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Potency of killed plague vaccines prepared from avirulent *Yersinia pestis**

JAMES E. WILLIAMS,[1] PATRICIA L. ALTIERI,[2] SANFORD BERMAN,[3] JOSEPH P. LOWENTHAL,[4] & DAN C. CAVANAUGH [5]

Killed plague vaccines prepared from avirulent strains A1122 and EV76S of Yersinia pestis *were more effective in mouse potency tests than samples of Plague Vaccine, USP, prepared from killed* Y. pestis *of the virulent strain 195/P. Manufacture of vaccine from avirulent* Y. pestis *would obviate requirements for the large containment facilities that are currently needed for producing Plague Vaccine, USP.*

Killed *Yersinia pestis* have been used as plague vaccines since 1897, when W. M. W. Haffkine, in India, inoculated himself with his experimental vaccine (*1*). In the United States of America, killed plague vaccine for human use (the so-called Army Vaccine) was first produced in 1946 (*2*). Progressive improvements (*3*) in the Army Vaccine have culminated in the Plague Vaccine, USP, E Medium, in current use (*4*). This vaccine was apparently instrumental in preventing plague in US personnel during the Viet Nam war (*5*).

Plague Vaccine, USP, E Medium is prepared from the highly virulent Indian strain 195/P of *Y. pestis*. Consequently, special laboratory facilities that ensure safe containment are required for its production. At present, there is only one company in the USA licensed to manufacture the vaccine, which is rather expensive.

Since additional regulations are now being placed on institutions involved in vaccine development and production (Good Laboratory and Manufacturing Practices, US Food and Drug Administration regulations, etc.), the preparation of plague vaccine from virulent organisms may become even more restricted and expensive. In the event of a sudden increase in demand for vaccine, new or additional production facilities may not be available or, at best, a considerable time may be required to make them operational. Therefore, alternative methods of manufacture that would reduce requirements for special containment facilities have been considered. Here we report the results of potency tests on experimental killed vaccines prepared from avirulent strains of *Y. pestis*.

MATERIALS AND METHODS

Vaccines

Three lots of Plague Vaccine, USP, E Medium[a] were used, containing killed *Y. pestis* of strain 195/P in saline with 0.4 ml of formalin and 5 ml of phenol per litre. Experimental killed plague vaccines were prepared from avirulent, encapsulated *Y. pestis* strains EV76S (e.g., strain EVS, EV76 Saigon, EV76-WR) (*6*) and A1122 (e.g., strain 11953)[b] (*7*).

Methods similar to those employed in the manufacture of Plague Vaccine, USP, E Medium were used to make the experimental vaccines. Lots EV76S (11 July 75) and A1122 (29 Aug. 75) were derived from *Y. pestis* grown on Bacto Blood Agar Base[c] (BAB). Lot A1122 (11 Feb. 77) was derived from organisms grown on an E Medium agar tested and found to be free of blood groups A and B substances,[d] as required by the US Department of Defense specifications for plague vaccine (*8*). In all instances, agar-grown cultures were harvested in physiological saline after 72 hours' incubation at 37 °C. Purity was determined by visual inspection of the growth on the agar before harvesting, by microscopic examination of Gram staining of the harvest pools, and by the examination of

* From the Departments of Hazardous Microorganisms and Biologics Research, Division of Communicable Disease and Immunology, Walter Reed Army Institute of Research, Washington, DC 20012, USA.

[1] Microbiologist/Ecologist, Plague Section, Department of Hazardous Microorganisms.

[2] Chief, Biochemistry and Freeze-Drying Section, Department of Biologics Research.

[3] Chief, Microbiology Section, Department of Biologics Research.

[4] Chief, Department of Biologics Research.

[5] Chief, Department of Hazardous Microorganisms.

[a] Cutter Laboratories, Berkeley, CA 94710, USA.

[b] American Type Culture Collection, Rockville, MD 20852, USA.

[c] Difco, Detroit, MI 48201, USA.

[d] MEYER, K. F. *Are plague vaccines—live or killed—effective prophylactics in man?* WHO unpublished document BD/PL/WP/69.2 (1969).

colonial morphology of samples from the pools grown on BAB. Immediately after sampling for determinations of purity, neutral formalin was added to each harvest pool to a final concentration of 0.5 ml/litre, in order to inactivate the bacilli. The formalinized pools were kept for four days at 22 °C with frequent shaking. Periodic plating of samples from the pools showed that inactivation was achieved 24 hours after the addition of formalin. The inactivated pools were stored at 4 °C until used. The total protein nitrogen content of these experimental vaccines, and of the USP lots studied, was determined with a micro-Kjeldahl technique followed by nesslerization.

Mouse potency tests

Three tests were conducted that generally followed the US Food and Drug Administration's (FDA) protocol for testing plague vaccine (*8, 9*), except that, in the third test, the recommended challenge dose was deliberately exceeded. In place of the standard vaccine employed by the FDA, lots of Plague Vaccine, USP, conforming to all FDA requirements for mouse potency, were used as controls.

In each potency assay, the experimental vaccines were prediluted with physiological saline to obtain a total nitrogen content equivalent to that of the USP control vaccine being tested. For inoculation of the mice,[e] the vaccines were further diluted in saline to 1:5, 1:15, 1:45, 1:135, 1:405, and 1:1215. The USP control vaccine was similarly diluted.

Each vaccine dilution was then inoculated intraperitoneally into 20–25-g ICR strain mice.[f] Two doses of 0.2 ml were given, one week apart. One week after the second injection of vaccine, the mice were challenged by subcutaneous inoculation of virulent *Y. pestis*, strain 195/P, cultured in broth at 37 °C for 24 hours. Concurrent titration of the challenge in unvaccinated mice was conducted to establish the mouse LD_{50}. Mortality data for the 14 days following the challenge were used to calculate, by the method of Reed & Muench (*10*), the dilution of vaccine protecting 50% of the mice (median protective dose or PD_{50}).

RESULTS

Killed vaccines prepared from avirulent *Y. pestis* strains A1122 and EV76S were, in every case, more potent than the USP vaccine (Table 1). Indeed, the poorest protection was always provided by the USP control vaccine against the homologous strain (195/P). With a challenge of 1700 *Y. pestis*, equivalent to 214 mouse LD_{50}s, PD_{50} values for experimental vaccines were approximately twice that for the USP vaccine. With more severe challenges of 2660 and 30 100 *Y. pestis* (985 and 10 030 mouse LD_{50}s, respectively), the PD_{50} values for experimental vaccines were approximately ten times those of USP lots.

[e] In conducting the research described in this report, the investigators adhered to the *'Guide for laboratory animal facilities and care'*, as promulgated by the Committee of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council.

[f] Walter Reed Army Institute of Research, Washington, DC 20012, USA.

Table 1. Potency of killed plague vaccines in mice

Assay	Challenge dose: No. *Y. pestis* 195/P	Challenge dose: No. mouse LD_{50}s	Vaccine	Nitrogen content (mg/ml)	Vaccine dilution for 50% protective dose (PD_{50})
1	1700	214	USP Lot K9703	0.36	1 : 175
			EV76S (11 July 75)	0.32	1 : 500
			A1122 (29 Aug 75)	0.34	1 : 340
2	2660	985	USP Lot M3793	0.39	1 : 22
			EV76S (11 July 75)	0.39	1 : 180
			A1122 (29 Aug 75)	0.39	1 : 210
3	30 100	10 030	USP Lot M5826	0.40	1 : 5
			EV76S (11 July 75)	0.40	1 : 58
			A1122 (29 Aug 75)	0.40	1 : 63
			A1122 (11 Feb 77)	0.40	1 : 49

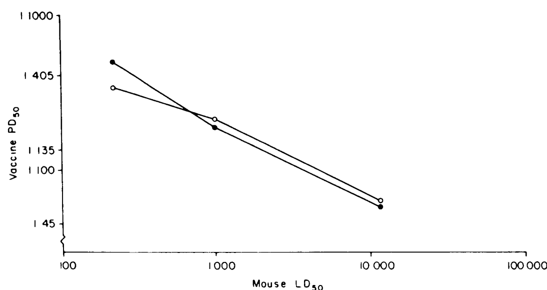

Fig. 1. The relationship of severity of challenge (no. of mouse LD_{50}s) to PD_{50} for vaccines EV76S (11 July 75) (●) and A1122 (29 Aug 75) (○). Axes of graph are log scale.

A clear relationship between mouse LD_{50} and mouse protection was found for the experimental vaccines (Fig. 1), in which the PD_{50} declined with increase in the challenge dose. The killed EV76S vaccine provided slightly more protection than the A1122 vaccine at the low-challenge dose, but otherwise the results with the two experimental vaccines were similar. Values for Plague Vaccine, USP, could not be included in Fig. 1 since different lots were used in the various assays; nevertheless a clear relationship between LD_{50} dose and mouse protection was evident (Table 1).

Two lots of killed A1122, prepared 18 months apart and tested concurrently in mice, demonstrated PD_{50} values that were almost identical (Table 1, Assay 3).

DISCUSSION

Extensive laboratory investigations and field evaluations have indicated that the currently manufactured USP vaccine is highly effective (*11*). Killed plague vaccines prepared from encapsulated, avirulent *Y. pestis* can equal or exceed the mouse-potency requirements for the USP vaccine. Our data corroborate the conclusion of Schütze (*12*), from early experiments with heat-killed broth vaccines, that 'virulent cultures do not result in more potent vaccines for either rats or mice than do avirulent ones'.

Immunity to typical plague infection in rats is directly related to the host's titre of antibody to the capsular F1 antigen of *Y. pestis* (*13*). The same is apparently true in primates and presumably also applies to man. Curiously, guinea pigs also produce significant F1 antibody titres but high-level F1 administration causes immunoparalysis (*14*). This is a possible reason why killed vaccines have failed to protect these animals in some studies. Taken together, the information available indicates that it is imperative, when producing a killed vaccine, to use a strain that elaborates F1 and to adjust the vaccine dosage to comply with the immunological idiosyncrasies of the recipient. The avirulent *Y. pestis* strains A1122 and EV76S used in our studies are well documented and produce considerable amounts of F1 antigen under suitable cultural conditions (*15*). Serological studies with laboratory animals administered killed vaccines of avirulent *Y. pestis* are now in progress.

Reactions to vaccination must be considered with any new vaccine. Specific information on reactogenicity of killed vaccines of avirulent *Y. pestis*, as well as on serological responses in man, must await suitable studies with human volunteers. However, killed vaccines from avirulent organisms should prove of no greater reactogenicity than the USP vaccine prepared from virulent *Y. pestis*. The currently used USP vaccine has produced local reactions in 11–24% and systemic reactions in 4–10% of vaccinees, but severe reactions have been rare (*3*).

The development of killed plague vaccines from avirulent organisms is desirable for several reasons. In an emergency, it would be relatively easy to increase vaccine production, since no special containment facilities would be required; the requisite potency tests could be done at a regional laboratory equipped for plague work. It is also an attractive alternative for developing nations that desire to use killed vaccines but cannot afford to purchase vaccine from abroad and are unable or unwilling to risk vaccine production from virulent organisms. The work reported here indicates that consistency in vaccine potency should be achieved quite easily. Furthermore, killed vaccines from avirulent organisms should be free from other problems frequently encountered with attenuated living vaccines, such as local variability in seed phenotype (*2*, *16*) and loss of viability during shipment or storage (*17*).

RÉSUMÉ

ACTIVITÉ DES VACCINS TUÉS ANTIPESTEUX PRÉPARÉS À PARTIR DE SOUCHES AVIRULENTES DE *YERSINIA PESTIS*

Le vaccin antipesteux tué USP (préparé à partir de la souche virulente 195/P de *Yersinia pestis*), qui est couramment utilisé aux Etats-Unis d'Amérique, en particulier par l'Armée, et s'est montré apparemment efficace pendant la guerre du Viet-Nam, présente toutefois le désavantage de nécessiter un laboratoire spécialement

équipé sur le plan de la sécurité pour sa production, qui est donc coûteuse. Si les circonstances requéraient une grande quantité de vaccin, les laboratoires spécialement équipés existants pourraient ne pas suffire à leur production et la mise en place de nouvelles installations pourrait prendre du temps. Les vaccins tués préparés à partir de microorganismes avirulents n'exigeraient pas, quant à eux, d'aménagements spéciaux pour assurer la sécurité de la production.

Des vaccins antipesteux tués expérimentaux ont donc été préparés à partir des souches encapsulées EV76S et A1122 de *Y. pestis* avirulent. Trois épreuves d'activité sur la souris ont été faites, et des lots de vaccin antipesteux USP ont été utilisés aux fins de comparaison. Après une première dilution en soluté salin pour obtenir une teneur en azote total équivalente pour les deux types de vaccin, des dilutions de raison 3 ont été préparées, puis inoculées aux souris.

Les vaccins tués utilisant *Y. pestis* avirulent ont fait preuve d'une plus forte activité que le lot de vaccin USP employé. Le vaccin tué EV76S a conféré aux souris une protection légèrement supérieure à celle obtenue avec le vaccin A1122 quand la dose d'épreuve était faible, mais les deux vaccins expérimentaux ont donné des résultats comparables dans les autres cas. Deux lots de vaccin A1122 tué, préparés à 18 mois d'intervalle et essayés concurremment, ont conféré aux souris une protection à peu près identique.

En cas de nécessité, la production à grande échelle de vaccin antipesteux à partir de *Y. pestis* avirulent pourrait être réalisée rapidement. Les épreuves d'activité requises pourraient être effectuées dans un laboratoire régional équipé pour les travaux sur la peste. On n'aurait pas à redouter, avec les vaccins tués préparés à partir de microorganismes avirulents, les problèmes divers qu'ont coutume de poser les vaccins vivants atténués, tels que la variabilité locale du phénotype de semence et la perte de viabilité lors du transport et du stockage. La production de vaccins tués à partir de microorganismes avirulents pourrait constituer une bonne solution pour les pays en développement désireux d'utiliser des vaccins tués, mais qui n'ont pas les moyens d'en acheter à l'extérieur et qui ne peuvent ou ne veulent assumer les risques de la production de vaccins à partir de microorganismes virulents.

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