PRINCIPLES GOVERNING THE APPEARANCE OF Cx-REACTIVE PROTEIN AND ITS CONNECTION WITH ANTIBODY FORMATION

(UDC 616.153.96-02 : 616.9-097.2)

I. B. Fryazinova

Rickettsioses Division, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow
(Presented by Active Member of the Academy of Medical Sciences of the USSR P. F. Zdrodovskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 61, No. 5, pp. 61-64, May, 1966
Original article submitted October 7, 1964

Considerable attention has recently been paid to the pathogenetic role of C-reactive protein, appearing in human blood in various pathological states. However, the causes of the appearance of this protein and its role still remain unexplained.

After the discovery in rabbits [4] of a substance analogous to C-reactive protein, and called Cx-reactive protein (CxRP), the experimental study of the conditions of its formation became possible. It was found [6, 7] that CxRP appears in rabbits' blood not only in inflammatory processes, but also immediately after injection of an antigen. A high degree of correlation was observed between the amount of CxRP formed and the titers of the antibodies developing later. It was suggested that this protein may be directly concerned with the processes leading to antibody formation.

Since it is well-known that the ability to form antibodies changes in the course of an organism's development [3, 5], in this investigation the dynamics of the formation of CxRP was studied at various stages of ontogenesis.

EXPERIMENTAL METHOD

Pneumococcus type 1 in the R form, obtained from the Department of Microbiology, USSR Academy of Sciences Institute of Epidemiology and Microbiology, Leningrad, was grown to obtain Cx-polysaccharide on a broth made in acid casein hydrolyzate, pH 7.6. The Cx-polysaccharide was obtained by the method of Anderson and McCarty [4], modified at the S. M. Kirov Military Medical Academy.

CxRP was obtained from rabbits' blood taken 24 h after subcutaneous injection of 0.2 ml turpentine in accordance with the same instructions. The only difference was in the method of removal of lipids from the preparation of CxRP, which was done not with chloroform, but by precipitation in the cold (−12°) in ten volumes of a 3 : 1 mixture of alcohol and ether, followed by three washes of the residue with cold ether and lyophilic drying. A higher yield of CxRP was obtained in this way.

The CxRP was not crystallized. Serum against CxRP was obtained by immunizing guinea pigs. CxRP (0.5 mg) in 1 ml of adjuvant was injected intramuscularly into the hind limb twice, with an interval of 20 days between injections. Blood was taken from the heart on the 7th day after the second injection. Revaccination was carried out every 3-4 months with a single injection of the same dose of CxRP in adjuvant. Blood was taken 5 days after revaccination.

Since the antiserum against CxRP still reacted with normal rabbit serum (0.5 mm of residue after precipitation in a capillary tube) after immunization with adjuvant, it was exhausted by this serum. For this purpose antiserum against CxRP was treated by the addition of a mixture of sera of 30 normal rabbits, not containing CxRP, incubated for 2 h, and kept overnight in a refrigerator, after which the residue was removed by centrifugation. The antiserum treated in this manner was used in experiments to determine CxRP by the method of precipitation in a

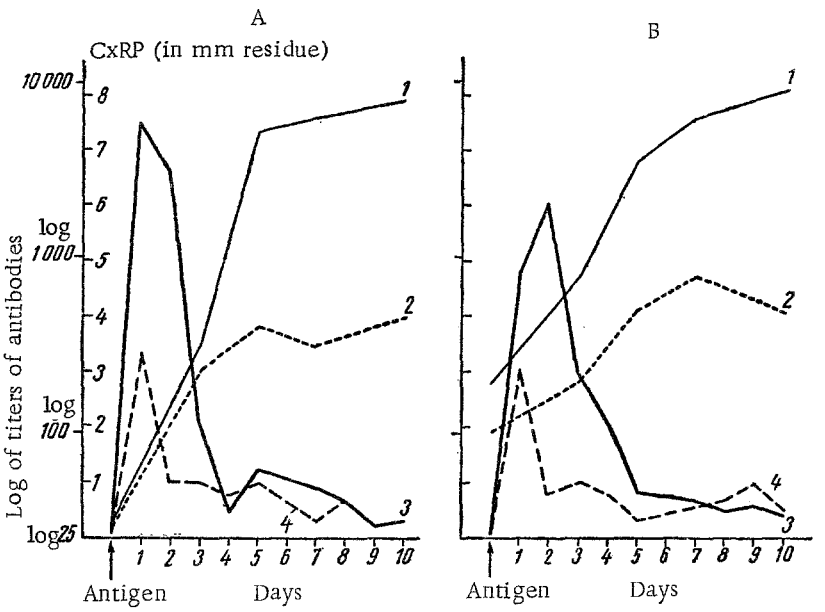

Fig. 1. Dynamics of accumulation of Cx-reactive protein and antibodies after a single injection of antigen into rabbits: A) typhoid vaccine; B) complete antigen obtained by Boivin's method. Titers of agglutinins: 1) active producers; 2) inert producers. Cx-reactive protein: 3) active producers; 4) inert producers.

capillary tube. The content of CxRP in the test serum was expressed in millimeters of precipitate (in accordance with the instructions for determination of C-reactive protein).

Experiments were conducted on adult rabbits weighing 2.5-3 kg and on animals aged 5 and 20 days.

The antigens used were typhoid vaccine strain No. 4446 and a complete antigen obtained from the same strain by Boivin's method. The antigens were given as a single intraperitoneal injection: the vaccine in a dose of 3 billion bacterial cells per rabbit, and the complete antigen in a dose of 2.5 mg/kg body weight. A nonspecific stimulus turpentine was injected intradermally into the animals.

The CxRP content in the sera was determined before injection, 5 h after injection, and daily for 10 days after the injection of antigen or turpentine. Titration of the antibodies was carried out by means of the agglutination reaction with typhoid diagnostic serum before, and 3, 5, 7, and 10 days after injection of the antigen.

RESULTS

In the first experiment nine adult rabbits were immunized with typhoid vaccine and seven with complete antigen (Fig. 1). After injection of the antigen CxRP was found within 5 h. Its content reached its maximum after 24 h, and then fell gradually, traces persisting throughout the period of observation.

Content of CxRP Protein in Serum of Rabbits after Intradermal Injection of Turpentine

Age of animals	Dose of turpentine (ml)	Content of CxRP in serum (in mm of precipitate)						
		before injection	24 h after	2 days after	3 days after	4 days after	5 days after	7 days after
Adult	0,2	0	6,4	6,4	3,0	1,7	1,5	0,8
»	0,1	0	4,3	3,7	2,6	1,8	—	—
»	0,05	0	3,2	2,9	1,2	0	0	0
5 Days	0,2	0	3,6	2,2	0,2	0,4	0	0
20 Days	0,2	0	4,6	3,1	1,6	0,4	0,2	0,6

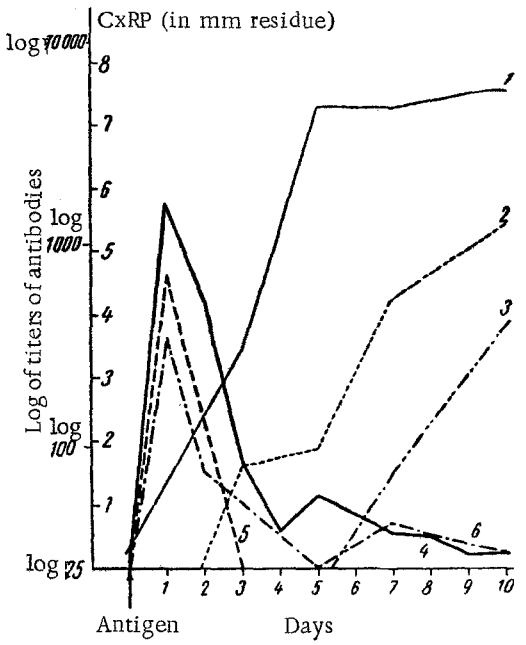

Fig. 2. Changes in dynamics of accumulation of Cx-reactive protein and of antibodies after a single injection of typhoid vaccine into rabbits at different stages of ontogenesis. Titers of agglutinins: 1) in adults; 2) in 20-day old; 3) in 5-day old rabbits; Cx-reactive protein: 4) in adults; 5) in 20-day old; 6) in 5-day old animals.

Five of the rabbits immunized with vaccine were found to have a high content of CxRP (5 mm or more of residue in the capillary tube) and high titers of agglutinins (1:3200-1:12,800). In three animals CxRP was found in small amounts (less than 5 mm) and agglutinins in low titers (1:200-1:800). One rabbit with a low CxRP content (3 mm) had high antibody titers.

Similar results were obtained after immunization with complete antigen.

In four rabbits a high CxRP content was accompanied by increased antibody production, while in three animals both the CxRP and the antibody titer were on a low level.

In the next experiment the relationship between the intensity of CxRP formation and the dose of the nonspecific stimulus was investigated. Rabbits were given an intradermal injection of turpentine: 10 animals received 0.2 ml each, 15 – 0.1 ml, and 12 – 0.05 ml. These experiments showed a direct relationship between the dose of turpentine and the amount of CxRP formed (see table).

To determine what changes take place in the ability to form CxRP in ontogenesis, two experiments were carried out on three age groups. Group 1 consisted of adult animals, which acted as controls, group 2 were 5 days old, and group 3, 20 days old. In the first experiment adult rabbits (9), 20-day old (5), and 5-day old (7) rabbits each received an intradermal injection of 0.2 ml turpentine. In the second experiment adult (10), 20-day old (4), and 5-day old (9) rabbits received typhoid vaccine.

The results given in Fig. 2 and in the table show that the ability to form CxRP varies with age. In both cases the lowest level of CxRP was found in the 5-day old animals and the highest in the adults. The 20-day old rabbits occupied an intermediate position.

Hence, in the overwhelming majority of animals (in 15 of 16) a correlation was observed between the amount of CxRP formed in the 1st day after injection of antigen and the level of the titers of the antibodies subsequently appearing. The ability to form CxRP also varied in ontogenesis parallel with the changes in immunological reactivity. This suggests a definite link between the formation of antibodies and of CxRP. The fact that CxRP appeared on the 1st day suggests that it is associated with the specific induction process. It has been demonstrated [1, 2] that protein metabolism is stimulated during the first hours after injection of antigens and of nonspecific stimulators, including Freund's adjuvant. According to other findings the injection of adjuvant and of its components stimulating antibody formation causes the appearance of large amounts of CxRP. It may therefore be postulated that CxRP formation is one of the manifestations of the activation of protein synthesis in the organism.

SUMMARY

It was found in a study of the conditions under which Cx-reactive protein forms in the rabbit body after a single injection of various antigens (typhoid vaccine or complete antigen produced from typhoid bacteria after Boivin) that the amount of Cx-reactive protein appearing immediately after antigen injection was correlated with the subsequent value of titers to this antigen. At the same time, the experiments showed that the capacity for production of Cx-reactive protein increases with the advent of age, just as the rabbit's capacity for immunogenesis. All this is evidence in favor of a definite connection existing between the formation of Cx-reactive protein and antibodies.

LITERATURE CITED

1. I. Ya. Uchitel' and É. L. Khasman, In book: Problems in Infectious Pathology and Immunology [in Russian], Moscow (1963), p. 55.

2. I. Ya. Uchitel' and É. L. Khasman, Vestn. Akad. Med. Nauk SSSR, No. 3 (1964), p. 23.
3. J. Sterzl, Uspekhi Sovr. Biol., Vol. 48, No. 3 (1959), p. 356.
4. H. C. Anderson and M. McCarty, J. Exp. Med., Vol. 93 (1951), p. 25.
5. J. V. Sparck, Acta Path. Microbiol. Scand, Vol. 46 (1959), p. 206.
6. H. F. Wood, J. Exp. Med., Vol. 98 (1953), p. 311.
7. Idem, Ibid., p. 321.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of the first issue of this year.
