EXPERIMENTAL STUDY OF ACUTE-PHASE REACTIONS

PART I. Cx-REACTIVE PROTEIN

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Serious attention has been paid of recent years to the so-called acute-phase reactions. These are related to the appearance in the blood of persons in the acute phase of various infectious and noninfectious diseases of a protein, which is not found in the blood of healthy persons. This protein has the property of reacting with the somatic pneumococcal polysaccharide, which is designated as C-polysaccharide, and for this reason it was called C-reactive protein.

The appearance and disappearance of C-reactive protein in the serum are closely connected with the exacerbation or extinction of inflammatory processes, and its content in the blood is an index of the activity of the morbid process.

The presence of C-reactive protein was first reported by W. Tillet and T. Francis [4] in pneumonia patients. The protein disappeared from the blood after the crisis had passed. The originally held view that C-protein is an antipneumococcal antibody was not confirmed. Shortly after, T. Francis [4] and T. Abernethy found this protein in the blood of patients suffering from acute rheumatism. C-reactive protein was first isolated from the blood serum of patients by C. MacLeod and O. Avery [3], and later by M. McCarty [2] from inflammatory exudates, and prepared in crystalline form.

Immunologically, this protein differs from other serum proteins. Immune serums obtained from rabbits which had been immunized against this protein contained the corresponding antibody, but they did not react with normal human serum. This finding permitted of the detection of C-reactive protein in the serum of patients.

G. Löfström's finding [1] that a protein analogous to C-reactive protein was present in the blood of rabbits suffering from acute inflammatory processes was of great importance for the study of acute-phase reactions. The rabbit protein did not react with pneumococcal C-polysaccharide, but it reacted with a related polysaccharide, which was prepared by rapid lysis of the bacteria, followed by removal of proteins. This polysaccharide was called Cx-polysaccharide, and it is thought to be a polymeric form of the somatic pneumococcal polysaccharide, termed C-polysaccharide (M. McCarty).

It was found that the serums of patients reacted with both of these polysaccharides, whereas those of infected rabbits reacted only with the Cx-polysaccharide. For this reason, the corresponding protein component of acute-phase rabbit serums was called Cx-reactive protein.

The above findings made it possible to study acute-phase reactions in animal experiments, and to attempt to use Cx-reactive protein antiserum clinically for the detection of C-reactive protein in the blood of patients.

The present research was undertaken with the following objects: 1) to ascertain the conditions and the

dynamics of appearance of Cx-protein in the blood of rabbits in response to various stimuli; 2) to attempt to obtain the corresponding antiserum; 3) to find whether the Cx-proteins formed under different conditions are immunologically identical.

EXPERIMENTAL METHODS

In our first series of experiments intracutaneous injections of a virulent culture of Type I hemolytic streptococcus were given to a group of 40 rabbits. We used an 8-hour meat broth culture, which was given undiluted in some cases (from 1 to 6 injections of 0.2 ml each), and in other cases at dilutions of 1:10 and 1:100. A marked inflammatory reaction was seen within 16-18 hours at the site of injection, where necrotic changes appeared subsequently. Some of the animals, which had been given undiluted culture, died within a few days, with symptoms of generalized sepsis. In the second series of experiments (17 rabbits) the animals received intracutaneous injections of live H. pertussis organisms, the inoculum of 16 billion microbial bodies being administered in a course of 20 injections; other animals of this series were given toxic whooping cough vaccine, at the same dosage levels. Pronounced hemorrhagic inflammatory reactions, followed by necrosis, were seen at the injection sites. In some of the experiments the animals were given diphtheria toxin, in doses of 1.5 Mld (guinea pig), in 5 injections. These injections too, were followed by a marked localized inflammatory reaction and by skin necrosis. The Schwartzman phenomenon was produced in 5 rabbits, by giving them 5 intracutaneous injections of E. coli filtrate (each 0.2 ml), followed after 24 hours by an intravenous injection of the same filtrate, at a dosage level of 2 ml per kg body weight. Finally, 2 rabbits were given intravenous injections of a pyrogenic preparation (Pyrogenal), in a dose of 5μg per kg body weight, which caused a transient febrile reaction.

TABLE 1

Times of Appearance and Disappearance of Cx-Protein in the Blood of Rabbits Inoculated Intracutaneously with Streptococcus Culture

Time of taking blood sample (hours)	Rabbits inoculated with culture in 1:10 dilution (5 injections)					Rabbits inoculated with culture in 1:100 dilution (5 injections)				
	1	2	3	4	5	6	7	8	9	10
4	++	+	.	.	.	.	.	±	±	+
8	++	+	.	.	.	.	.	+	±	.
18	.	++	++++	+++	++++	++	++	.	.	+
24	.	+++	+++(+)	++	+++	++	++	+++	++	+++
48	.	+	++	++	++	++	+++	++	+++	+
72	.	—	++	+(+)	+	++++	.	++	++	+
4 day	.	—	+	+(+)	+	++	.	+	+(+)	+
5—6 days	.	—	—	—	±	—	.	.	—	—

Explanation of symbols in this and in subsequent Tables; +++ and ++++ designate strongly positive reactions; ++ positive reactions; + weakly positive reactions; . tests not performed.

Repeated blood examinations were made, at times from 4 hours after beginning the experiments up to 6 days. We applied the complement-fixation test (prolonged fixation at +2°). The antigen was C-polysaccharide, prepared from a pneumococcal culture either by hydrolysis with HCl (by Lancefield's method), or by McCarty's method, with the difference that lysis of the pneumococci was achieved by adding ox bile instead of sodium desoxycholate. The Lancefield antigen was taken in 1:20 dilution; the McCarty antigen was dissolved to give a concentration of 1 mg/ml, and this solution was then diluted 1:50. The blood serums were used in dilutions of from 1:10 to 1:80.

The results of the tests were designated as positive (++) when total inhibition of hemolysis was found with not less than $1\frac{1}{2}$ doses of complement, and as strongly positive (++++) when a double dose of complement was taken. Blood samples taken from the same rabbits before the experiment served as controls.

EXPERIMENTAL RESULTS

Cx-reactive protein was found in 36 of 40 rabbits in which localized inflammatory reaction had been produced by injection of streptococcal suspensions. Of 87 blood serums examined, negative results were obtained in 12, and definite or strongly positive reactions (++ or ++++) in 61 cases; weakly positive results were obtained in 14 cases.

TABLE 2

Specificity of Cx-Reactive Protein Antiserums

Antigens ("acute-phase" rabbit serums)	Experiments using non-neutralized immune serum	Experiments with neutralized immune serum	
		Normal rabbit serum	"Acute-phase" rabbit serum
№ 31	++	++	—
№ 33	++	++	—
№ 37	++	++	—
№ 6	++++	++++	—
№ 7	++++	++++	—

Cx-reactive protein was found in the blood of all 17 of the animals of the second series of experiments. Negative reactions were found with 29 of 86 blood samples, definite or strongly positive reactions with 36 samples, and weakly positive reactions with 21 samples. The larger number of negative reactions found in the second series may be ascribed to the tests being performed at both earlier (4 hours) and later (5-6 days) times than in the first series.

TABLE 3

Reactions of Native and Neutralized Serums with Heterologous and Homologous Cx-Proteins

Antigens ("acute-phase" serums)	Immune serums from rats immunized against "acute-phase" rabbit serums											
	No. 2 37 and 34				No. 31 and 33				No. 6 and 7			
	non-neutralized	neutralized serums			non-neutralized	neutralized serums			non-neutralized	neutralized serums		
		№ 37 and 34	№ 31 and 33	No. 6 and 7		№ 37 and 34	№ 31 and 33	No. 6 and 7		№ 37 and 34	№ 31 and 33	No. 6 and 7
From rabbits inoculated with streptococcal culture — serums:												
№ 2	++	—	—	—		—	—	—	++	—	—	—
№ 37		—	—	—	++++	—	—	—	+++	—	—	—
№ 34	++	—	—	—	++++	—	—	—		—	—	—
From rabbits given H. pertussis culture — serums:												
№ 31	++	—	—	—	++++	—	—	—	+++	—	—	—
№ 33	++	—	—	—	+++	—	—	—	++	—	—	—
From rabbits showing the Schwartzman phenomenon — serums:												
№ 6	++	—	—	—	++++	—	—	—	+++	—	—	—
№ 7	++	—	—	—	+++	—	—	—	+++	—	—	—

The times of appearance and accumulation in the blood of Cx-reactive protein corresponded with the evolution of the inflammatory and febrile reactions. Thus Cx-reaction protein appeared soonest in the serum of rabbits which had been given Pyrogenal. Rise in temperature was seen after 1 hour, and a definite positive (++) reaction for Cx-protein was obtained after 4 hours, remaining at this level for a day. In animals suffering from localized inflammatory-necrotic processes due to intracutaneous introduction of H. pertussis culture or vaccine, or of diphtheria toxin, the highest contents of Cx-reaction protein were found after 24-72 hours, i.e at the times taken for development of the skin lesions; most of the rabbits giving the Schwartzman phenomenon showed maximum concentrations of Cx-reactive protein within 24-48 hours. The times of appearance of Cx-protein in the blood of rabbits inoculated with streptococcus culture, and of its subsequent disappearance, are shown by the data of Table 1.

For the preparation of serum immune with respect to Cx-reactive protein we immunized rats with rabbit serum in which this protein was present. Four groups, each of 10-25 rats, were immunized with serum obtained from rabbits which had been inoculated intracutaneously with streptococcal culture. The animals of another 4 groups were immunized respectively with serum taken from rabbits which had been inoculated intracutaneously with live H. pertussis culture, diphtheria toxin, and Pyrogenal, and in which the Schwartzman phenomenon had been produced.

Immunization consisted of 6 weekly courses of 4 daily injections of antigen, with a 3-day interval between the courses. Intraperitoneal injections of antigen were given, in doses of 0.01 ml per injection during the first week, and of 0.015 ml per injection in subsequent weeks. Blood samples were taken 10 days after completing immunization; they were tested for complement fixation with the "acute-phase" serums, and also with normal rabbit serum. In most cases we were thus able to obtain serums which gave a sufficiently definite reaction with "acute-phase" serum, while giving only a low titer with respect to normal rabbit serum.

Apart from this, specificity of the results was ensured by preliminary neutralization of the immune sera with normal rabbit serum. Examples of this are given in Table 2.

We performed 2 series of experiments with the object of ascertaining whether or not the Cx-proteins appearing in the blood of rabbits suffering from inflammatory conditions due to the action of various agents are immunologically identical. In the first series we tested various immune serums against "homologous" and "heterologous" Cx-proteins. In the second series we saturated immune serums with various "heterologous" Cx-proteins, and then tested the neutralized serums. Examples of these experiments are given in Table 3.

We found that each of the rat immune serums gave an equivalent reaction with all the rabbit "acute-phase" serums, irrespective of the nature of the agent responsible for the inflammatory reaction in both groups of rabbits. The corresponding antibodies were removed from the immune serums after saturation with any of the "acute-phase" serums. It may hence be concluded that the Cx-proteins appearing in the blood of rabbits suffering from inflammatory conditions caused by various agents are immunologically identical.

SUMMARY

The ability of acute-phase rabbit serums to react with somatic pneumococcal polysaccharide has been confirmed. The complement-fixation test is applicable to the detection of this reaction.

Immune serums were obtained from rats, which reacted specifically with rabbit acute-phase serums, but only weakly with normal rabbit serum. The Cx-reactive protein appearing in the blood of rabbits in the acute phase of inflammatory reactions to inoculation with various agents (pneumococcus and H. pertussis cultures, diphtheria toxin, E. coli culture filtrates, pyrogens) was immunologically identical, irrespective of the nature of the agent used.

LITERATURE CITED

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