THE ROLE OF THE LYMPHOID ORGANS IN ANTIBODY PRODUCTION

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After extirpation of the thymus or the artificial production of hyperactivity of the thymus, many workers have observed the development of various changes in animals. It has been possible to show in this way a connection between the function of the thymus and the assimilation of vitamins [8], the influence of the thymus on the nervous system [9] and the musculature [2] and also a connection between this gland and other glands of internal secretion [1, 6, 10, 11, 12]. No unanimity yet exists, however, concerning the role of this gland in the body as a whole.

The problem of the role of the thymus as a lymphoid organ is of interest in connection with research in recent years into the importance of lymphoid tissue in immunogenesis. There is an extensive literature on the role of the spleen in the process of antibody formation, but so far as the thymus is concerned very little has been written on this subject. There are some observations on the lowering of the level of immunity in animals subjected to partial or total thymectomy, as revealed by lower morbidity and higher mortality in these animals by comparison with controls [1]. On the basis of experiments in which dry powdered thymus was fed to rabbits and mice, we know that hyperthymization inhibits the growth of mice and lowers the immune properties of rabbits. Finally there are results [5] showing a fall in the titer of antityphoid agglutinins in rabbits after receiving thymus extract.

The aim of the present research was to ascertain the effect of removal of the thymus and spleen on antibody production in rabbits and to discover the relationship between these two organs in immunogenesis.

EXPERIMENTAL METHODS

Investigations were carried out on rabbits weighing from 2 to 3 kg. Thymectomy and splenectomy were performed under ether anesthesia. The thymus was removed first, and the spleen 48 hours later.

The antigen used for immunization was a vaccine prepared from typhoid bacilli (strain 495), killed by heating to 56-58° for 1 hour.

The rabbits were vaccinated intravenously by means of triple immunization with doses of 1.2 and 2×10^9 bacterial cells at 7-day intervals between injections. Revaccination was by a single injection of 2×10^9 bacterial cells of the same vaccine.

The immunological response was judged by the rise in the agglutinin titer in the sera of the animals. Blood samples were taken before each injection of antigen and then subsequently once a week. The observations were prolonged until a fall in the titers, after which the animals were revaccinated in order to study the anamnestic reaction.

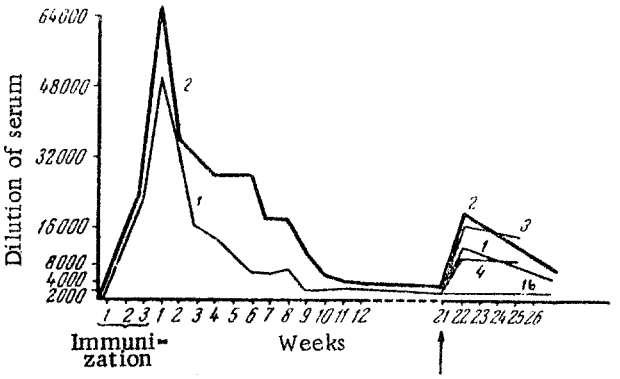

Fig. 1. The effect of thymectomy on the agglutinin titer in rabbits after triple immunization and revaccination. 1) In controls, 2) after removal of the thymus, 3) after removal of the thymus at the time of revaccination, 4) after removal of the thymus 48 hours after revaccination, 1b) not revaccinated. The arrow (↑) indicates the time of revaccination.

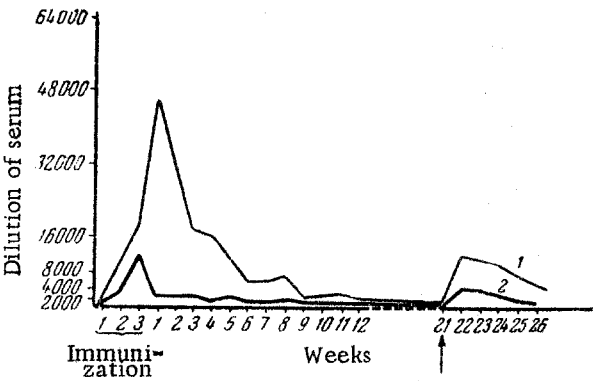

Fig. 2. The effect of splenectomy on the agglutinin titer in rabbits after triple immunization and revaccination. 1) In controls, 2) after splenectomy. The arrow indicates the time of revaccination.

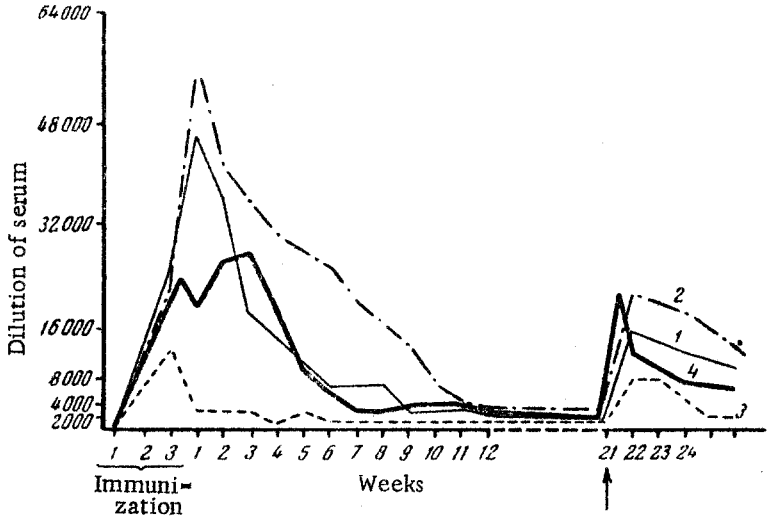

Fig. 3. The effect of removal of the thymus and spleen on the agglutinin titer in rabbits after immunization and revaccination. 1) In controls, 2) after removal of the thymus, 3) after removal of the spleen, 4) after removal of spleen and thymus. The arrow (↑) indicates the time of revaccination.

The agglutination reaction was carried out by the usual method. The antigen used in the reaction was a suspension of the same culture, killed with formalin. The preliminary results of the reaction were read after incubation of a mixture of serum and antigen for 2 hours at 37° and the final results on the day following standing of the mixture for 18-20 hours at room temperature. The intensity of the positive reaction was indicated by the number of plus signs.

Certain modifications of the method are given in the description of the experiments.

EXPERIMENTAL RESULTS

In the first series of experiments 15 rabbits were used, in 5 of which the thymus was removed one week

before the beginning of immunization, and 10 rabbits acted as controls. All the rabbits were immunized by means of three injections of typhoid vaccine.

The blood of all the animals was investigated before immunization for the presence of normal antibodies. No antibodies were found in serum diluted 1 : 100.

The blood was subsequently investigated before each injection of antigen, and once a week after the conclusion of immunization.

As may be seen from Fig. 1, the mean titer in the control animals throughout the entire period of observation was slightly lower than in the thymectomized animals (2). Revaccination on the 21st day showed that the antibody titer was increased still further in response to the repeated injection of antigen both in the rabbits from which the thymus was removed before the primary immunization (2) and in those from which the thymus was removed at the time of revaccination (3). If, however, the thymus was removed 48 hours after revaccination (4), the antibody titer in these animals was equal to the titer in control rabbits not undergoing operation (1). The antibody titer in the rabbits which were not revaccinated stayed at the previous level (Fig. 1, 1b).

These experiments showed that the presence of the thymus in an animal depresses to some extent the reaction of the animal in response to the injection of an antigen. This was shown by the lower degree of accumulation of antibodies in the blood in control rabbits by comparison with thymectomized rabbits.

In the second series of experiments observations were made on the antibody titers of 5 rabbits after splenectomy. Five unoperated animals were used as controls. The conditions of immunization and revaccination were the same as in the previous series.

These experiments revealed a clear difference between the accumulation of antibodies in the unoperated and splenectomized animals (Fig. 2). The agglutinin titer of the control animals (1) was many times higher than that of the splenectomized animals (2). This relationship was maintained after revaccination.

This difference in the titers was not, however, observed when the animals were immunized subcutaneously. These findings are in agreement with clinical observations on patients with splenomegaly [7]. It has been observed, for instance, that when patients after splenectomy are immunized intravenously with typhoid vaccine, the antibody titer is much higher in these patients than in healthy persons. This difference was not observed after subcutaneous vaccination.

The next series of experiments was conducted on animals from which the thymus and spleen were removed simultaneously. The experiments were carried out on 10 unoperated rabbits, 5 without the thymus, 6 without the spleen, and 5 without both thymus and spleen.

In spite of the opinion of some authors [4] that animals from which the thymus and spleen are removed simultaneously are not viable, we were able to make observations on such animals for a period of 1 year.

The following observations, shown in Fig. 3, were made after immunization and revaccination respectively. The largest accumulation of antibodies in the blood was observed in the group of thymectomized animals (2) and the smallest in the splenectomized group (3) by comparison with the control group (1). The titers in the group of animals without both thymus and spleen occupied an intermediate position (4).

The experiments to study the reaction of rabbits in response to injection of typhoid antigen after removal of the thymus, spleen, or both organs thus showed that these organs, along with other organs and tissues, take part in immunogenesis, but that the parts they play are different. Whereas removal of the thymus not only does not lower the content of circulating antibodies in the blood stream but even increases their accumulation, splenectomy lowers the antibody titer in response to intravenous immunization to a considerable degree, a fact which indicates the antagonistic relationship between these two organs in immunogenesis.

The immunological response of normal and thymectomized animals differs only slightly, but the spleen has only to be removed for the suppressing action of the thymus on the accumulation of antibodies to be sharply expressed. Evidence for this is given by experiments in which the thymus was removed from splenectomized animals (see Fig. 3), when the titers in these animals rose by comparison with those in the animals from which only the spleen was removed.

It may be assumed from these findings that the difference in the immunological response depends on the functional state of the lymphoid organs at the moment of immunization, and in particular of the thymus and spleen.

Preliminary experiments in which thymus homogenate was administered in the course of immunization confirmed the hypothesis that the thymus depresses the response reaction of the body to the injection of antigen. These findings are in agreement with the reports in the literature [1, 3, 5] that hyperthymization leads to a fall in the immunological response of the animal.

These results suggest that in the thymectomized animal both the accumulation of antibodies in the blood in response to injection of antigen, and the liberation of antibodies from the organs and tissues, take place to a greater degree, as is shown by the more pronounced anamnestic reaction in the thymectomized animals.

SUMMARY

Simultaneous removal of the thymus gland and spleen in rabbits does not affect the viability of these animals. Together with the other organs the thymus gland and the spleentake part in the response of the animal to the administration of the typhoid antigen. This participation is manifested in various forms: thymectomy promotes greater accumulation of the antibodies in the blood; splenectomy on the contrary reduces the titre: Simultaneous removal of the thymus and spleen results in a greater accumulation of antibodies than in the case of splenectomized animals, although it is slightly less than in the normal ones.

Thus one may assume that the thymus has a depressing effect on the mechanisms responsible for the accumulation of the antibodies in the blood; this effect is especially clearly manifested in the absence of the spleen. This is also confirmed experimentally by the increased titer of circulating antibodies in splenectomized rabbits after thymectomy, as well as by experiments with administration of thymus homogenate. The depressive effect of the thymus gland is manifested only during the first 48 hours after the administration of the antigen.

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