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Measuring the efficacy of vaccination in affording protection against plague *

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The relationship of F1 antibody titre to protection against plague was investigated by subjecting seropositive laboratory rats to virulent challenge and observing for survival. The passive haemagglutination (PHA) test in microtitre was employed for serology. Rats vaccinated with live vaccine EV76(51f), killed U.S.P. vaccine, or F1 antigen and challenged by subcutaneous inoculation of 1×10^3 *to* 5×10^5 Yersinia pestis *survived at similar rates that, overall, equalled 6% at titres less than 1:16, 46% at titres of 1:32–1:64, 90% at titres of 1:128–1:256, and 96% at titres of 1:512–1:1024. Rats vaccinated with F1 antigen and rats that had been infected previously were challenged intranasally with* 8.9×10^4 Y. pestis *and subsequently demonstrated similar rates of survival that was zero at titres less than 1:128, 86% at titres of 1:128–1:256, and 100% at titres of 1:512–1:1024. The significance of titre of F1 antibody as a measure of seroimmunity against acute bubonic or pneumonic plague is discussed for rats, monkeys, and man.*

The plague bacillus *(Yersinia pestis)* characteristically possesses the ability to elaborate a specific envelope or capsular antigen known as fraction 1 (F1) that functions as an important determinant of virulence (*1–3*). The F1 content of a vaccine has a direct effect on the degree of immunity to acute plague infection produced in laboratory animals (*4*, *5*). Exposure to F1, either through vaccination or infection, stimulates the production of antibodies to the antigen that can be measured quantitatively (*6*). At the present time, the passive haemagglutination (PHA) and haemagglutination-inhibition techniques (*7*) are most widely employed for this purpose because these procedures are convenient and exhibit great sensitivity and specificity (*8*). Although the occurrence of antibody to F1 in man or animals suggests that some degree of protection against reinfection has been acquired (*9–12*), the relationship between the serological titre of F1 antibody and immunity to plague has not been clearly defined. The work reported here investigated the correlation between titre and protection with reference to the PHA procedure for measuring F1 antibody.

MATERIALS AND METHODS

The WR strain of laboratory rat *(Rattus norvegicus)*, derived from the Wistar strain, was employed in the experiments,[a] as unvaccinated individuals characteristically exhibit a high susceptibility to plague infection (LD_{50} <20 virulent organisms) regardless of age, sex, or season of the year.

Challenge infections were done by subcutaneous inoculation or nasal instillation of virulent *Y. pestis* of the 195/P strain. Plague bacilli to be inoculated subcutaneously were cultured at 25°C to obtain phagocytosis-sensitive *Y. pestis* similar to those transmitted by fleas, whereas intranasal challenge employed encapsulated phagocytosis-resistant plague bacilli grown *in vivo* to simulate organisms implicated in interhuman transmission of pneumonic plague (*13*). Intranasal challenge employed *Y. pestis* 195/P taken from the peritoneum of a moribund WR rat by the technique of Cavanaugh & Randall (*13*) and administered by the method of Meyer et al. (*14*).

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[a] In conducting the research described in this report, the investigators adhered to the *Guide for the care and use of laboratory animals*. US Department of Health, Education and Welfare. DHEW Publication No. (NIH) 73-23, 1972.

Table 1. Experiments with laboratory rats

Method of challenge	Treatment before challenge	F1 antibody titre at challenge [a]	Percentage survival (survivors/total)	Average day of death
Subcutaneous inoculation of 1×10^3 to 5×10^5 *Y. pestis* 195/P [c]	Vaccination with live avirulent *Y. pestis* strain EV 76(51f)	None [b]	0 (0/23)	2.8
		⩽1 : 16	0 (0/24)	4.2
		1 : 32–1 : 64	46 (6/13)	5.4
		1 : 128–1 : 256	91 (10/11)	6
		1 : 512–1 : 1024	100 (6/6)	—
	Vaccination with killed whole-organism plague vaccine U.S.P.	None [b]	0 (0/6)	3.2
		⩽1 : 16	9 (6/70)	4.3
		1 : 32–1 : 64	50 (3/6)	13.3
		1 : 128–1 : 256	89 (8/9)	10
		1 : 512–1 : 1024	80 (4/5)	13
	Vaccination with the F1 antigen of *Y. pestis*	None [b]	0 (0/5)	4.2
		1 : 32–1 : 64	40 (2/5)	9.0
		1 : 512–1 : 1024	97 (34/35)	11
Nasal instillation of 8.9×10^4 *Y. pestis* 195/P [d]		None [b]	0 (0/7)	3.0
	Vaccination with the F1 antigen of *Y. pestis*	1 : 32–1 : 64	0 (0/4)	9.2
		1 : 128–1 : 256	50 (1/2)	13
		1 : 512–1 : 1024	100 (13/13)	—
	Infection with virulent *Y. pestis* 195/P and antiserum injected 2 days later	⩽1 : 16	0 (0/3)	3.7
		1 : 32–1 : 64	0 (0/4)	6.2
		1 : 128–1 : 256	100 (5/5)	—
		1 : 512–1 : 1024	100 (4/4)	—

[a] Final serum dilutions in the PHA test.
[b] Unvaccinated controls.
[c] Simulated flea challenge with phagocytosis-sensitive *Y. pestis*.
[d] Simulated pneumonic challenge with phagocytosis-resistant *Y. pestis*.

Three groups of seropositive rats, with appropriate controls, were challenged subcutaneously. Rats in the first group had been vaccinated with 10^8–10^7 *Y. pestis* of the live avirulent strain EV76(51f) and were challenged either 26 days later with 5×10^5 *Y. pestis* 195/P or 10 weeks later with 2×10^5 *Y. pestis* 195/P. The second group consisted of rats that had received killed whole-organism plague vaccine U.S.P.,[a] with injections of either 1 ml on days 1, 32, and 49 followed by 0.2 ml on day 83 or 0.5 ml on days 1, 32, and 49 followed by 0.1 ml on day 83. These rats were challenged 100 days after vaccination with 10^8, 10^4, or 10^5 *Y. pestis* 195/P. The third group contained rats vaccinated with F1 antigen (Walter Reed lot 4, July 1971) in Freund's complete adjuvant. The rats received an initial subcutaneous injection of 500 μg of F1 followed by booster injections of 200 μg of F1 at 7 and 14 days. These rats were challenged 6 weeks later with 3.5×10^3 *Y. pestis* 195/P.

Two groups of rats were challenged concurrently by nasal instillation of 8.9×10^4 phagocytosis-resistant *Y. pestis* 195/P. One group consisted of rats vaccinated with F1 antigen, as outlined above, 6 weeks earlier. The other group contained rats that had been inoculated subcutaneously with 48 virulent *Y. pestis* 195/P and, 2 days later, given an intraperitoneal injection of antiplague serum-globulin [b] equivalent to 15 ml of rabbit serum. Rats surviving subcutaneous infection without discernable buboes were challenged intranasally after 6 months when it could be reasonably assumed, from previous studies (*15*), that the antiserum administered had been eliminated and any titre present was derived from active immunization.

Serum titres of specific F1 antibody were determined before and after every challenge using the PHA microtitration test (*15*). To provide continuity with earlier studies (*9–12*), the titres reported are the highest final dilutions of serum giving clear-cut 4+ haemagglutination. As a further precaution to provide the best data possible, new microtitration loops were employed and these were recalibrated before use to confirm accuracy.

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RESULTS

Whereas unvaccinated rats always died after challenge infection, many vaccinated rats survived subcutaneous challenge with phagocytosis-sensitive *Y. pestis* or intranasal challenge with phagocytosis-resistant *Y. pestis*. Survival among vaccinated rats was in direct proportion to the titre of F1 antibody present at the time of challenge (Table 1). In vaccinated rats that died, death was delayed and the average time before death was directly correlated with the F1 antibody titre at the time of challenge.

Patterns of survival following subcutaneous challenge were similar in groups of rats vaccinated with avirulent live vaccine, whole-organism killed vaccine, or F1 subunit vaccine. Overall survival in rats challenged subcutaneously was 6% (6/94) at F1 antibody titres $<1:16$, 46% (11/24) at titres of 1:32–1:64, 90% (18/20) at titres of 1:128–1:256, and 96% (44/46) at titres of 1:512–1:1024.

Patterns of survival after intranasal challenge were similar in groups of rats that had derived F1 antibody titres from vaccination with F1 antigen or from a previous infection with virulent *Y. pestis*. However, unlike those challenged subcutaneously, rats with titres $<1:64$ never survived intranasal challenge, reflecting the high virulence of phagocytosis-resistant *Y. pestis*. An increased mortality was not evident in rats possessing higher levels of antibody at the time of challenge. Overall survival from intranasal challenge was 86% (6/7) in rats with F1 antibody titres of 1:128–1:256 and 100% (17/17) at titres of 1:512–1:1024.

DISCUSSION

The results of our studies with laboratory Norway rats suggest that the PHA titre of F1 antibody is a significant indicator of protection from acute bubonic plague in this species. In fact, retrospective analyses of published data for bubonic plague in wild-caught Norway rats, and also in laboratory monkeys, showed strikingly similar results (Table 2): in all cases, greater numbers of animals survived experimental infections where the levels of F1 antibody present at the time of challenge were higher. In Norway rats and monkeys, the majority of animals possessing final F1 antibody titres $\geq 1:128$ (e.g., initial serum dilutions $\geq 1:64$ in the PHA test) survived severe challenges with plague bacilli of classical virulence. Some protection was evident even in vaccinated animals that succumbed to experimental plague, including individuals that had no measurable titre at challenge, as death was delayed. Possibly very small amounts of antibody to the F1 antigen were present that were not detected by the serological method used, or perhaps other immunological mechanisms had functioned for a time to delay death. Alternatively, some protection against virulent challenge may have been derived from exposure to other antigens of the plague bacillus such as antigen T (toxin) (*16*, *17*) or antigen L (*18*).

Table 2. Experiments with wild rats and laboratory monkeys

Species of animal and treatment before challenge	Method of challenge	F1 antibody titre at challenge [a]	Percentage survival (survivors/total)	Average day of death	Source of data
Wild Norway rats (*R. norvegicus*) from Oahu, Hawaii infected with 1.2 × 10^4 *Y. pestis* 195/P and survived spontaneously	Subcutaneous inoculation of 7.7 × 10^7 *Y. pestis* 195/P	≤1 : 16	14 (1/7)	Not reported	Chen & Meyer (*12*)
		1 : 32–1 : 64	50 (1/2)		
		1 : 128–1 : 256	80 (4/5)		
		1 : 512–1 : 2048	100 (10/10)		
Hanuman langurs (*Presbytus entellus*) vaccinated with killed whole-organism plague vaccine U.S.P. or living *Y. pestis* strain EV Saigon	Subcutaneous inoculation of 6.4 × 10^4 to 2.3 × 10^5 *Y. pestis* 195/P	None [b]	0 (0/11)	6	Meyer (*9*); Chen et al. (*11*)
		≤1 : 16	0 (0/2)	12	
		1 : 32–1 : 64	17 (2/12)	11	
		1 : 128–1 : 256	67 (6/9)	19	
		1 : 512–1 : 2048	100 (5/5)	—	
African green vervets (*Cercopithecus aethiops*) vaccinated with living *Y. pestis* strain EV 51f	Subcutaneous inoculation of 8.8 × 10^3 *Y. pestis* strain F357	None [b]	0 (0/2)	7	Hallett et al. (*10*)
		1 : 32–1 : 64	0 (0/2)	9	
		1 : 128–1 : 256	86 (6/7)	12	

[a] Final serum dilution in the PHA test.

[b] Unvaccinated controls.

Similarly, a good correlation between F1 antibody titre and survival was found from our experiments with intranasal challenge of laboratory rats. Meyer (*14*) estimated that only 10% of the bacilli administered intranasally reach deeper respiratory passages (e.g., 8900 *Y. pestis* in the present study), and infection of the larynx, tonsils, or other parts of the upper respiratory tract may also result. Although intranasal challenge is not as reliable as other methods (e.g., Henderson apparatus) for inducing a relatively uncomplicated plague pneumonia, our data indicated intranasal challenge to be much more severe than subcutaneous challenge, with no survivors among intranasally challenged rats possessing F1 antibody titres lower than 1:128. The experimental data suggest that F1 antibody titre may be a good measure of immunity to pneumonic as well as to bubonic plague.

Although anti-F1 PHA titre in Norway rats is an indicator of protection against acute plague, there is good evidence that this relationship does not extend to protection from chronic plague in these rats (*19*). Indeed, F1 antibody may have some influence on the development of chronic disease (*2*).

The vaccines employed in our study with rats gave essentially identical results in terms of the correlation between F1 antibody titre and protection. However, this relationship may be altered with some experimental vaccines or animal species. Chen et al. (*11*) found that the administration of a combined plague–cholera–typhus vaccine to Hanuman langurs and silver leaf monkeys (*Presbytus cristatus*) considerably enhanced survival in animals with very low titres of specific F1 antibody. Fournier et al. (*20*) observed that two baboons with titres of 1:32 and 1:64 after exposure to the living vaccine strain EV40 succumbed to plague, while a baboon vaccinated with EV76(51f) that did not produce a detectable titre of F1 antibody survived virulent challenge.

The mouse protective antibody index has been employed to measure the immune response of man to vaccination against plague (*21*). A strong correlation between the PHA titre of F1 antibody in human serum and the protection afforded mice injected with serum just prior to virulent challenge has recently been reported (*22–24*). These results suggest that the relationship between immunity from plague and the titre of antibody to F1 found in rats and monkeys may also apply to man.

RÉSUMÉ

ÉVALUATION DE LA PROTECTION CONFÉRÉE PAR LA VACCINATION ANTIPESTEUSE

La relation entre le titre d'anticorps F1 et la protection contre la peste a été étudiée en exposant des rats de laboratoire séropositifs à des formes virulentes du bacille et en observant les taux de survie. Pour les examens sérologiques, on a employé le microtitrage par hémagglutination passive. Des rats ayant reçu le vaccin vivant EV76(51f), le vaccin tué U.S.P. ou l'antigène F1 et infectés ensuite par inoculation sous-cutanée de *Yersinia pestis* à raison de 1×10^3 à 5×10^5 organismes ont présenté un taux de survie uniforme pour des valeurs déterminées du titre d'anticorps F1, soit 6% pour des titres $<1:16$, 46% pour des titres compris entre 1:32 et 1:64, 90% pour des titres compris entre 1:128 et 1:256 et 96% pour des titres compris entre 1:512 et 1:1024. Des rats vaccinés avec l'antigène F1 et des rats qui avaient été infectés antérieurement ont reçu des aérosols de *Y. pestis* à raison de $8{,}9 \times 10^4$ organismes, et ils ont également présenté des taux de survie uniformes — mais très inférieurs à ceux constatés chez les rats infectés par voie sous-cutanée — soit 0% pour des titres d'anticorps $<1:64$, 86% pour des titres compris entre 1:128 et 1:256 et 100% pour des titres se situant entre 1:512 et 1:1024. La valeur du titrage des anticorps F1 en tant que moyen de mesurer l'immunité sérologique contre la peste bubonique aiguë ou la peste pulmonaire est examinée ici chez les rats et les singes, mais il a sans doute une signification analogue chez l'homme.

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