3.8	10^6	1,510	274	5.5
13	265	150	43	3.5	10^6	3,200	720	4.4
12	265	132	43	3.1	10^6	3,200	720	4.4
7	104	340	110	3.1	3.7×10^5	692	483	1.4
8	104	260	110	2.4	3.7×10^5	1,000	483	2.1
26	337	72	31	2.3	10^6	1,700	274	6.2
3	571	540	247	2.2	4.6×10^5	2,100	400	5.3
2	201	650	350	1.8	5.7×10^5	2,150	516	4.2
1	201	600	350	1.7	5.7×10^5	2,900	516	5.6
16	633	160	98	1.6	10^6	3,170	860	3.7
24	291	39	26	1.5	10^6	2,080	643	3.2
27	531	52	38	1.4	10^6	2,153	843	2.5
17	270	76	57	1.3	10^6	3,484	1,135	3.0
5	571	295	247	1.2	4.6×10^5	690	400	1.7
20	235	154	133	1.2	10^6	1,420	348	4.1
23	270	71	57	1.2	10^6	2,600	643	4.0
6	104	115	110	1.0	3.7×10^5	845	483	1.7
18	270	119	133	0.9	10^6	2,200	1,135	1.9
9	288	260	300	0.87	8.1×10^5	1,460	470	3.1
10	288	260	300	0.87	8.1×10^5	660	470	1.4
15	633	83	98	0.85	10^6	2,700	860	3.1
4	571	194	247	0.8	4.6×10^5	850	400	2.1
21	235	100	133	0.75	4.8×10^5	1,660	966	1.7
22	305	58	105	0.55	4.8×10^5	2,370	966	2.4
19	235	67	133	0.5	10^6	1,795	348	5.1
11	288	140	300	0.47	10^6	2,000	720	2.8

[a] Median lethal dose.
[b] Median effective dose.

TABLE 2. *Statistical comparison of the immunogenicity of plague vaccines tested in mice and guinea pigs*[a]

Determination	Mice		Guinea pigs	
	Range	Mean[b]	Range	Mean[b]
ED_{50}[c] of test vaccine	39 to 650	196.6 ± 62.9	660 to 3,484	1,913 ± 326.5
ED_{50} of reference vaccine	26 to 350	128 ± 70.4	274 to 1,135	638.2 ± 170.6
Potency ratios of vaccine[d]	0.47 to 5.8	1.82 ± 0.50	1.2 to 6.2	3.22 ± 05.6

[a] Comparison of values shown in Table 1.
[b] Mean ± 95% confidence limits.
[c] Median effective dose.
[d] A comparison of the difference in mean potency ratios of vaccine between mice and guinea pigs shows a probability of <0.01.

TABLE 3. *Evaluation of two plague vaccines in mice and guinea pigs*

Vaccine	Trial no.	Mice				Guinea pigs			
		LD_{50}[a] *Pasteurella pestis* per mouse	ED_{50}[b]		Potency ratio	LD_{50}[a] *Pasteurella pestis* per pig	ED_{50}[b] Test		Potency ratio
			Test	Reference			Test	Reference	
A	1	593	180	71	2.5	1.8×10^5	1,560	377	4.1
	2	362	285	81	3.5	2.6×10^5	268	340	0.8
	3	332	380	280	1.4	2.1×10^5	448	149	3.0
B	1	593	152	71	2.1	1.8×10^5	1,140	377	3.0
	2	362	78	81	0.96	2.6×10^5	1,280	340	3.3
	3	332	760	280	2.7	2.1×10^5	647	149	4.3

[a] Median lethal dose.
[b] Median effective dose.

TABLE 4. *Effects of challenge dosage on the median effective dose (ED_{50}) of plague vaccine in guinea pigs*

Vaccine	Challenge no. LD_{50}[a] per guinea pig	ED_{50}	Control[b] survivors out of total
C	1.1×10^6	1,000	0/10
	4.4×10^2	2,260	0/10
D	8.5×10^5	516	0/16
	8.5×10^2	774	0/16
E	1.1×10^6	1,470	0/16
	5.4×10^2	1,240	0/16

[a] Median lethal dose.
[b] Controls were injected with adjuvant only on day of vaccination and challenged 3 weeks later.

vaccine A and 2.8-fold for vaccine B. In guinea pigs, a 5.1-fold range for vaccine A and a 1.4-fold range for vaccine B was obtained.

Response of vaccinated guinea pigs to different challenge doses of P. pestis. To determine the effect of varying the challenge dose on the resistance of vaccinated guinea pigs, groups of animals given the same amounts of vaccine were infected with either a high or low dose of *P. pestis*. The results (Table 4) show that similar protection was afforded whether the animals were challenged with as few as 440 or with as many as 1.1×10^6 LD_{50}.

Response of guinea pigs to vaccine without adjuvant. As shown in Table 5, animals receiving one or two intramuscular doses of plague vaccine without adjuvant required more than 1,000 times as much vaccine for protection as the animals receiving vaccine with adjuvant.

It was also possible to protect guinea pigs against low levels of challenge by giving one dose of vaccine without adjuvant by either the subcutaneous or intraperitoneal routes. The data in Table 6 show that similar protection against 88 *P. pestis* cells was obtained when either one or two doses of vaccine were injected. Even with higher challenge levels, the proportion of protected animals remained about the same.

DISCUSSION

The present study sought to determine if guinea pigs offered advantages over the commonly used mouse for plague vaccine evaluation.

From Table 1, it is evident the potency ratios differ between the mouse and guinea pig. The mouse values fall in a 12-fold range, whereas those of the guinea pigs cover a 5-fold range. The statistical evaluation of these values (Table 2) indicated a significantly greater precision with guinea pigs. And yet, in repeated tests with two vaccines (Table 3), the guinea pig gave better reproducibility than the mouse with one vaccine although it was poorer with the other.

In the studies using vaccine with and without adjuvant (Tables 5 and 6), our findings confirm the observations reported by Chen et al. (1), Smith and Packman (5), and Spivack et al. (6) in that without adjuvant guinea pigs required up to 1,000 times more vaccine for protection. An important limitation in using guinea pigs for vaccine evaluation is that they require adjuvant, which is not used in man, to develop a well-defined immune response. Without adjuvant, the response was minimal, which made if difficult to establish criteria for the evaluation of any particular plague vaccine.

Since both species as test hosts showed considerable variation, and the guinea pig, additionally, required adjuvant to respond, we concur with the present practice of employing mice for evaluation of plague vaccines. The test time is shorter, and they are cheaper and easier to handle in large numbers than guinea pigs. Furthermore, there is no reason to believe that the guinea pig is a better analogue for man than is the mouse.

TABLE 5. *Effects of dosage and adjuvant on the immunogenicity of plague vaccine in guinea pigs*

Dose	Vaccine			Survivors[a]	ED_{50}[b]
	Milliliter/guinea pig	Adjuvant	Dilution tested		
1	0.5	With	100	7	
			1,000	6	
			10,000	1	1,000
1	1.0	With-out	Undiluted	3	
			10	3	
			100	1	Undiluted
2[c]	0.5 each	With-out	Undiluted	6	
			10	3	
			100	0	2.5
0	0	Only	0	0	

[a] Ten guinea pigs per group; challenged subcutaneously with 3.2×10^5 median lethal doses of *Pasteurella pestis* strain 195/P.
[b] Median effective dose.
[c] One week apart.

ACKNOWLEDGMENTS

This work was supported by a contract between the University of California Regents and the Office of Naval Research with funds provided by the Division of Biologics Standards of the National Institutes of Health, Bethesda, Md.

TABLE 6. *Effects of dosage and routes of vaccination on the immunogenicity of plague vaccine without adjuvant in guinea pigs*

Vaccination			*Pasteurella pestis* in subcutaneous injection[a]	Controls[b]	ED_{50}[c]
Dose	Milliliter per guinea pig[b]	Route			
1	0.5	Subcutaneous	88.5		45
1	0.5	Intraperitoneal	88.5		35
2[d]	0.5 each	Subcutaneous	88.5		25
2[d]	0.5 each	Intraperitoneal	88.5	0	50
1	0.5	Subcutaneous	885		15
1	0.5	Intraperitoneal	885		13
2[d]	0.5 each	Subcutaneous	885		38
2[d]	0.5 each	Intraperitoneal	885	0	39
1	0.5	Subcutaneous	8,850		21
1	0.5	Intraperitoneal	8,850		5
2[d]	0.5 each	Subcutaneous	8,850		30
2[d]	0.5 each	Intraperitoneal	8,850	0	33

[a] The median lethal dose was <8.85 colony-forming units per guinea pig. Animals were challenged 2 weeks after the last vaccination.
[b] Ten guinea pigs per group.
[c] Median effective dose.
[d] Vaccinations were 1 week apart.

LITERATURE CITED

1. Chen, T. H., L. E. Foster, and K. F. Meyer. 1961. Experimental comparison of the immunogenicity of antigens in the residue of ultrasonated avirulent *Pasteurella pestis* with a vaccine prepared with killed virulent whole organisms. J. Immunol. **87**:64–71.
2. Gerard, G. 1955. Plague. Annu. Rev. Microbiol. **9**:253–276.
3. Keppie, J., E. C. Cocking, and H. Smith. 1958. A non-toxic complex from *Pasteurella pestis* which immunizes both guinea pigs and mice. Lancet **1**:246–247.
4. Reed, L. J. and H. Muench. 1938. A simple method for determining fifty per cent end points. Amer. J. Hyg. **27**:493–497.
5. Smith, H., and L. P. Packman. 1966. A filtered non-toxic plague vaccine which protects guinea pigs and mice. Brit. J. Exp. Pathol. **47**:25–34.
6. Spivack, M. L., L. Foster, A. Larson, T. H. Chen, E. E. Baker, and K. F. Meyer. 1958. The immune response of the guinea pig to the antigens of *Pasteurella pestis*. J. Immunol. **80**: 132–141.
7. U. S. Public Health Service. 1945. Minimum requirements: plague vaccine. National Institutes of Health, Bethesda, Md