

The mechanism of immunity in animals to diphtheria and tetanus

1890 • *Emil von Behring and Shibasaburo Kitasato*

von Behring, Emil, and Kitasato, Shibasaburo. 1890. Ueber das Zustandekommen der Diphtherie-Immunität und der Tetanus-Immunität bei Thieren. *Deutsche Medizinische Wochenschrift*, Vol. 16, pages 1113-1114.

IN THE STUDIES WHICH WE HAVE BEEN carrying out for some time on diphtheria (von Behring) and tetanus (Kitasato), we have also considered questions of therapy and immunization. In both infectious diseases, we have been able to cure infected animals, as well as to pretreat healthy animals so that later they will not succumb to diphtheria or tetanus.

In what way the therapy and immunization have been obtained will only be stated here in enough detail to demonstrate the truth of the following sentence: "The immunity of rabbits and mice, which have been immunized against tetanus, depends on the ability of the cell-free blood fluid to render harmless the toxic substance which the tetanus bacillus produces."

This explanation of immunity has not been considered in any of the works on the immunity question

which have appeared in recent years.

Aside from the studies on phagocytosis, which seek to explain immunity in terms of the vital activities of the cells, others have considered the bactericidal action of the blood and the acclimatization of the animal body to the toxin.

When one of these explanations has been found not acceptable, then it has been believed that this exclusion of one explanation is an argument for the other. Thus Bouchard stated: "Let us no longer speak of the action of the leucocytes or the adaptation of the nerve cells to the bacterial toxin: this is pure rhetoric." and "It is actually the bactericidal action which is responsible for vaccination or acquired immunity."

This positive statement derives from that which Roger expressed as follows: "Vaccination induces in the or-

ganism chemical modifications which make the fluids and tissues less favorable to the growth of the microbe which has been used to immunize the animal."

However, one of us (von Behring) could determine in his studies on rats immune to diphtheria and on immunized guinea pigs, that none of the theories mentioned above could explain the immunity of these animals, and he realized that it was necessary to look for another principle to explain these phenomena. After many negative experiments, it was discovered that the blood of immune animals had the ability to neutralize the diphtheria toxin, and this discovery revealed the reason for the insensitivity of these animals to diphtheria. But it was only by applying this concept to tetanus that we were able to achieve results which, so far as we can tell, are completely conclusive.

The experiments to be outlined below show:

1. The blood of rabbits immune to tetanus has the ability to neutralize or destroy the tetanus toxin.

2. This property exists also in extravascular blood and in cell-free serum.

3. This property is so stable that it remains effective even in the body of other animals, so that it is possible, through blood or serum transfusions, to achieve an outstanding therapeutic effect.

4. The property which destroys tetanus toxin does not exist in the blood of animals which are not immune to tetanus, and when one incorporates tetanus toxin into nonimmune animals, the toxin can be still demonstrated in the blood and other body fluids of the animal, even after its death.

As proof of these statements, we present the following extensive series of experiments:

A rabbit was immunized against tetanus by a method which will be reported in detail later. The degree of immunity of this animal was such that it would stand a dose of 10 cc. of a bacteria-containing culture of virulent tetanus bacilli, while a normal rabbit would always die from a dose of 0.5 cc. Every rabbit remained completely healthy after this injection.

This was not only true of the infection with living tetanus bacilli, but also by injection with tetanus toxin, for each immune rabbit would tolerate without symptoms a dose of toxin 20 times that which would kill normal rabbits.

From these rabbits, carotid blood was removed.

Before coagulation, the fluid blood was injected into the abdominal cavity of mice, 0.2 cc. and 0.5 cc. After 24 hours these two mice were inoculated along with two control mice with virulent tetanus bacilli. The inoculation was strong enough so that the control mice became sick after 20 hours and died after 36 hours. However, both treated mice remained perfectly healthy.

Most of the blood from the immunized rabbits was allowed to stand until it had coagulated and the serum had formed.

Six mice were injected with this serum in the abdominal cavity. The infection 24 hours later had no effect on these, while the control mice died from tetanus after 48 hours.

The serum could also be used for therapeutic treatment, in which the mice were infected first, and then the serum was injected intraperitoneally afterward.

Also we have done experiments with the serum, which show the enormous toxin destroying activity of it.

A ten day old tetanus culture was filtered to render it free of bacteria. 0.00005 cc. of this was sufficient to

cause death of mice after 4–6 days, and 0.0001 cc. caused death after less than 2 days.

Now we mixed 5 cc. of serum from rabbits that were immune to tetanus with 1 cc. of this toxin-containing culture and allowed the serum to act on the toxin for 24 hours. We injected 0.2 cc. of this mixture into each of four mice. This would correspond to 0.033 cc. of culture fluid, or more than 300 times the lethal dose for mice. All four mice remained perfectly healthy. The control mice, however, died 36 hours after an injection of 0.0001 cc. of culture.

The mice from all of the experiments that had received either serum alone or serum with toxin were rendered permanently immune, so far as

one can tell. Repeated injections at a later time with virulent tetanus bacilli caused not a trace of illness in them. . . .

Naturally we have performed every experiment with control blood and serum from non-immune rabbits. Such blood and serum are neither therapeutic nor active against tetanus toxin.

The same is true of serum from cows, calves, horses, and sheep. . . .

In earlier times blood transfusions were considered to be effective . . . methods for the treatment of diseases. Recently it has been believed that physiological saline can exert the same effects. The results of our experiments remind us forcibly of these words: "Blut ist ein ganz besonderer Saft," ["Blood is a very unusual fluid."]

Comment

The science of serology can be said to have begun with this paper. It presented the first evidence that there were substances formed in the serum in response to infection which were able to neutralize foreign materials. It was probably only with tetanus that the facts could first have been demonstrated so clearly.

All of the symptoms of tetanus are due to a toxin that is elaborated by the causal organism. This toxin had been discovered earlier by Kitasato. It is produced in artificial culture media. When this toxin is injected into mice, they die rapidly, and a reproducible curve relating dose and number of deaths can be obtained. By a method not mentioned by the authors, it was possible to immunize rabbits against tetanus. We know now that this immunity is due to the production of antibodies by the rabbit in response to the toxin. These antibodies are able to neutralize the toxin and completely prevent the symptoms of the disease. Because a rabbit is a much larger animal than a mouse, it is possible to inject into the mouse serum from immunized rabbits in sufficiently large amounts so that

there is sufficient antibody present in the mouse to neutralize all of the tetanus toxin injected. In this way the mouse is passively immunized. We know now that this immunity is not permanent, but gradually wears off. This is because the rabbit antibodies are gradually destroyed by the mouse. von Behring and Kitasato missed this point, for a number of rather complex reasons which need not concern us here.

Note that the substance in the serum of immunized rabbits is highly specific, neutralizing only tetanus toxin and none other. This point is quite important, since it demonstrates that antibodies are highly specific. This specificity is at the base of a number of immunological procedures.

Note also that this discovery opens up the possibility of specific therapy for diseases through the injection of immune serum. It also opens up the possibility of specific prevention of diseases through the induction of specific antibody production in a potential host. This is one of the most important contributions that has been made to medicine by microbiology.