DETECTION OF CIRCULATING "AUTOANTIBODIES" IN EXPERIMENTAL SCHISTOSOMIASIS JAPONICA

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In schistosomiasis japonica of man, as well as of experimental animals, serum γ-globulin markedly increases, lymphocytes and plasma cells aggregate in certain tissues and the response to cortisone is conspicuous. These clinical findings seem to indicate that some syndrome of schistosomiasis may possibly be explained as a manifestation of "autoimmunization" with the corresponding tissues. In this point of view, the possibility of appearance of "autoantibodies" in blood serum has been studied on the rabbits experimentally infected with schistosoma japonicum.

MATERIAL AND METHODS

A. Method for precipitin reaction

1. Antigen

The liver or the colon of the rabbits were homogenized and extracted with absolute alcohol and successively with benzol. The precipitate of the extract after aceton treatment was further extracted with petroleum ether. The sediment, obtained with the latter process, was dissolved in concentration of 0.5 g/dl with physiological saline.

2. Antiserum

Serum of the rabbits at the 2nd to the 12th week of schistosome infection was taken and inactivated immediately before use by keeping it at a temperature of 56°C for 30 minutes.

3. Procedure for precipitation reaction

The antigen solution in volume of 0.1 ml was overlapped on each (0.25 ml) of a series of two-fold dilution of serum solutions. The solutions were kept at room temperature and the appearance of precipitin ring was observed at 30 minutes, one hour and 3 hours later.

B. Methods for passive hemagglutination test

1. Suspension of sensitized erythrocytes

Rabbit erythrocytes were sensitized, for two hours at 37°C, with 0.5 % solution of the liver or the colon extract diluted with physiological saline. The final concentration of the suspension was 0.25 %.
2. Antiserum
The test serum was inactivated at 56°C for 30 minutes and was serially diluted with phosphate buffer of pH 7.2.

3. Procedure for agglutination reaction
The suspension of sensitized erythrocytes (0.25%) in volume of 0.1 ml was added to each (0.25 ml) of a two-fold dilution series of antiserum. After keeping the mixtures at 37°C for 2 hours and successively at room temperature for 18 hours, they were observed with an agglutinoscope.

C. Recording method for the movement of isolated rabbit intestine
Rabbits with or without experimental infection of schistosomes were killed by bleeding. The ileum was then isolated and strips of approximately 1.5–2.0 cm length were made. The movements of the strip were recorded according to Magnus method in 40 ml of Tyrode solution which was constantly oxygenated at 37°C. The amount of test substances added to the bathing solution was always 0.4 ml.

Bacteriologic examination of the flora in used intestines was also carried out, because the flora may exert some influence upon reactivity of the intestine strip.

RESULTS

A. Precipitin reaction
The specific precipitating antibody ("autoantibody") against the extract of the liver or the colon of the normal rabbits (0.5%) appeared in blood serum during the course of experimental schistosomiasis. The titer of such a precipitin at various times after experimental infection of schistosome is shown in Fig. 1 (A and B). As can be clearly seen in the figure, the appearance of the precipitin could be recognized 3 to 4 weeks after infection. The precipitation reaction showed the maximum titer at the 6th to 8th week and recovered to the original value by the 12th week.

The susceptibility of the precipitin to the extract of the liver or the colon taken from the same infected rabbits instead of the normal ones is now under investigation.

B. Passive hemagglutination
The specific antibody ("autoantibody") which agglutinates the rabbit erythrocytes sensitized with the liver extract of normal rabbits was also produced in experimental schistosomiasis. Fig. 2-A shows the temporal changes of the titer of such a hemagglutinin in blood serum of infected rabbits. The titer of hemagglutinin increased at the 4th to the 5th week of infection and reached its maximum at the 7th to 9th week. Then it tended to decrease but remained higher than the original value at the 11th to the 12th week.

The "autoantibody" which agglutinates the rabbit erythrocytes sensitized with the extract of normal rabbit colon (0.5%) also appeared in blood serum of infected rabbits. The changes of its titer are shown in Fig. 2-B. The entire time course was similar to that of the above-mentioned hemagglutinin.

The autoantibody of infected rabbits which agglutinates the erythrocytes sensitized with the liver or the colon extract of the same infected rabbits is now under study.

C. Effects of rabbit serum on the motility of isolated rabbit intestine
Precipitin reaction

Fig. 1. Changes in titer of serum precipitin, which reacts with the extracts of the liver (A) and the colon (B) of the normal rabbits, during the course of experimental schistosomiasis. Ordinate: Titer. Abscissa: Week after infection.

The influence of blood serum taken from the normal and schistosomiasis rabbits on the motility of rabbit ileum was investigated with Magnus method. The results obtained are illustrated in Fig. 3 and 4. Very little affect on the motility of the intestine isolated from the normal rabbits was detected by application of the normal rabbit serum (Fig. 3-A, Fig. 4-A), but noticeable change was noted with the application of the infected rabbit serum (Fig. 3-B). On the contrary, the intestine isolated from infected rabbits showed a marked increase in its tonus as well as spontaneous contractions after the application of the normal rabbit serum (Fig. 4-B). Such an accelerating effect was usually preceded by a temporal depression of motility.
Fig. 2. Changes in titer of hemagglutinin against the erythrocytes sensitized with the extracts of the liver (A) and the colon (B) of the normal rabbits. Ordinate: Titer. Abscissa: Week after infection. See details in text.

SUMMARY

In the rabbits infected with a moderate number of schistosomes, an “autoantibody”, which shows precipitation reaction with the liver or colon extract of the normal rabbits, is 3 to 4 weeks after the infection. Furthermore, at the 4th to the 5th week of infection, another “autoantibody” which agglutinates the erythrocytes sensitized with the extract of the liver or the colon was proved to appear in blood serum. The titer of these circulating “autoantibodies” reached its maximum by the 8th or the 9th
Fig. 3. Effects of rabbit serum on the motility of normal rabbit intestine

Fig. 4. Effects of normal rabbit serum on the motility of rabbit intestine
week of infection.

The results obtained with Magnus method on the normal and the infected rabbit's ileum also seemed to suggest the presence of "autoantibody" in the intestine or serum of the infected rabbits.

REFERENCES

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