CONCERNING THE VARIABILITY OF THE PLAGUE VACCINE STRAIN EV

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Over a period of many years the plague microbe vaccine strain EV has been used successfully for the preparation of dry live vaccine. It has proven itself as the most stable vaccine strain, preserving its initial properties under favorable conditions (in a freeze dried state). There is great interest in data indicating the possibility of the directed mutability of this strain and in conditions furthering the development of hereditary changes in its cultural and immunogenic properties. Cases have been described in the literature when under the influence of plague bacteriophage mutants have been obtained which differed sharply from the initial culture of the plague microbe based on their cultural-biochemical properties. (Pokrovskaya, 1934; Zhukov-Verezhnikov and Khvorostuhkina, 1936; Korobkova, 1937). It is also known that after prolonged maintenance on nutrient media there may be a lowering in the immunogenicity of cultures of the EV vaccine strain. At the present time the NIEG variant is the most full-valued of the various variants of this strain.

In this paper a study is made of the properties of two variants of the EV strain which originated under completely different conditions.

In 1963 during the process of controlling the dry live plague vaccine from the EV NIEG strain, prepared under aeration conditions in a reactor at the "Mikrob" Institute, we isolated an achromogenic variant of this strain. When the vaccine was inoculated on an agar surface, along with the typical chromogenic colonies of the EV strain several colonies grew which were achromogenic, semihyaline, and strongly tuberous with wavy peripheries. This variant, which we named "EV achromogenic 1963" (EV achr.-63), based on its cultural-morphological properties was similar to the achromogenic variant obtained by us in 1954 (Ivanov et al.,
1956) artificially by means of the action of P$_{32}$ on the EV vaccine strain and named "EV achromogenic 1954" (EV achr.-54).

We made a comparative study of both of these variants and the standard EV strain of the NIIEG line. It was established that the standard NIIEG EV strain grows on nutrient agar in the form of chromogenic rough colonies, typical for the plague causative agent. In contrast to the achromogenic variants, in passing light the colonies had a dark color, were less tuberous, and had even edges and somewhat smaller dimensions. The bacterial mass was compact. When taken with a loop the colonies were removed from the agar easily. It was not possible to obtain a homogeneous suspension in a physiological solution.

When the EV standard strain was incubated in broth a surface film was formed, bottom growth in the form of a precipitate was observed, and the broth did not become turbid. When the EV achr.-54 was cultivated, a uniform turbidity was observed, bottom growth and surface film were absent. EV achr.-63 turned the broth slightly turbid, and an insignificant bottom growth and a small film on the surface of the broth were observed. Based on the nature of growth in broth the achromogenic variants were similar to the smooth forms of the plague causative agent.

The EV standard strain and its achromogenic variants formed a capsule only during the process of incubation at 37°C.

In smear imprints from colonies of a 24-hour culture of EV achr.-54 and EV achr.-63 the microbes were the same in size. In microcolonies they were arranged singly, some paralllely by 2--3's, while the cells of the EV standard strain were arranged in a chain in microcolonies and were somewhat larger.

The inoculating dose of achromogenic variants of the EV plague microbes was less than the standard strain. When the same number of plague microbes (based on the GKI turbidity standard) from a 2-day agar culture of EV NIIEG was inoculated, 75 colonies grew, while for EV achr.-54 it was 132 and for EV achr.-63 it was 142 colonies. In dishes with nutrient agar the colonies of the standard strain grew isolatedly, and the colonies of achromogenic variants were arranged in groups.

The biological activity of the standard strain and its achromogenic variants was checked on Hiss media with the following carbohydrates: Glucose, maltose, saccharose, ramnose, lactose, arabinose (with Andrade indicator). Only the achromogenic variant -54 decomposed ramnose on the 17th day. These variants did not decompose other sugars and also glycerin.

They did not liquefy gelatin. The results were considered beginning 8...24 hours and up to 21 days.
The achromogenic variants preserved a sensitivity to polyvalent plague bacteriophage, but during titration by the Appelman method a culture of achromogenic variants was lysed worse by plague phage than a culture of the standard strain. In 2 tests, yielding similar results, the EV standard strain was lysed by phage in dilutions up to 10^{-11}, EV achr.-54 - up to 10^{-5}, and EV achr.-63 - up to 10^{-6}.

The ability of the tested variants to "take" in the organism of animals was checked by means of introducing a suspension of a 48-hour agar culture containing 2 billion microbial cells under the skin of guinea pigs. The animals were sacrificed on the 5th, 10th, 15th and 20th day (2 pigs for each period). Inoculations in Hottinger broth and dishes with nutrient agar were made from tissue from the site of administration, the lymph nodes and parenchymatous organs of the pigs.

From the guinea pigs inoculated with the achromogenic variants the plague microbe was isolated only on the 5th day from the site of administration, the regional node, liver and spleen. In latter periods a culture of the variants was not detected in the seedings. From guinea pigs inoculated with the EV standard strain a culture of the plague microbe was sown in even later periods - on the 10th day after inoculation (in seedings from the spleen). It should be noted that when the organs of the animals were inoculated in Hottinger broth the plague microbe was sown more often than when inoculated on nutrient agar.

We also studied the toxicity of cultures of the achromogenic variant EV achr.-63 and compared it with the toxicity of the EV standard strain. An 0.5 ml suspension of a 2-day agar culture of the EV standard strain and its variant EV achr.-63 was administered to mice (weight 16 - 18 grams) intraperitoneally in various concentrations.

It can be seen from the table that when small doses were used the toxicity of the achromogenic variant was somewhat lower than the EV NITEG standard strain.

The variants under study possessed a various capability for causing an allergic reorganization, exposed by the intracutaneous administration of the GKI pest allergen, in animals inoculated with them. The EV standard strain caused a well expressed allergic reorganization in guinea pigs inoculated with 1,000,000 and 10,000 microbial cells. The EV achr.-54 did not lead to an allergic reorganization in guinea pigs following immunization with the stated doses, and the EV achr.-63 caused a significantly less expressed allergic reorganization in the pigs than the EV standard strain.

Both achromogenic variants of the EV strain possessed low immunogenic properties. This was established in preliminary tests in mice.
Immunogenicity was studied with the help of the method based on the use of cortisone (Blyakher, 1958, 1960). The test, conducted on 600 mice, showed that both achromogenic variants possessed lower immunogenic properties than the EV NIIEG standard strain.

These data were supported in a test on guinea pigs with their controlled infection with a virulent EV culture. EV achr.-54 and -63 in doses of 10,000; 100,000 and 1 million microbial cells did not protect the animals from infection with 200 Dcl of a virulent strain of the plague causative agent, while in the stated doses the standard strain guaranteed complete nonsusceptibility.

The stability of EV achr.-54 was sufficiently high: In the course of 11 years (period of observation) it preserved the cultural-morphological features acquired by it as a result of the action of P32. Over this period 20 passages in guinea pigs were made, 5 passages in white mice, and more than 200 sub-cultures on nutrient media, of which 5 sub-cultures were on a medium with antiphage serum. Three times the culture was subjected to freeze drying but the strain rigidly preserved the cultural-morphological features.

The EV achr.-63 variant, isolated from plague vaccine, also preserved the properties acquired by it persistently, but its period of observation was considerably less (2 years).

The opinion exists that the mutability of the plague microbe is connected with the presence of specific phage. Therefore we turned our attention to this aspect of the problem. Lysogenicity of the achromogenic variants (presence of prophage) was studied by means of a thorough study of the structure of the colonies, appearance of the Twort phenomenon, formation of daughter colonies after repeated cultivation under conditions of aeration (3 passages), and a study of the filtrate of old cultures, and also on agar with antiphage serum.

Not in one of the methods used were we able to establish the presence of phage in the achromogenic variants of the EV strain.

Thus during the process of the commercial preparation of live plague vaccine we disclosed the formation of achromogenic variants of the EV vaccine strain which were sufficiently stable and differed from the standard strain by their cultural-morphological features and a reduced ability to take root in guinea pigs and to cause allergy and immunity in inoculated animals. These circumstances again confirm the necessity for the constant systematic study of the biological characteristics of the commercial vaccine strain.
Literature


Pokrovs'kaya, M. P., Ibid., 1934, No 1, p 3.
Comparative study of the toxicity of cultures of the standard EV strain and its variant EV achr.-63 in mice

<table>
<thead>
<tr>
<th>Dose of culture (number of microbial cells)</th>
<th>Standard strain EV NIEB</th>
<th>Achromogenic variant -63</th>
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<tbody>
<tr>
<td>Number of mice in test</td>
<td>Death of mice</td>
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<td></td>
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