

THE POSITION OCCUPIED BY VACCINE "ATTENUATED" STRAINS OF BACTERIA IN THE  
SYSTEM OF MICROORGANISMS

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## THE POSITION OCCUPIED BY VACCINE "ATTENUATED" STRAINS OF BACTERIA IN THE SYSTEM OF MICROORGANISMS

[Following is the translation of an article by R. A. Saltykov, State Control Institute of Medical and Biological Preparations imeni Terasevich, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology), No 9, 1965, pages 126-127. It was submitted on 15 Aug 1964, Translation performed by Sp/7 Charles T. Ostertag, Jr.]

Live vaccines are being used all the more extensively in the struggle with infectious diseases. Against a background of a many-sided experimental study of these preparations, problems connected with the rational nomenclature of vaccine strains of bacteria, with the establishment of their taxonomic category and position in the system of microorganisms, have not been worked out sufficiently. Apart from theoretical interest, the solving of these problems has already become a requirement in the production and application of live vaccines. The disorderliness in the nomenclature of the bacteria under discussion leads, on the one hand, to the disregarding of the main differences between the pathogenic strains of the causative agent and the vaccine strains, which are not capable of causing a specific disease in an inoculated organism. On the other hand, vaccine strains are used as certain static variants, described and characterized once and for all, without taking into consideration the possibility of their mutability. As a result, in different laboratories microorganisms are recorded under the same classification number of a vaccine strain and in actuality they are different: No less than 9 substrains of the BCG are known, several lines of the EV plague strain, no less than 4 essentially different tularemia strains of Gayskiy No 15, and non-uniform populations of the STI-1 anthrax strain. Such a position is fraught with serious errors in the comparative evaluation of various vaccine strains and vaccines when it is desired to select the best preparations.

It is possible to consider the taxonomic category of only certain concrete microorganisms, and not vaccine strains in general. In the present report we have attempted to establish the taxonomic category and rational nomenclature of 3 vaccine strains: Anthrax STI-1 Ginsburg, tularemia No 15 Gayskiy, and the EV Plague of Girard and Robic. As a basis here we used the data of our 20 year experience in working with the stated strains of bacteria and the data from the literature.

The fundamental difference of vaccine strains from the initial forms of the causative agents of infectious diseases is found in the qualitative change of their pathogenicity (Ginsburg, 1946, 1960, Korobkova, 1956; Kravchenko, 1948; Saltykov, 1964). Therefore, the terms "avirulent", "attenuated" and "strain with a residual virulence", which are used in

literature for characterising the vaccine strains, cannot be accepted as successful, since they do not express exactly the qualitative peculiarities of the microorganism.

The vaccine strains under study are apathogenic for man and farm animals, for the immunization of which the corresponding live vaccines are used. Under any known conditions of administration (subcutaneous, cutaneous and inhalation), even in massive doses, live cultures of these bacteria are not capable of causing a clinically expressed infectious disease - anthrax, tularemia or plague. Convincing data has been gathered which indicated that the apathogenicity of vaccine strains for man and farm animals cannot be overcome by the magnitude of the "infecting" dose -- the qualitative peculiarities of the vaccine culture cannot be compensated by its quantity.

For certain laboratory animals -- guinea pigs, white mice, these vaccine strains are pathogenic and are able to cause an expressed disease in them, sometimes with a lethal outcome. But, in these cases the manifestation of the so-called residual virulence characterizes qualitative, and not just quantitative differences in the strains from the initial pathogenic forms of causative agents.

As is known the virulence of separate strains of causative agents of plague, tularemia and anthrax may fluctuate within considerable limits. But during the infection of animals with cultures of these strains, the quantitative difference in their virulence may be compensated for by the selection (under titration) of the infecting doses. In animals, infected with a selected dose, a clinically and pathologoanatomically expressed specific illness is observed. However, those same white mice and guinea pigs endure the parenteral administration of vaccine cultures in amounts which by a million times exceed the lethal doses of the pathogenic initial forms. But even with the administration of such massive doses it is practically impossible to establish the minimum lethal dose of the vaccine culture. Consequently in this case also the qualitative difference in the pathogenicity of the vaccine strains cannot be compensated for by the quantitative variation of the infection dose. If as the result of the administration of a vaccine strain an animal becomes ill, the pathological process proceeds uniquely, differently from the process which is caused in the same species of animals by the initial pathogenic forms of causative agents (Pokrovskaya, Kaganova, 1947; Chalisov and Tamarin, 1946; Chalisov and Spasskaya, 1948).

In strains of pathogenic bacteria with a lowered virulence the latter is increased relatively easily by passaging through the organism of animals which are susceptible to infection. In contrast to this, comprehensive and diverse experimental investigations, including tests on numerous passaging through the organism of animals of various species, ages, and degree of anti-infectious resistance (Yemelyanova, 1963; Motornaya, 1953; Saltykov, 1946), conclusively demonstrated the impossibility of any significant increase in the virulence of the STI-1, EV and the tularemia No 15 strains.

Up to the present time materials from many years of work have accumulated on the results of vaccination of many millions of people in the Soviet Union and abroad with live vaccines from these strains (EV strain), and also the annual multimillion vaccination of farm animals (STI-1 strain). This huge experience in practice together with experimental data testifies unconditionally to the fact that the theoretical discussion, which has increased in due course, concerning the possibility of the unexpected recovery of virulence in vaccine strains, and the danger of their reversion into the initial pathogenic form of the causative agent, preserves only an historical interest at the present time (Ginsburg, 1947; Shmalgauzen, 1946).

The loss by these microorganisms of the specific pathogenicity which is inherent to their initial forms, is a persistent, hereditarily secured feature of vaccine strains.

Another existing peculiarity of the vaccine strains is their preservation of the ability to "take root," to multiply in the inoculated organism of man, causing a unique process -- vaccinal infection, which is characterized by the subclinical course like a type of inapparent infection, without the development of an infectious disease, by the benignancy and reversibility of the pathomorphological changes, and by the development of an immunity of a high intensity (Ginsburg, 1946; Zdrodovskiy, 1953; Zilber, 1958; Zhdanov, 1958, and others).

Together with the persistent loss by the vaccine strains of the pathogenicity which is inherent to the initial forms, the ability of these strains to take root in the organism of man or animal is a less persistent feature. When it loses its ability to cause a vaccinal infection in the organism, a bacterial strain is deprived of immunogenic properties and loses its importance as a vaccine. Mutability of vaccine strains in the direction of saprophytization and the lowering of immunogenicity are furthered by the changing of generations of cultures on artificial nutrient media. Vaccine strains are most successfully stabilized by being switched to an anabiotic condition (with a minimum number of sub-cultures). Based on the data of our laboratory, a dry spore culture of the STI-1 strain preserved its original characteristics following 18 years of storage, the EV NIEG strain remained highly immunogenic for no less than 15 years, and a dry culture of tularemia strain No 15 (variant NIEG) preserved its original properties for 10 years (the periods of observation are indicated).

Thus the experimental data of various authors, including our material and that of our co-workers, and also the many years of experience in using live vaccines, conclusively testify to the fact that the anthrax STI-1, the plague EV, and the tularemia No 15 strains possess hereditarily fixed qualitative differences from the initial pathogenic forms of microorganisms. Acceptance of such a position requires a determination of the position of vaccine strains in the system of microorganisms, their taxonomic category and relation to the typical representatives of the species of causative agent. This will also make it possible to

establish a rational nomenclature for these microorganisms. The term "strain" is very widespread and indefinite in microbiology. As a rule, it is used without any taxonomic significance (Krasilnikov, 1949). The term "variant" which is used in the literature only indicates a deviation in the characteristics of the organism from those which are typical for the specific species, without specifying either the degree or the persistence of this deviation.

In 1952 some works appeared in which the authors considered it possible to recognize certain vaccine strains as new independent species of bacteria. Muromtsev considered the anthrax vaccine strains as new species, stemming from the fact that these strains lost their pathogenicity; Mikhaylov recognized the feasibility of isolating the tularemia strain No 15 into a new species of bacteria on the basis of experiments in which strain No 15, following numerous passages on a nutrient medium in a mixture with a virulent strain of the tularemia causative agent, dislodge the latter, which in the opinion of the author was a display of interspecies antagonism. In experimental investigations with strain STI-1 (Kolesov, 1955) and with strain EV (Shmuter, 1958) no antagonistic relations were observed between these vaccine strains and the typical forms of the corresponding causative agents of anthrax and plague. However, Lysenko's proposed criterion of antagonistic relations, supposedly characterizing only interspecies relations, will be subject to serious revision and its reliability is doubtful (Davitashvili, 1957; Kudlay, 1958; Shevelev, 1958). A group of authors from the Saratov Antiplague Institute "Mikrob", in describing species forming mutability of the plague microbe, do not regard the EV strain as a new species of bacteria (Lenskaya, 1951). Zhukov-Verezhnikov and Sokolov (1960) point out that "Forms of a microbe which are connected with species forming mutability are not suitable for use as vaccines." Yelin (1954), easily recognizing the possibility of species formation of microbes under laboratory conditions, does not rank vaccine strains of bacteria to new species. Ginsburg (1946, 1960), Grinbaum (1949), Togunova (1960), Imshenetskiy (1962), Kudlay (1962) and others regard vaccine strains of bacteria to one or the other categories of intraspecies subdivisions.

In stressing the qualitative differences of vaccine strains, we still do not consider them sufficient for the isolation of these forms of bacteria into new independent species. As is known, immunological specificity is a very fine criterion for the degree of affinity of organisms, and its test is often used for establishing phylogenetic bonds between various living creatures. A characteristic peculiarity of the vaccine strains under consideration is their ability to cause a vaccinal infection in the inoculated organism. This creates an immunity of a high intensity against the typical representatives of plague, anthrax or tularemia microbes. This circumstance testifies unconditionally to the extreme closeness (if not identical) of the main antigenic properties, and consequently the principle similarity of metabolism in the initial pathogenic forms and vaccine strains, which determines the species specificity of organism. In immunology there are no known reliable facts of immunity against one causative agent which was conditioned by infection with another species of microorganism. No sharp biological break -- an "hiatus" which is characteristic for interspecies differences -- is observed between the initial pathogenic forms and the vaccine strains derived from them.

On the basis of the above data and considerations, we suggest that the vaccine strains STI-1, EV and tularemia No 15 should be taxonomically specified as subdivisions within the species of the corresponding anthrax, plague and tularemia microbes, as varieties or subspecies of them. Therefore, we consider it feasible to speak of typical representatives of the above species of pathogenic bacteria and their vaccine varieties. With such a taxonomic definition and in accordance with the nomenclature which is used in the systematization of bacteria, it is proposed that the microorganisms under consideration be named in the following manner: *Bacillus anthracis* varietas *Sti-1*, Ginsburg, 1940; *Pasteurella pestis* var. EV, Girard et Robic, 1928; *Francisella tularensis* var. No 15, Gaysky, 1942.

Under actual conditions the stated varieties of bacteria exist in the form of individual cultures -- populations with possible quantitative differences between them, caused by the appearance in the cultures of new variants -- dissociants which disrupt the genetic homogeneousness of the population. In respect to these various culture-populations we suggest the preserving of the use of the term "strain" as a practical and industrial one, but not as a taxonomic one. As an example, it is possible to speak of various strains of the EV variety of the plague microbe: Such strains are known as EV-NIIEG, EV76, EV-Parisian, EV hyaline and others. A strain which is genetically homogeneous and typical for a given variety is accepted as standard. Only such a strain should be utilized in comparative experimental investigations and for the production of live vaccine. In as much as the laboratory maintenance of microbes on nutrient media usually leads to a disruption of the genetic homogeneousness of the population, the preservation of the typicalness and full-value of the standard strains requires the special selection of conditions for their storage and systematic selective work with them.

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