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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

Passive immunization of mice with anti-capsular anthrax immune serum.

by J. Tomcsik and G. Bodon.

Translated from: Kolle, Probleme der Bakteriologie, usw., 1935, p. 114-116.

Brief report.

Our concepts of the nature of anthrax immunity have experienced little change since Pasteur's times. The endeavors of the past years to render active immunization of animals more successful and less dangerous by the use of saponin vaccines, do not really represent a new principle. Ever since Pasteur, immunity to anthrax has been considered to be an immunity due to super-infection. We know little about its true nature; although we possess a feasible anthrax serum, we designate its specifically active component simply as protective substance.

Investigations started by one of us about 10 years ago have directed attention to another area. After exhaustive analyses of the antigenic structure of anthrax bacilli, the assumption was expressed that a considerable immunity to anthrax could be evoked with a chemically definable constituent of killed, corporeal anthrax bacilli.

According to studies by Tomcsik and Szongott (1), the corporeal anthrax bacillus contains two essential antigenic components. The specific constituent of the bacterial soma is a polysaccharide that produces the socalled thermoprecipitation reaction with currently known precipitating serum. The bacterial capsule, on the other hand, contains a specific protein. As is well known, the situation is quite different in the other capsular bacteria, where the capsular substance that determines type specificity is composed of different carbohydrates (Avery and his coworkers).

It was demonstrated further that the immunization of rabbits with killed corporeal anthrax bacilli produces an immune serum which gives a strictly specific precipitation and complement fixing reaction with purificd capsular substance in dilutions of several millions.

The effect of this immune serum on live corporeal bacteria may also be shown microscopically, as recently observed by us (2). The capsule of the corporeal anthrax bacillus is invisible in the ordinary moist preparation (hanging drop), even if a loopful of normal serum or polysaccharide-precipitating anthrax immune serum is added. The microscopic picture changes radically, however, when our specific protein immune serum is admixed. The capsule appears as a yellow positive substance due to altered light refraction.

This leads to a clear understanding of the fact that the anti-capsular serum produces a strictly specific agglutination only with encapsulated bacteria, as we have demonstrated recently (3).

These findings, essentially of theoretical interest, allow the formulation of a number of practically significant working hypotheses. The most important question seems to be the following: Is it possible to bind the anthrax bacillus protein specifically in vivo with our immune serum and to influence the disease favorably in this manner? We tried to solve this problem by passive immunization of different animal species. At this time only the test with mice will be discussed briefly, since these have contributed the most important findings to the clarification of anthrax immunity. It is highly unusual that the clearest results were achieved in experiments with this species which, according to past experience, has proved itself least suited to the study of the specifically protective power of anthrax immune serum.

In our preliminary tests we were obliged to find a strain of anthrax that possessed attenuated virulence even for mice, although it would cause a positively lethal outcome when instilled in sufficient dosages. The strain found to be suitable for these tests (A 15) originally had been isolated in 1900 from ahuman case of anthrax and subsequently had been maintained on agar media. Its virulence had diminished spontaneously to such an extent that rabbits could not be killed even with increased dosages, whereas mice succumbed to the infection within 2-6 days. This was a typical, well-sporulating anthrax strain that never produced capsules on artificial nutrients.

In all of our tests the serum was dispensed as follows: 1 cc prophylactically 24 hours prior to infection, 015 cc ½ hour before inoculation subcutaneously. Aside from our anti-protein serum (anti-capsular serum), the following sera were tested:

1. A polysaccharide-precipitating serum that only reacted with anthrax carbohydrate (anti-C).

2. Protective serum No. 33, produced by inoculation of horses with viable cultures.

3. An immune serum (anti-aggressin) containing anti-bacterial immune substances and anti-aggressins, distinguished by good results in practice.

The table indicates that protective serum No. 33 and the one ontaining anti-aggressins did not have a protective effect in the first test series. It is evident, on the other hand, that our anti-protein serum developed complete immunity.

The infective dosis (1:100,000 slant agar culture) used in the second test series proved to be too weak, since 2 controls survived. It is evident from the table, however, that no other immune serum except anti-protein had any kind of protective effect.

Since the infective doses in the 3rd test series were 100 times larger than the previous ones, the results are correspondingly more lucid. With the exception of one control animal, all animals immunized with protective serum 33, anti-aggressin and anti-C serum, died. All animals inoculated with our serum survived, thus proving to be completely protected against 100 lethal doses. It is particularly significant for the understanding of the mechanism of anthrax immunity that the protective effect of this serum was neutralized by additon of purified protein substance.

Prophylactic protective effect of different anthrax sera against an attenuated strain of anthrax (A 15).

Test series	Serum	Infective dose in slant agar cultures	Number of inoculated mice	Number of dead mice	Average survival in days	Number of surviving mice
I.	---	1/1,000	2	2	3.5	-
	---	1/100,000	2	2	4.5	-
	Protect. serum 33	1/1,000	2	2	2.5	-
	Protect. serum 33	1/100,000	2	2	2	-
	Anti-agressin	1/1,000	2	2	3	-
	Anti-agressin	1/100,000	2	2	3	-
	Anti-protein serum	1/1,000	4	-		4
	Anti-protein serum	1/100,000	4	-		4
II.	---	1/100,000	4	2	4	2
	Protect. serum 33	1/100,000	4	4	4	-
	Anti-agressin	1/100,000	4	4	3	-
	Anti-C	1/100,000	4	2	3.5	2
	Anti-protein serum	1/100,000	8	-		8
III.	---	1/1,000	4	3	2	1
	Protect. serum 33	1/1,000	4	4	2	-
	Anti-aggressin	1/1,000	4	4	2	-
	Anti-C	1/1,000	4	4	3	-
	Anti-protein serum	1/1,000	4	-		4
	Anti-protein serum w/absorbed protein	1/1,000	4	4	2.5	-
IV.	---	1/1,000	4	4	1	-
	Anti-C	1/1,000	4	4	2	-
	Anti-protein serum	1/1,000	4	-		4
	Anti-protein serum w/adsorbed protein	1/1,000	4	4	2.5	-

The results summarized in the 4th test series completely confirmed previous experiences.

The experiments point out that the anti-capsular immune substance prepared by us possesses a reliable protective effect against infection with spontaneously attenuated anthrax bacilli, far superior (at least in tests with mice) to the influence of other anthrax immune sera proved feasible in practice.

Literature.

1. J. Tomcsik and H. Szongott, Z. Immunforschg. 77, p. 86 (1933).
2. J. Tomcsik and G. Bodon, Proc. Soc. exper. Biol. a Med. in press.
3. G. Bodon and J. Tomcsik, Proc. Soc. exper. Biol. a. Med. in press.