Studies on the mechanism of immunity to diphtheria in animals

1980 • Emil von Behring


In No. 49 of this Journal, Kitasato and I reported on experiments which show that the immunity to tetanus of experimental animals resides in the ability of the blood to render harmless the toxic products of the tetanus bacillus.

The same mechanism was advanced in that paper for diphtheria immunity, without actually reporting experiments which supported this idea. It is the purpose of this paper to present this data.

Lössler, as well as Roux and Yersin, have shown that there are animals which are naturally immune to diphtheria. I have found myself that mice and rats fall into this category, and that these animals can be injected with cultures which have no effects on their health, while equal volumes of these cultures are fatal to guinea pigs, rabbits, and sheep.

A broth culture of diphtheria bacteria isolated from the membrane of a child which had died in January of this year from diphtheria can kill a guinea pig in 3-4 days after an injection of only 0.05 cc. In rabbits, 0.3 cc. subcutaneously kills after 2-4 days. 2.0 cc. kills a mature sheep after 50 hours. From the same culture, rats can receive 2 cc. and mice 0.3 cc. without showing any disease symptoms.

It is also possible to take animals which originally are sensitive to diphtheria and make them immune, and this can be done in different ways.

1. One of these immunization methods, which I can state from my own work is very reliable, has been described by Prof. C. Fränkel. Guinea pigs are inoculated with cultures that have been sterilized, and after 10-14 days of such inoculations, they are insensitive to doses which would ordinarily kill them.

2. I have also immunized guinea pigs in the following way: I take 4 week old cultures and add iodine trichloride in concentrations of 1/500 and allow this to act for 16 hours. Then I inject two guinea pigs with 2 cc. of this treated culture in the abdominal cavity.

After 3 weeks I inject these guinea pigs with 0.2 cc. of a diphtheria culture, which has grown for four days in broth containing 1/5500 iodine trichloride. The control animals die after 7 days; the treated animals remain alive.
After another 14 days the two treated animals receive an injection of a fully virulent culture, which is sufficient to kill normal guinea pigs in 36 hours.

In both of these methods, it is the metabolic products which are produced by the diphtheria bacilli, which induce the immunity.

4. Another method which has not been reported previously is based on the metabolic products of the bacteria.

It consists of first infecting the animals and then alleviating the deleterious effects through therapeutic treatment.

This method is similar to that which occurs in humans when they recover from an infection. Of the many chemical agents which could be effective, the best seems to be iodine trichloride. Eight guinea pigs which had been infected subcutaneously with 0.3 cc. of culture were divided into two groups. The two controls died after 24 hours. The other four animals were treated immediately after the infection with 2 cc. of a solution of iodine trichloride (two smaller animals received a 1% solution, two larger received 2%) at the point of infection. These remained alive. With two other animals the treatment was begun 6 hours after infection, and one animal died after four days while the other remained alive. All animals received new injections of iodine trichloride on the next three days. When the treatment was delayed more than six hours after infection, I have had no success. Also, I have been unsuccessful in immunizing animals when they receive so light an infection with the bacteria that the controls do not die until four days later.

I have animals treated with iodine trichloride, and Dr. Boer has animals treated with gold sodium chloride, which have recovered from the infection, and these guinea pigs are able to stand injections of fully virulent diphtheria bacteria which would kill control animals in 36 hours.

5. Another method for the induction of complete resistance in guinea pigs against infections, and in rabbits against the lethal effects of culture fluids, is not related to the metabolic products of the diphtheria bacillus. This is through the use of hydrogen peroxide.

Hydrogen peroxide is an excellent disinfectant in certain cases, and I originally used it with the idea that it might have a therapeutic effect in diphtheria. However, animals which were treated after infection with hydrogen peroxide became diseased much faster than the controls. Hydrogen peroxide also seemed to increase the virulence of the culture when added to it.

However, when I treated the animals with hydrogen peroxide several days before infection, I found that they had achieved a more or less pronounced degree of immunity.

Of the five different immunization methods which have been mentioned, the first four are basically those methods which we have been acquainted with by Pasteur. The fifth, which produces immunity through completely heterogeneous material of a simple chemical nature, has no analogy in previous work.

In my opinion, all five methods of immunizing against diphtheria could not be useful in humans, at least in the form they have been described here.

However, from the scientific viewpoint, and especially for the solution to the problem of the mechanism of diphtheria immunity, these methods may serve a very useful purpose.

No matter how the immunity is induced, and I include here also natural immunity, diphtheria-immune animals
have certain characteristics in common, which differentiate them from non-immune animals.

First, all living immune animals are protected not only against the infection with living diphtheria bacilli, but also against the harmful effects of that toxic substance which is produced by the diphtheria bacilli in culture and in the animal body. . . .

For my purposes, it was not necessary to separate or purify the diphtheria toxin or toxins from the culture fluid. Old filtered cultures furnished me with suitable material.

I prepared cultures in alkaline broth and found that after 10 weeks these contained so much toxic substance, that after filtering to make them bacterial-free, 1 cc. of this broth injected into middle-sized guinea pigs induced typical symptoms of diphtheria toxification, that did not completely disappear until after three to four weeks. Three to four cc. was sufficient to kill a large guinea pig in three to eight days. Regularly necrosis of the skin appeared, and not only at the injection site, but quite distant from it, most frequently on the abdomen.

All guinea pigs which had a solid immunity, so that they could stand repeated infections of bacteria without any diseased symptoms, could also stand injections of 3 to 5 cc. of this toxic broth without showing any toxic symptoms or only a localized reaction. . . .

These observations and considerations led me nearer to the question of whether the cause of the toxin resistance was possibly due not to a characteristic of the living cellular part of the animal, but to a special property of the cell-free blood.

In order to answer this question, I took rats which had received intraperitoneally a large amount of diphtheria toxin and removed, three hours later, their blood and injected it or the serum from it intraperitoneally into guinea pigs. There was no sign of toxic symptoms, while blood from diphtheria-sensitive animals, which had received an equal amount of diphtheria toxin, was injected in equal amount (4 cc.) into guinea pigs, and although it did not kill them, it made them distinctly sick.

A further fact in support of this argument is that the extravascular blood from animals immune to diphtheria possesses the ability to render diphtheria toxin harmless. The intensity of this effect as well as the therapeutic possibilities of blood from immunized animals will be taken up in a further communication. . . .

After I had made these observations regarding the mechanism of immunity to diphtheria, Herr Kitasato and I performed similar experiments with tetanus. In the earlier communication we were able to offer uncontestable proof that the toxin-destroying property of the blood of tetanus-immune animals is a sufficient explanation for tetanus immunity.

In tetanus we had the happy situation that we were able to obtain large quantities of blood and serum from immune rabbits and then inject this into very small mice. This made it possible to demonstrate completely and conclusively the therapeutic consequences of this treatment. Perhaps the therapeutic possibilities of the transfusion of blood from tetanus-immune rabbits was not sharply enough expressed in our earlier communication. If not, I would like to mention especially here that mice that have received blood from tetanus-immune rabbits are not merely immunized, and are not merely protected from some future attack of the disease. Even when the mice have been infected in several extremities with tetanus, and would,
from past experience, die within a few hours, it is even then possible with high certainty to produce a cure by serum injection. And this cure takes place so rapidly that even after a few days there are no signs of the illness left.

The possibility for the cure of very acute diseases can therefore no longer be denied.

Comment

This work is an extension of von Behring and Kitasato's paper, just preceding. It presents observations on diphtheria which seem to indicate that a similar situation exists here as in tetanus. Diphtheria was more important medically than tetanus, since it was at one time one of the great killers of children. This statement is no longer true largely because of the work of von Behring.

Like tetanus, diphtheria is also a disease in which all of the symptoms are due to the action of a toxin which is elaborated by the causal organism. Once successful results had been obtained with tetanus, it was natural to attempt to extend this work to diphtheria. von Behring has apparently done so in this paper, although the data are not completely convincing. But it is true that, with the help of Ehrlich and others, he was able to develop this discovery into a practical therapeutic method for diphtheria. Within the next ten years, serum containing antibodies against diphtheria (diphtheria antitoxin) was available commercially and was being used widely to cure children of the disease. Also available later were vaccines which could be used to induce formation of diphtheria antitoxins in potential hosts, and thus eventually eliminate diphtheria as an important disease. Today cases of diphtheria are so rare that they receive widespread attention when they occur.

Leucocytes and the active property of serum from vaccinated animals

1895 • Jules Bordet


In consequence of the extensive researches of recent years, the theory of phagocytosis seems capable of explaining entirely the phenomenon of immunity.* The intervention of phagocytes constitutes a regular process in the defense by animals against parasites, and their cooperation is a powerful aid in the recovery of infected organisms. There have been cases, nevertheless, where the importance of phagocytes has been considered as a secondary factor, and the essential thing for the destruction of the infec-