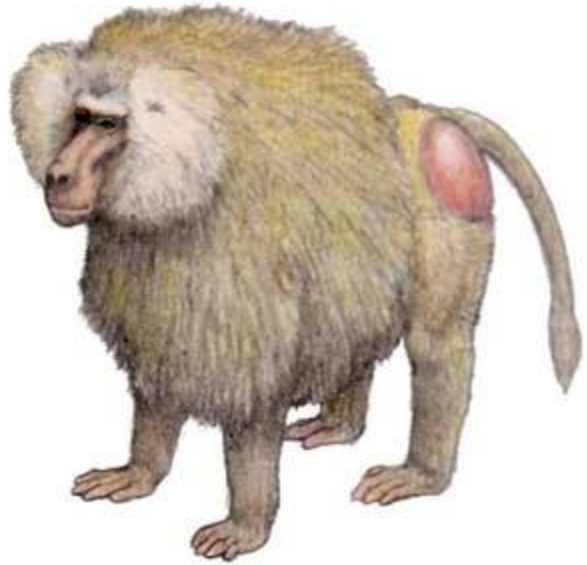
Animal models for plague vaccine testing

Andrey P. Anisimov

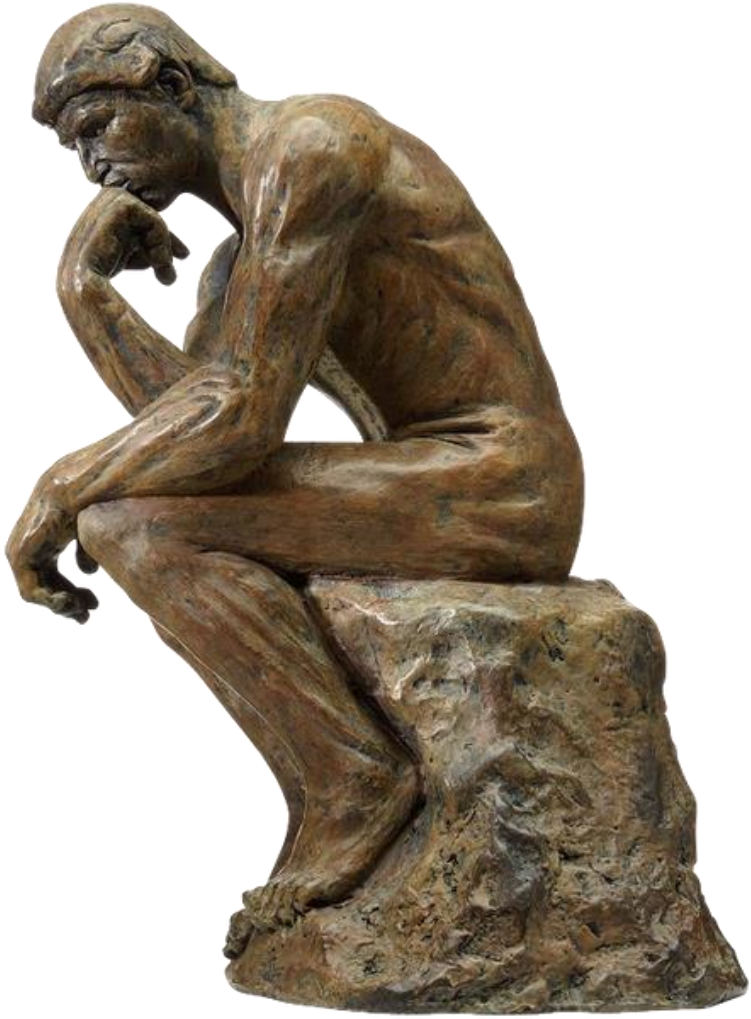
(State Research Center for Applied Microbiology and Biotechnology, Russia)

Our work is supported by the Russian Science Foundation (grant number 23-15-00132).

Main requirements for vaccine strains of the plague pathogen:
Methodological Guidelines MU 3.3.1.1113-02



Hamadryads baboon
(*Papio hamadryas*)



102-108 | ACTA NATURAE | VOL. 7 № 1 (24) 2015

Selective Protective Potency of *Yersinia pestis* Δ*nlpD* Mutants

S. V. Dentovskaya[1], S. A. Ivanov[1], P. Kh. Kopylov[1], R. Z. Shaikhutdinova[1], M. E. Platonov[1], T. I. Kombarova[1], T. V. Gapel'chenkova[1], S. V. Balakhonov[2], A. P. Anisimov[1]*

[1]State Research Center for Applied Microbiology, Obolensk, Moscow Region, 142279, Russia

[2]Irkutsk Antiplague Research Institute of Siberia and Far East, Trilissera Str., 78, 664047, Irkutsk, Russia

*E-mail: anisimov@obolensk.org, a-p-anisimov@yandex.ru

Received: 12.06.2014

ABSTRACT It has recently been shown that the NlpD lipoprotein is essential to *Yersinia pestis* virulence and that subcutaneous administration of the *nlpD* mutant could protect mice against bubonic and pneumonic plague better than the EV vaccine strain [PLoS One 2009. V. 4. № 9. e7023]. In this study, similar Δ*nlpD* mutants were generated on the basis of other *Y. pestis* parent strains, including strains from the subspecies *microtus*, which is avirulent to guinea pigs and humans. Comparative testing confirmed that immunization of mice with Δ*nlpD* mutants induces immunity 10^5 times more potent than the one induced by the administration of the EV vaccine strain. At the same time, NlpD$^-$ bacteria failed to protect guinea pigs in the case of a subcutaneous challenge with *Y. pestis*, inducing a 10^6 times less potent protection compared with that conferred by immunization with the EV vaccine strain. The possible causes of the observed phenomena are discussed.

KEYWORDS *Yersinia pestis*, Δ*nlpD* mutant, selectivity of protective potency, live plague vaccine.

of the immunizing action of whole-cell plague vaccines. At the same time, there are publications in which the protectiveness of some of the aforementioned antigens is denied.

3. Animal Models for Testing Potency of Plague Vaccines

The above results were obtained mostly on animals of the same species—mice. The presence of such antigens, apparently, will not be sufficient for the formation of immunity in guinea pigs and primates, or many of them may not be protective at all. Currently, real attempts to design a subunit plague vaccine are associated with the use of only F1 and V antigens. The effectiveness of immunization with these two antigens was evaluated in experiments not only on mice, but also on animals of other species—rats, guinea pigs, non-human primates. As it turned out, the protective potency of antigens depended on the species of laboratory animals [52,53,89]. Several plague subunit vaccine candidates have been going through various stages of clinical trials for some years [90,91], but only one of them, the plague molecular microencapsulated vaccine, has now received a registration certificate in Russia [92]. After two subsequent vaccinations of volunteers, 67% of them developed specific antibody titers equal to or exceeding the threshold level, while 33% of the subjects did not develop specific antibodies to either V or F1 antigens. Seronegative individuals differed from responding ones by alleles of single nucleotide polymorphisms in 14 of immune response genes [4].

Table 1. Protective potency of *Y. pestis* native antigens for laboratory animals infected with the plague pathogen.

Antigen or Vaccine	Challenge Technique	Protective Potency for		
		Mice	Guinea Pigs	Nonhuman Primates
F1 (Caf1)	percutaneous [1]	+ [52]	+ [52,56] [2]	+ [57]
	respiratory	+ [58]	+ [27]	+ [53,59] [2]
V (LcrV)	percutaneous	+ [60]	+ [28]	?
	respiratory	+ [60]	?	− [61] [3]
«Murine» toxin	percutaneous	+ [62]	− [63]	?
	respiratory	?	?	?
Superoxide dismutase	percutaneous	?	+ [64]	?
	respiratory	?	?	?
YopD	percutaneous	+ [65]	?	?
	respiratory	?	?	?
YpkA	percutaneous	+ [65] [4]	?	?
	respiratory	?	?	?
YscF	percutaneous	+ [66] [5]	?	?
	respiratory	?	?	?
OppA	percutaneous	+ [67] [4]	?	?
	respiratory	?	?	?
YadC	percutaneous	?	?	?
	respiratory	+ [68]	?	?
PsaA	percutaneous	− [69]	?	?
	respiratory	+ [70]	?	?
YP00606	percutaneous	+ [71]	?	?
	respiratory	?	?	?
YPO1914	percutaneous	+ [71]	?	?
	respiratory	?	?	?
YPO0612	percutaneous	+ [71]	?	?
	respiratory	?	?	?
YPO3119	percutaneous	+ [71]	?	?
	respiratory	?	?	?
YPO3047	percutaneous	+ [71]	?	?
	respiratory	?	?	?
YPO1377	percutaneous	+ [71]	?	?
	respiratory	?	?	?
YPCD1.05c	percutaneous	+ [71]	?	?
	respiratory	?	?	?
YPO0420	percutaneous	+ [71]	?	?
	respiratory	?	?	?
YPO3720	percutaneous	+ [71]	?	?
	respiratory	?	?	?
"B antigen" (OMVs)	percutaneous	− [72]	+ [72,73]	?
	respiratory	?	+ [72]	+ [72,74] [6]
EV live vaccine [7]	percutaneous	+ [75]	+ [76]	+ [77]
	respiratory	?	+ [78]	+ [79]
USP killed vaccine [7]	percutaneous	+ [75]	+ [75]	+ [80]
	respiratory	− [81]	+ [27]	− [53]

[1] Percutaneous mode of infection means the subcutaneous, intramuscular, or intradermal challenge. [2] Lebedinsky et al. [56] and Byvalov et al. [59] presented experimental evidence for the protective potency of the F1 antigen as a booster for animals primarily immunized with the EV live vaccine. [3] Li and Yang [61] gave indirect data on the V antigen low protection of primates based on the assessment of the effectiveness of their immunization with a F1+V complex preparation. [4] In these studies, the protective effect after inoculation with YpkA and OppA was recorded only by extending the life span to death in immunized animals after infection. [5] Protection of mice by intraperitoneal immunization with YscF is indicated for the intravenous route of infection. [6] The significance of the B antigen in immunogenesis is evidenced by the data on the protective potency of the complex of F1 and B antigens and lack of protection (under the conditions of this experiment) of the single F1 antigen administered at the same dose. [7] Prior plague vaccine attempts are described in [3,37,82,83]. "+"—antigen is protective; "−"—non-protective; "?"—no data.

biomolecules

MDPI

Review

Yersinia Outer Membrane Vesicles as Potential Vaccine Candidates in Protecting against Plague

Andrey A. Byvalov [1,2,*], Ilya V. Konyshev [1,2], Vladimir N. Uversky [3,4,*], Svetlana V. Dentovskaya [5] and Andrey P. Anisimov [5,*]

vaccines

MDPI

Article

Peptidoglycan-Free Bacterial Ghosts Confer Enhanced Protection against *Yersinia pestis* Infection

Svetlana V. Dentovskaya [1], Anastasia S. Vagaiskaya [1], Mikhail E. Platonov [1], Alexandra S. Trunyakova [1], Sergei A. Kotov [2], Ekaterina A. Krasil'nikova [1], Galina M. Titareva [1], Elizaveta M. Mazurina [1], Tat'yana V. Gapel'chenkova [1], Rima Z. Shaikhutdinova [1], Sergei A. Ivanov [1], Tat'yana I. Kombarova [3], Vladimir N. Gerasimov [2], Vladimir N. Uversky [4] and Andrey P. Anisimov [1,*]

- As a rule, the majority of plague vaccine candidates are developed on the basis of the two antigens that are highly protective for mice, F1 and V, which protect other animal species to a lesser extent [13]. The F1-V fusion protein vaccine protected cynomolgus macaques, but largely failed to protect African green monkeys [30], raising concerns that humoral immunity targeting F1 and V might be inadequate in protecting people from pneumonic plague. **According to Li *et al.*, in the case of post-infection immunity, the seroprevalence to the F1 antigen in all patients recovered from plague was 78.5% [**Li, B. *et al.* Vaccine Immunol. 2012, 19, 228–234**], while in the case of the V antigen it was only 28.6% [**Li, B. *et al.* Microbes Infect. 2008, 10, 45–51**].**

ELSEVIER

Vaccine

Volume 28, Issue 6, 10 February 2010, Pages 1655-1660

Comparison of mouse, guinea pig and rabbit models for evaluation of plague subunit vaccine F1+rV270

Zhizhen Qi [a 1], Lei Zhou [b 1], Qingwen Zhang [a], Lingling Ren [a], Ruixia Dai [a], Benchuan Wu [c], Tang Wang [b], Ziwen Zhu [b], Yonghai Yang [a], Baizhong Cui [a], Zuyun Wang [a], Hu Wang [a], Yefeng Qiu [b], Zhaobiao Guo [b], Ruifu Yang [b], Xiaoyi Wang [b]

Show more

https://doi.org/10.1016/j.vaccine.2009.02.078

was evaluated for protective efficacy using mouse, guinea pig and rabbit models in comparison with the live attenuated vaccine EV76. Complete protection against challenging with 10^6 colony-forming units (CFU) of virulent *Yersinia pestis* strain 141 was observed for mice immunized with the subunit vaccines and EV76 vaccine. In contrast, the subunit vaccine recipes VII (F1-20μg+rV270-10μg) and IX (F1-40μg+rV270-20μg) and EV76 vaccine provided 86%, 79% and 93% protection against the same level of challenge in guinea pigs and 100%, 83% and 100% protection in rabbits, respectively. The immunized mice with the vaccines had significantly higher IgG titres than the guinea pigs and rabbits, and the immunized guinea pigs developed significantly higher IgG titres than the rabbits, but the anti-F1 response in guinea pigs was more variable than in the mice and rabbits, indicating that guinea pig is not an ideal model for evaluating protective efficacy of plague subunit vaccine, instead the rabbits could be used as an alternative model. All the immunized animals with EV76 developed a negligible IgG titre to rV270 antigen. Furthermore, analysis of IgG subclasses in the immunized animals showed a strong response for IgG1, whereas those receiving EV76 immunization demonstrated predominant production of IgG1 and IgG2a isotypes. The subunit vaccine and EV76 vaccine are able to provide protection for animals against *Y. pestis* challenge, but the subunit vaccines have obvious advantages over EV76 in terms of safety of use.

Complete protection against challenging with 10^6 CFU of virulent *Yersinia pestis* strain 141 was observed for mice immunized with the subunit vaccines and EV76 vaccine. In contrast, the subunit vaccine recipes VII (F1-20 μg + rV270-10 μg) and IX (F1-40 μg + rV270-20 μg) and EV76 vaccine provided 86%, 79% and 93% protection against the same level of challenge in guinea pigs and 100%, 83% and 100% protection in rabbits, respectively. The immunized mice with the vaccines had significantly higher IgG titres than the guinea pigs and rabbits, and the immunized guinea pigs developed significantly higher IgG titres than the rabbits, but the anti-F1 response in guinea pigs was more variable than in the mice and rabbits, indicating that guinea pig is not an ideal model for evaluating protective efficacy of plague subunit vaccine, instead the rabbits could be used as an alternative model. All the immunized animals with EV76 developed a negligible IgG titre to rV270 antigen. Furthermore, analysis of IgG subclasses in the immunized animals showed a strong response for IgG1, whereas those receiving EV76 immunization demonstrated predominant production of IgG1 and IgG2a isotypes. The subunit vaccine and EV76 vaccine are able to provide protection for animals against *Y. pestis* challenge, but the subunit vaccines have obvious advantages over EV76 in terms of safety of use.

vaccines

MDPI

Article

Peptidoglycan-Free Bacterial Ghosts Confer Enhanced Protection against *Yersinia pestis* Infection

Svetlana V. Dentovskaya [1], Anastasia S. Vagaiskaya [1], Mikhail E. Platonov [1], Alexandra S. Trunyakova [1], Sergei A. Kotov [2], Ekaterina A. Krasil'nikova [1], Galina M. Titareva [1], Elizaveta M. Mazurina [1], Tat'yana V. Gapel'chenkova [1], Rima Z. Shaikhutdinova [1], Sergei A. Ivanov [1], Tat'yana I. Kombarova [3], Vladimir N. Gerasimov [2], Vladimir N. Uversky [4] and Andrey P. Anisimov [1,*]

The outcomes of bacterial infections and the immune response to bacterial vaccines are highly variable in different animal species and even in diverse intraspecies groups [34–36]. At least a partially successful solution to this problem is generated by replacing very popular inbreds of one mammalian species with outbred biomodels of two or three species [2,37,38], or with carefully selected panels of several inbreds [39]. Both of these approaches aim to reproduce the diversity of clinical pictures and immune responses detected in the natural animal and human populations. However, this approach will only provide an understanding of what proportion of the immunized remained unprotected, but in no way will protect seronegatives and/or T cell-negatives. To ensure the protection of that part of the immunized that does not respond to the antigens already included in the vaccine, it is necessary to introduce into the vaccine composition of conservative protective antigens, characterized by minimal polymorphism of their molecular structure and epitopes, as well as the uniformity of the protective immune response in different genotypes of immunized individuals.