THE REPRODUCTION OF PARADOXICAL SENSITIVITY TO STAPHYLOCOCCAL AND CLOSTRIDIUM WELCHII TOXINS

I. N. Morgunov and V. V. Khatuntsev

Kiev Institute of Epidemiology and Microbiology (Director — Candidate Med. Sci. S. N. Terekhov)

(Presented by Active Member AMN SSSR V. N. Chernigovskii)
Translated from Byulleten' éksperimental' noi biologii i meditsiny Vol. 49 No. 3, pp. 73-76, March, 1960
Original article submitted April 18, 1959

The phenomenon of paradoxical sensitivity to toxins, known under the name of Behring's phenomenon, has been produced by many toxins (diphtheria, tetanus, type A and B botulinus and dysentery) tested by various research workers [1- 4]. It has become accepted that summation of the toxic stimulus is possible by the injection of any exotoxin.

In order to confirm the correctness of this view, we made an attempt to reproduce Behring's phenomenon by means of daily injections of sublethal doses of staphylococcal and Clostridium welchii toxins. This parallel study was carried out because the chemical, biological and even the immunological properties of these toxins are very similar.

METHOD AND RESULTS

Experiments were conducted on mice. The toxins were injected daily, intravenously, at intervals of 24 hr. The results of the experiments with different sessional doses of staphylococcal toxin are shown in the table.

It may be seen from the figures given that not one of the 20 mice died after the repeated daily injection of 1/10 MLD. of toxin. After the injection of 1/5 and 1/3 MLD., 1 and 3 mice, respectively, died. The absence of the characteristic signs of staphylococcal toxemia, with the slow onset of death, did not allow death from other causes unconnected with the action of the toxin to be confirmed or excluded. It must be emphasized that in some experiments with a large number of injections of toxin, not one mouse died.

Similar experiments were carried out with Cl. welchii toxin. In two series of experiments, 15 mice were given daily injections of 1/5 MLD of toxin for 12 days. Not one mouse died, although each animal received more than 2 MLD.

We were thus unable to reproduce Behring's phenomenon with the two toxins tested, even in those cases when large doses of the toxins were given. The death of four mice, even if connected with the action of the toxin at all, must be explained by cumulation.

It may be concluded that, so far as their ability to cause the phenomenon of summation of toxic stimuli is concerned, staphylococcal and Cl. welchii toxins differ from those previously studied, after injection of which the phenomenon of paradoxical sensitivity was comparatively easily reproducible.

The inability of the tested toxins to cause disease (lethal poisoning) when injected in fractional doses could be attributed to two reasons.

The absence of deaths among the mice could be the result of a rapidly developing immunity. This explanation seemed to be most likely in the case of the staphylococcal toxin. The blood of laboratory animals very often contains natural antitoxin, which is evidence that these animals in the past have been exposed to the action of a similar antigen, and that their immunological structure has been modified. In these conditions, the frequent injection of fractional doses of toxin may accelerate the formation of immunity, which will develop more quickly than a lethal toxemia will develop. This hypothesis was tested in a small number of experiments. In one of these, ten mice received injections of 1/5 MLD of staphylococcal toxin. After five such injections, all the animals received 1 MLD of the same toxin. Five mice died and the remaining five survived, i.e. immunity developed in half the mice. In another experiment with the same dose

The Results of Experiments With Staphylococcal Toxin

Experiment No.	Sessional dose (MLD)	Number of series of experiments	Total number of animals	Number of injections of toxin	Result of experiment	
					no. of animals dying	no of animals surviving
1	1/3	2	22	18	3	19
2	1/5	2	20	18	1	19
3	1/10	2	20	18	—	20

of toxin (1/5 MLD) the number of injections was increased to 16, counting on a more intensive immunogenesis. When the immunity was tested, however, a dose of 2 MLD caused death of all the animals. In the same experiment five mice received 24 injections of 1/5 MLD of toxin, and then were given a dose of 2 MLD. Two of these mice died and the remaining three survived.

An analogous experiment was performed on 10 mice, which received daily injections of 1/10 MLD of staphylococcal toxin. After 17 injections, the immunity of four mice was tested by the injection of 1.5 MLD of toxin. Only one was found to be immune. The remaining six mice were tested with toxin (1.5 MLD) after 23 injections. In this group, four mice survived and two died.

In subsequent experiments it was found that immunity was regularly reproduced in the mice only after 16-24 daily injections of 1/3 MLD of toxin.

Our findings thus gave no grounds for explaining the impossibility of reproduction of Behring's phenomenon with staphylococcal toxin other than by the development of immunity. It was obvious that the disease did not develop, although the animals showed no immunity to the toxin.

The second reason for the impossibility of reproduction of Behring's phenomenon in response to injection of staphylococcal and Cl. welchii toxins could be the following. Behring's phenomenon is evidently a manifestation of the principle of summation of stimuli (in this case, toxic). If it is assumed that each injection of toxin causes a transient excitation (for less than 24 hr), summation of stimuli obviously cannot take place.

We tested this hypotesis experimentally. Experiments were carried out with staphylococcal toxin. Two groups of animals, with ten mice in each group, received injections of 1/5 and 1/10 MLD of toxin at hourly intervals. In the first case five injections were given and in the second, ten. None of the mice died, either during the experiment or the subsequent period of observation lasting a few days. Consequently, the shortening of the intervals between injections, taking account of the transient nature of the stimulation with staphylococcal toxin, had no effect.

At the same time it was found that no cumulation of the action of the staphylococcal toxin took place; the injection of 1 MLD in the course of 5 hr did not cause death of the animals.

We thus were unable to explain the conditions favoring the summation of toxic stimuli and the second hypothesis could not, consequently, be regarded as proved.

In order to discover the causes of the difference between staphylococcal and Cl. welchii toxins on the one hand and many tested bacterial toxins on the other, it is evidently necessary to pay attention to the properties which distinguish them most from other exotoxins. The hypothesis that all toxins act as enzymes has not, in fact, been confirmed in the majority of cases. Only with staphylococcal and Cl. welchii toxins are the properties of enzymes reliably found, and a substrate for their action (lecithin) identified. It may thus be accepted that the distinctive "behavior" of these toxins during the reproduction of Behring's phenomenon is associated with their distinctive type of action. This may possibly be due to the fact that the substrate of these toxins is present in large quantities in the plasma, and the toxin, when given in small doses (as in experimental conditions), acts on the dissolved lecithin and combines with it, which prevents (or attenuates) its action on sensitive cells. The significance of these properties has not been tested experimentally and we are merely putting forward a hypothesis.

It must be pointed out that, despite the absence of summation of toxic stimulation, in the experiments with staphylococcal toxin it was comparatively easy to produce summation of antigenic stimulation.

The experiments with staphylococcal toxin in fact showed that fractional administration leads to the development of immunity in at least half the animals, although the total dose of antigen injected was small (equivalent to 1-2 MLD of toxin). A single injection of such a dose of antigen (in the form of toxoid) elicits no reaction. The development of immunity thus takes place only as a result of the fractional injection of toxin, i.e. it is connected with the summation of antigenic stimulation. After the administration of tetanus toxin the opposite state of affairs is observed: It is easy to obtain summation of the toxic stimulation and impossible to obtain summation of the antigenic stimulation. We have postulated [5] that this is due to the actual amount of the substance itself in the dose of toxin injected, since antigenic stimulation may be revealed when there is an adequate amount of antigen, of which there is only little in active tetanus toxin.

It is not yet possible to explain the differences in the rate of summation of toxic stimulation. Besides the properties of the toxins themselves, many other factors may also be important: different rates of destruction or neutralization, or of excretion of the toxin from the body, the accessibility of sensitive cells and the possible rate of reaching them, the ease of compensation of the function disturbed by the toxin and the comparative strength of the toxin as a stimulus.

SUMMARY

The authors attempted to reproduce the Behring phenomenon by repeated administration of sublethal doses of staphylococcal and Cl. welchii toxins. This phenomenon could not be reproduced either with a single dose of 1/5 MLD or with a total dose exceeding 2 MLD. No cumulation of the toxin was noted. The fact that there occurred neither the development of the disease nor the death of the animals had nothing to do with the development of antitoxic immunity or the brevity of stimulation: test injections with 1-MLD resulted in the death of the animals, while more frequent administration thereof failed to provoke the development of the disease. The authors suggest

that the impossibility to reproduce Behring's phenomenon with the toxins under investigation is conditioned by their exzymatic nature and the presence of a free substrate (lecithin) in the blood plasma.

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