Immunogenicity of Plague Vaccines in Mice and Guinea Pigs

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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 $2 \times 10^9$ particles per ml, with phenol as preservative.

A lyophilized reference standard vaccine was supplied by the Division of Biologic 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 monolaurate) obtained from Hill Top Laboratories, Inc., Cincinnati, Ohio. Three weeks later each animal was challenged subcutaneously with $P$. pestis strain 195/P $0.85 \times 10^6$ to $3.2 \times 10^6$ 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.
TABLE 1. Immunogenicity of plague vaccines in NAMRU strain mice and Hartley strain guinea pigs

| Vaccine no. | Challenge no. LD₅₀ | ED₅₀ᵇ | Potency ratio | Challenge no. LD₅₀ | ED₅₀ᵇ | Potency ratio |
|-------------|--------------------|-------|---------------|--------------------|-------|---------------|
|             |                    |       |               |                    |       |               |
| 28          | 531                | 220   | 38            | 5.8                | 10⁶   | 1,000         |
| 14          | 265                | 180   | 43            | 4.2                | 10⁶   | 1,980         |
| 25          | 337                | 118   | 31            | 3.8                | 10⁶   | 1,510         |
| 13          | 265                | 150   | 43            | 3.5                | 10⁶   | 3,200         |
| 12          | 265                | 132   | 43            | 3.1                | 10⁶   | 3,200         |
| 7           | 104                | 340   | 110           | 3.1                | 10⁶   | 3,200         |
| 8           | 104                | 260   | 110           | 2.4                | 10⁶   | 3,200         |
| 26          | 337                | 72    | 31            | 3.8                | 10⁶   | 3,200         |
| 3           | 571                | 540   | 247           | 2.2                | 10⁶   | 3,200         |
| 2           | 201                | 650   | 350           | 1.8                | 10⁶   | 3,200         |
| 1           | 201                | 600   | 350           | 1.7                | 10⁶   | 3,200         |
| 16          | 633                | 160   | 98            | 1.6                | 10⁶   | 3,200         |
| 24          | 291                | 39    | 26            | 1.5                | 10⁶   | 3,200         |
| 27          | 531                | 52    | 38            | 1.4                | 10⁶   | 3,200         |
| 17          | 270                | 76    | 57            | 1.3                | 10⁶   | 3,200         |
| 5           | 571                | 295   | 247           | 1.2                | 10⁶   | 3,200         |
| 20          | 235                | 154   | 133           | 1.2                | 10⁶   | 3,200         |
| 23          | 270                | 71    | 57            | 1.2                | 10⁶   | 3,200         |
| 6           | 104                | 115   | 110           | 1.0                | 10⁶   | 3,200         |
| 18          | 270                | 119   | 133           | 0.9                | 10⁶   | 3,200         |
| 9           | 288                | 260   | 300           | 0.8                | 10⁶   | 3,200         |
| 10          | 288                | 260   | 300           | 0.8                | 10⁶   | 3,200         |
| 15          | 633                | 83    | 98            | 0.85               | 10⁶   | 3,200         |
| 4           | 571                | 194   | 247           | 0.8                | 10⁶   | 3,200         |
| 21          | 235                | 100   | 133           | 0.75               | 10⁶   | 3,200         |
| 22          | 305                | 58    | 105           | 0.55               | 10⁶   | 3,200         |
| 19          | 235                | 67    | 133           | 0.5                | 10⁶   | 3,200         |
| 11          | 288                | 140   | 300           | 0.47               | 10⁶   | 3,200         |

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 ED₅₀ 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

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*Median lethal dose.
*b Median effective dose.
TABLE 2. Statistical comparison of the immunogenicity of plague vaccines tested in mice and guinea pigs

| Determination                                      | Mice | Guinea pigs |
|---------------------------------------------------|------|-------------|
|                                                  | Range | Mean b      | Range     | Mean b      |
| ED₉₀ of test vaccine                             | 39 to 650 | 196.6 ± 62.9 | 660 to 3,484 | 1,913 ± 326.5 |
| ED₉₀ of reference vaccine                        | 26 to 350 | 128 ± 70.4  | 274 to 1,135 | 638.2 ± 170.6 |
| Potency ratios of vaccine                         | 0.47 to 5.8 | 1.82 ± 0.50 | 1.2 to 6.2  | 3.22 ± 0.56  |

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. | LD₅₀° Pasteurella pestis per mouse | ED₉₀ ° | LD₅₀° Pasteurella pestis per pig | ED₉₀ ° Test | Potency ratio |
|---------|-----------|-----------------------------------|-------|---------------------------------|------------|--------------|
|         |           | Test                              | Reference | Potency ratio                  | Test       | Reference     |
| A       | 1         | 593                               | 180     | 71                             | 2.5        | 1.8 × 10⁶     | 1,560        | 377         | 4.1         |
|         | 2         | 362                               | 285     | 81                             | 3.5        | 2.6 × 10⁶     | 268          | 340         | 0.8         |
|         | 3         | 332                               | 380     | 280                            | 1.4        | 2.1 × 10⁶     | 448          | 149         | 3.0         |
| B       | 1         | 593                               | 152     | 71                             | 2.1        | 1.8 × 10⁶     | 1,140        | 377         | 3.0         |
|         | 2         | 362                               | 78      | 81                             | 0.96       | 2.6 × 10⁶     | 1,280        | 340         | 3.3         |
|         | 3         | 332                               | 760     | 280                            | 2.7        | 2.1 × 10⁶     | 647          | 149         | 4.3         |

a Median lethal dose.
b Median effective dose.

table 4. Effects of challenge dosage on the median effective dose (ED₉₀) of plague vaccine in guinea pigs

| Vaccine | Challenge no. LD₅₀° per guinea pig | ED₉₀ | Control survivors out of total |
|---------|-----------------------------------|------|-------------------------------|
| C       | 1.1 × 10⁶                         | 1,000| 0/10                          |
|         | 4.4 × 10²                         | 2,260| 0/10                          |
| D       | 8.5 × 10⁵                         | 516  | 0/16                          |
|         | 8.5 × 10²                         | 774  | 0/16                          |
| E       | 1.1 × 10⁶                         | 1,470| 0/16                          |
|         | 5.4 × 10²                         | 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 × 10⁶ LD₉₀.

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 vaccines 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 it 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.

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

| Dose | Milliliter/guinea pig | Vaccine | Survivors | ED₉₀
|------|----------------------|---------|-----------|------|
| 0.5  | With 100             |         | 7         |      |
| 0.5  | With 1,000           |         | 6         |      |
| 0.5  | With 10,000          |         | 1         | 1,000|
| 1.0  | Without Undiluted    |         | 3         |      |
| 2    | Each Undiluted       |         | 6         |      |
| 0    | Only                 |         | 0         | 2.5  |

a Ten guinea pigs per group; challenged subcutaneously with 3.2 × 10⁶ median lethal doses of Pasteurella pestis strain 195/P.
b Median effective dose.
c One week apart.

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

| Dose | Milliliter/guinea pig | Route | Pasteurella pestis in subcutaneous injection | Controls | ED₉₀
|------|----------------------|-------|---------------------------------------------|----------|------|
| 0.5  | Subcutaneous         |       | 88.5                                        |          | 45   |
| 0.5  | Intraperitoneal      |       | 88.5                                        |          | 35   |
| 0.5 each | Subcutaneous     |       | 88.5                                        |          | 25   |
| 0.5 each | Intraperitoneal    |       | 88.5                                        |          | 50   |
| 0.5  | Subcutaneous         |       | 885                                         |          | 15   |
| 0.5  | Intraperitoneal      |       | 885                                         |          | 13   |
| 0.5 each | Subcutaneous     |       | 885                                         |          | 38   |
| 0.5 each | Intraperitoneal    |       | 885                                         |          | 39   |
| 0.5  | Subcutaneous         |       | 8,850                                       |          | 21   |
| 0.5  | Intraperitoneal      |       | 8,850                                       |          | 5    |
| 0.5 each | Subcutaneous     |       | 8,850                                       |          | 30   |
| 0.5 each | Intraperitoneal    |       | 8,850                                       |          | 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.
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