TRANSLATION NO. 1953

DATE: 1 February 1967

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

STUDIES ON THE MECHANISM OF ANTIBODY FORMATION WITH SPECIAL REFERENCE TO ANTIBODY PRODUCTION PROMOTING FACTOR (A.P.P.)

PART II. INFLUENCE OF THE A.P.P. ON THE COMPLEMENT FIXING ANTIBODY PRODUCTION IN RABBITS

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Japan Arch. Internal Medicine
Vol 12, No. 11, 1965. pages 627-31

When the rabbits had been immunized by the mixture of extract of typoid bacilli and A.P.P., the complement fixing antibody against the extract of typhoid bacilli always increased more rapidly and to higher in its titer. and remained longer than in the controls immunized by the extract of typhoid bacilli alone, even though some individuals differences could be seen. From these results the effectiveness of A.P.P. on the promotion of complement fixing antibody production was demonstrated.

CHAPTER I. INTRODUCTION

The complement fixing reaction was first reported by Bordet and Gengou[1] in 1901. This reaction is one of the antigen and antibody reactions, and the presence of complement fixing antibody has been recognized by many students. This reaction has been classified into two groups: one of a special nature and the other of normal characteristics. The reaction of the former is more sensitive than those of other antigens and antibodies. Due to the stronger and more specific nature of the former, it is used for distinguishing different organisms, proteins, and also in diagnosing diseases. The Wassermann test represents the normal reaction, and, today, it occupies an important position in the diagnosis of syphilis. Therefore, the search for a complement

which would promote the production of an antibody has a significant role in the science of immunology. Much work has been done in the past on different suspensions of various organs of living bodies which promote the formation of agglutins and precipitins. Little work, however, has been done on the influence of these substances on the formation of complement fixing antibody. Boku, Hirai and Ken[2] reported that the spleen hormone obtained by extracting spleen tissue of a rabbit with water, alcohol, and acetone promoted complement fixing antibody production against tuberculosis organisms in a rabbit. Takaishi[3] reported that the injection of a similar white corpuscle into a rabbit promoted complement fixing antibody production. Other than the above-mentioned reports, no information is available.

Fukase[4] proved that when a mixture of A.P.P. of lymph gland of rabbit and an antigen was injected into a rabbit, it accelerated albumen formation, increased its production, and shortened the period of latency. The author undertook this study with a thought that the A.P.P. may have the property to promote complement fixing antibody production. The following are the results of this study.

CHAPTER II. EXPERIMENTAL MATERIALS AND METHODS

1) Experimental Animals and Physiological Salt Solution. For this study, rabbits and physiological salt solution similar to those used in the first study and reported in Part I were used.

2) Hemolysin. This was obtained by immunizing rabbits with red corpuscle of mountain goat. Injections were made into the vein of external ear of the rabbits as follows: two injections at three- to four-day intervals with 8 cc of 20%, red corpuscle solution in an aseptic physiological salt, followed by 7 cc of 50% solution and finally by 20 cc of 50% solution. Ten days after the final injection, a small quantity of blood was taken from each animal, and the hemolysin titer was measured. Only those animals whose blood showed a titer of a minimum of 1,000 times were selected; they continued to receive injections up to 14 days, at which time all of the blood of each animal was collected. From this blood, serum was separated as quickly as possible. The hemolysin serum thus obtained was inactivated by treating it for 30 minutes at 56°C, adding carbolic acid in the ratio of 0.5% of the serum, bottling in dark bottles, and storing in a refrigerated room.

3) Suspension of Red Corpuscle. Blood of a mountain goat from which fiber was separated was washed three times with physiological salt water and was centrifuged for ten minutes at 3,000 r.p.m. With this red corpuscle, 2.5% corpuscle suspension was made with physiological salt water.

4) Sensitized Blood Corpuscle Suspension. To 0.25 cc of the 2.5% corpuscle suspension prepared in the manner mentioned above, hemolysin serum containing three units of antihemolysin of mountain goat was added. This mixture was used for the experiment after having been left at room temperature for 15 minutes.

5) Complement. Serum separated from the blood of three healthy fur seals was stored in a refrigerated room overnight. Then, the complement titer of this serum was measured, either with the addition of an antigen or without. To 0.25 cc of this serum, 0.25cc of the above-mentioned sensitized corpuscle suspension was added. This mixture was then diluted to the strength to contain 2 units of complement factor.

6) Antigen. Typhus organism H901W was cultured on an ordinary agar culture media for 24 hours at 37°C. This cultured organism was mixed with physiological salt water in proportion of 5mm/cc on wet weight basis. This mixture was boiled for 15 minutes at 100°C, and was centrifuged for 60 minutes at 3,000 r.p.m. To the upper suspension of this mixture, 0.5% carbolic acid was added, and it was kept in a refrigerated room for future use. For each experiment, the complement titer of the antigen was measured, and 0.25 cc of the above solution diluted to 20 times with physiological salt water was used as antigen for this experiment. It was found that this strength of the antigen did not cause hemolysin.

7) Method of Preparation of A.P.P. The A.P.P. used in this experiment was prepared according to the method employed by Fukase[4], using healthy rabbits which had been checked in advance for the absence of any complement fixing antibody. The method of preparation is the same as described in Part 1.

8) Sensitization of Experimental Animals and Sensitized Serum. Sensitization of the experimental animals was accomplished by using a mixture of equal amounts of antigen mentioned above (typhus organism extract) and lymph gland suspension (A.P.P.). Two cc of this mixture were injected two to four times into the abdomens of the rabbits. At the same time, the control rabbits were sensitized by giving

them injections of either 1 cc of typhus organism extract or 1 cc of A.P.P. in the same manner as the experimental rabbits.

Ten to 12 blood collections were made 20 to 23 days starting after the first injection from both groups either from the external ear or from the heart. Serum was separated from the blood, and was kept in a refrigerated room for experimental use.

9) Experimental methods. In this experiment, the method of sensitized serum reduction was employed. The sensitized serum of both experimental and control animals was treated for 30 minutes at 56°C; 0.25 cc of this serum was diluted to double its volume with physiological salt water. To this, 0.25 cc of antigen solution diluted to 20 times and two units of fresh complement serum were added. At this time, in order to control the antigen and the antiserum, the following solutions were added to the above solution: (1) 0.5 cc of the immunized serum diluted two times and two units of the complement; (2) 0.25 cc of salt solution; and (3) 0.5 cc of the antigen diluted to 20 times and two units of the complement. These mixtures were thoroughly agitated for one hour in a hot water tank at 37°C.

To each of the above mixtures, 0.25 cc of sensitized corpuscle suspension which had been kept for 15 minutes at room temperature was added, and then they were heated for one hour at 37°C.

Observations were made on these groups at the predetermined hours. It was observed that in the groups in which the antigen and antiserum complement were controlled, a complete hemolysin took place, while no hymolysin occurred in the groups in which only the antiserum was controlled. The highest serum dilution point at which hemolysin stopped completely was used as the antibody titer of the complement fixing antibody.

CHAPTER III. EXPERIMENTAL RESULTS

A. Results of Four Immunizations at Two- to Four-day Intervals

For the study on the influence of the A.P.P. on the production of complement fixing antibody, 2 cc of a mixture of antigen and the A.P.P. were injected into the experimental rabbits, while the control rabbits were given injections of

either 1 cc of antigen or 1 cc of the A.P.P. A total of four injections were given to each group at two- to four-day intervals. Twelve blood collections were made from each animal during 21 days before and after immunization at 24- to 72-hour intervals. Serums of both groups were measured on the antibody titer, and comparisons were made.

As shown in Figure 1, the two experimental groups showed an increase in the antibody titer four to eight times respectively on the third day after the second injection. Until the day after the third injection (eighth day after the first injection), this titer was kept without any further increase. Forty-eight hours after the fourth injection (11th day after the first injection), however, one of the two showed a 64-time increase which was maintained for a few days more, dropping to 16 to 32 times by the 21st day. The other group showed an increase to eight times on the eighth day after the first injection, but it died of a sudden convulsion. Later it was found that the cause of death was an internal hemorrhage of the heart.

The two control groups had a slower increase in the antibody titer in comparison with the experimental groups. One of the two showed an increase to eight times on the eighth day after the first injection, and this continued until the 11th day. It increased to 18 times on the 13th day of the first injection, dropping to four times on the 21st day. The other control group had a slower increase, and reached only four times on the 13th day (fourth day after the fourth injection). It reached to 16 times, which was the highest, on the 15th day, declining gradually until it reached to four times on the 21st day after the first injection. In other words, the experimental groups in comparison with the control group had an earlier appearance of antibody, a shorter latency period, arrival at maximum increase two to three days earlier, and a titer four times that of the control groups. Furthermore, the speed of decline was much slower in the experimental than in the control groups. The group which received only the A.P.P. injection had no antibody formation.

According to these results, it is thought that the A.P.P. seems to promote significantly the production of complement fixing antibody against the typhus organism extract in rabbits.

B. Results of Three Injections at Three- to Four-Day Intervals

A study was made on the influence of the A.P.P. on the antibody production by changing the number of injections

and the days of blood collection. Figure 2 shows the results.

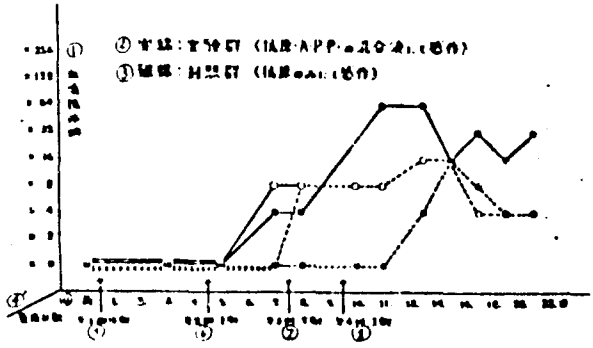

Fig. 1. Influence of the A.P.P. on Complement Fixation Reaction

[Legend]: 1) Serum antibody titer; 2) Solid line - Experimental group (sensitization with mixture of A.P.P. and antigen); 3) Broken line - Control (sensitization with antigen alone); 4) Before; 4') No. of Days of Immunization; 5) First injection; 6) Second injection; 7) Third injection; 8) Fourth injection.

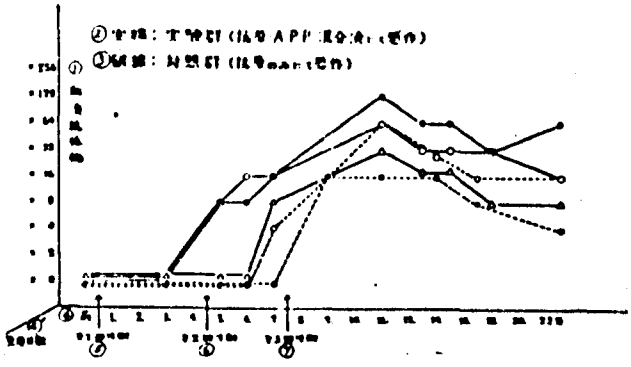

Fig. 2. Influence of A.P.P. on Complement Fixation Reaction

[Legend]: 1 to 7 are the same as in Figure 1.

In this study, three experimental and two control groups were injected three times at three- to four-day intervals. Blood collections were made every 24 hours until the seventh day after the second injection. Afterwards, they were made every two to six days until the 23rd day after the first injection. The collected blood was tested for antibody titer, and comparisons were made. In two of the three experimental groups, the antibody titer reached to eight times on the fifth day after the first injection (24 hours after the second injection), and it reached to 16 times on the seventh day after the first injection. In the other group, the increase was slower, but it reached to eight times on the seventh day. On the 11th day, these three groups showed maximum titers of 128 times, 64 times, and 32 times respectively. They decreased gradually until the 23rd day after the first injection.

In the two control groups (injected with antigen alone), there was no increase of antibody titer until the sixth day after the first injection. In one of the two groups, it increased to four times on the seventh day while in the other it reached to 16 times on the ninth day. In the former, the increase continued until it reached to 64 times on the 11th day, followed by a slow decline to 16 times on the 23rd day. In the latter group, the increase never went above 16 times, followed by the start of decline on the ninth day and continuing to the 14th day. According to these results, two of the experimental groups had the titer increase two to four days earlier than the control groups. One of the former groups had the appearance of titer increase at about the same as the control groups. In general, however, the experimental groups had a stronger increase than the control. While two of the experimental groups had a titer increase of 16 and 8 times on the seventh day of the first injection, the control groups had four times to zero at this time. Consequently, it is thought that the A.P.P. seems to promote the complement fixing antibody production.

C. Results of Three Injections at Three-day Intervals

Again, a study was made of the influence of the A.P.P. on the formation of complement fixing antibody under changed dates of blood collection and injections. As in the previous experiments, both the experimental and control groups were given immunizations three times at three-day intervals. This time, however, changes in the antibody titer were observed closely both before and after the injections. Blood was collected either every other day or every five days, and up to

the 20th day of injection, tests were made on the movenent of the antibody titer. Figure 3 shows the results.

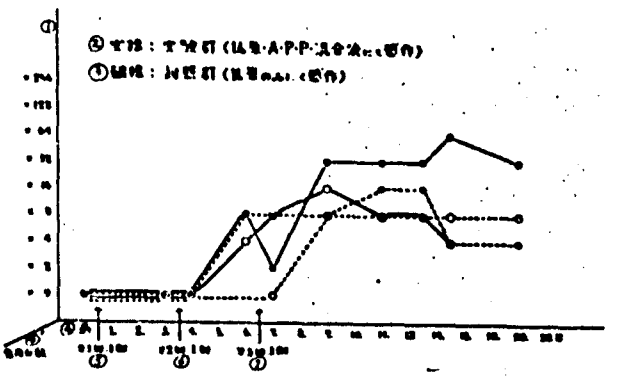

Fig. 3. Influence of A.P.P. on Complement Fixation Reaction

[Legend]: 1) Serum antibody titer; 2) Solid line - Experimental group (sensitization with mixture of A.P.P. and antigen); 3) Broken line - Control group (sensitization with antigen alone); 4) Before; 4') No. of Days of Immunization; 5) First injection; 6) Second injection; 7) Third injection.

Two of the three experimental groups did not show any increase of antibody titer until 24 hours after the second injection (four days after the first injection). On the sixth day after the first injection (third day after the second one), and one of the two had an increase of eight times while the other had only a four-fold increase. In the former, it dropped suddenly to two times 24 hours after the third injection (seventh day after the first one), but it increased to 32 times on the third day after the third injection. It rose again to 64 times on the 15th day, maintaining a level of 32 times even after 20 days after the first injection. The third group had an increase to eight times on the seventh day, and 16 times on the ninth day after the first injection. This group died of diarrhea on the 15th day of the experiment, at which time the titer was four times.

The two control groups did not show any increase in antibody titer until four days after the first injection. One of the two groups had an increase to eight times on the sixth day of the first injection, but there was no change even after the third injection. It reached to the highest

point of 16 times on the 11th day after the first injection. After this, there occurred a rapid change, and it reached to four times on the 15th day, continuing until the 20th day. In the other group, the increase was slow, showing an increase on the ninth day of the first injection. This level was held until the 20th day.

According to the above results, both the experimental and the control groups started the formation of antibody after four days of the first injection, but the former groups appeared to have started it earlier than the latter. Although both groups showed much increase in antibody formation after the third injection, which was given after the antibody formation had already started, the increase was speedier than that in the control groups according to the tests made on the ninth day after the first injection. There was no significant difference between the two 20 days after the first injection.

These results seem to indicate that the A.P.P. has the tendency to promote antibody formation.

CHAPTER IV. SUMMARY AND DISCUSSION

The above experimental results seems to indicate that when the A.P.P. is used in combination with typhus organism extract, it accelerates antibody formation and shortens its latent period in comparison with the use of antigen alone. Fukase reported that the use of the A.P.P. alone had no influence on the complement antibody formation. Also, the use of the combination of the A.P.P. and typhus organism extract caused a faster and a larger quantity formation of antibody in comparison with the control groups.

The difference between the experimental and the control groups was not due to the difference in the conditions of the animals because much care was given in selecting them in order to prevent this situation. All of the animals used in this experiment were of the same kind, their weights were about the same, and all of the experimental conditions were similar.

The production of complement fixing antibody by the use of the A.P.P. by Fukase was proven through the author's experiment in which precipitin was produced. The author thinks that A.P.P. accelerates the formation of antitoxin, hemolysin, and agglutinin as reported by Fukase[4].

It is not clear to the author what Takaishi[3] meant by "same kind of white corpuscle" in his report. It is impossible for the author to judge whether or not the A.P.P. he used for his experiment is the same as that which the author used because of the difference in the methods of preparation. Boku[2] found complement fixing antibody promoting function in a rabbit carrying tuberculosis. Boku[5] reported that the "hormone" of spleen had no influence on the formation of antibody against so-called liquid form antigen and that boiling alcohol extract of spleen tissue for 30 minutes at 100°C had no prohibitive effect. These facts show that these substances differ from the A.P.P. It is, however, interesting to note that spleen tissue, which is the source of antibody, also contairs hormone. These facts reveal that the antibody producing mechanism must be very complex.

In this experiment, the function of the A.P.P. was not clear, as was the case in the previous experiment on the production of precipitin. As shown in the previous experiment on precipitin, the A.P.P. showed an influence on the destruction and renewal lymph corpuscles. From this, it is thought that the A.P.P. may have a close relationship in the production of an antibody.

CHAPTER V. CONCLUSION

The author made a study on the influence of the A.P.P. to the complement fixing antibody formation in rabbits, and obtained the following result.

1) When the A.P.P. was injected into rabbits with antigen, it promoted significantly the production of complement fixing antibody against typhus organism extract and prolonged its latency period greatly in comparison with control.

In concluding, the author wishes to express his sincere appreciation to Professor Kikuchi (at present emeritis professor) and to lecturer Fukase (now assistant professor) for their guidance and for editing this paper.

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