

STUDIES ON THE Cx-REACTIVE PROTEIN

II. INHIBITION OF THE Cx-REACTIVE PROTEIN RESPONSE IN RABBITS BY BLOCKADE OF THE RETICULO-ENDOTHELIAL SYSTEM

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Despite extensive clinical and descriptive studies of the human C-reactive protein and definition of the experimental model, the rabbit Cx-reactive protein, nothing is known about the site or sites of origin of these acute phase proteins (1). It has been postulated that they appear as the direct result of tissue damage, but no evidence has been advanced to support this hypothesis. Earlier studies of the C-reactive protein and of the Cx-reactive protein related to their possible function or functions, but no studies indicating a possible cellular origin of the acute phase proteins have been reported (2, 3).

The present work is concerned with an investigation of the reticulo-endothelial system as the probable cellular system responsible for elaboration of the Cx-reactive protein. The investigation stems from the observation reported in the preceding study that the characteristic inflammatory response caused in rabbits by the intracutaneous injection of Cx-reactive protein incorporated in adjuvant can be prevented by previous intravenous administration of large amounts of thorotrast, a substance commonly used to "block" the reticulo-endothelial system (4). In earlier unpublished experiments done in this laboratory it was found that intravenously administered India ink and trypan blue dye, agents used to "block" the reticulo-endothelial system, caused the appearance of Cx-reactive protein in the blood of rabbits. Intravenously administered thorotrast proved to have this same property. It was found that thorotrast caused the appearance of Cx-reactive protein in the blood, but that repeated injections caused successively decreasing responses until finally further intravenous injections caused the appearance of little or no further Cx-reactive protein in the blood.

The goal of complete blockade of the reticulo-endothelial system cannot be readily achieved for several reasons: First, not all of the fixed elements of the system in various organs can be reached by intravenously administered "blocking agents," and although this unattainable portion represents only a minor

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part of the reticulo-endothelial system, it may still remain functioning (5). Secondly, new elements may be formed after blocking agents have been taken up and fixed by the available cells. Moreover, the elements of the reticulo-endothelial system which are not reached by intravenously administered blocking agents remain active.

Materials and Methods

Two types of experiments were carried out in the course of this study. In the first set of experiments thorotrast was given intravenously to determine its effect on the production of the Cx-reactive protein. In the second set of experiments rabbits which had received large amounts of intravenous thorotrast, amounts sufficient to "block" the reticulo-endothelial system, were challenged with Cx-reactive protein incorporated in adjuvant and with dermal infection with pneumococcus Type I.

Preparation of Cx-Reactive Protein for Incorporation in Adjuvant and Preparation of Adjuvant were carried out as in the preceding study (4).

Preparation of Sheep Antiserum to Cx-Reactive Protein and the Precipitin Tests with Rabbit Serum Employing Sheep Antiserum were carried out as in the preceding study.

Experimental Animals.—Rabbits weighing between 2500 and 3500 gm. were used in all experiments. A control bleeding was done on each animal to insure the absence of Cx-reactive protein in the serum at the beginning of each experiment.

Thorotrast.—Thorotrast, a 24 per cent to 26 per cent colloidal thorium dioxide in 25 per cent aqueous dextrin, was used in all experiments.¹ This substance has a direct toxic effect on rabbits, and the LD₅₀ varies from batch to batch. Very high doses of thorotrast were required to load the reticulo-endothelial system to an effective point. Thorotrast was given intravenously to sixteen rabbits. Eight rabbits received 41 cc. intravenously in four 9 cc. doses with a final dose of 5 cc.; the first three injections were given at intervals of 120 hours, the last two at intervals of 72 hours. Two of these eight rabbits died within 12 hours after the first injections; the remaining six tolerated the consecutive doses of thorotrast and none of them appeared ill during the course of the experiments.

An attempt to obtain an acute blockade of the reticulo-endothelial system by giving a first dose of 9 cc. and consecutive injections of 12 cc. every 12 hours failed and all eight of the rabbits died within 36 hours following the first injection.

RESULTS

Findings with Thorotrast-Treated Rabbits.—The findings in rabbits given serial injections of thorotrast are presented here.

All of the rabbits had previously been given 1 cc. of Cx-reactive protein in adjuvant intracutaneously to obtain control data. The first injection of thorotrast was given after Cx-reactive protein had been absent from the blood for 3 days. Following the injection of thorotrast Cx-reactive protein appeared in the blood in detectable amounts as early as 8 hours in all rabbits. This is the earliest consistent time at which the Cx-reactive protein has been observed to appear in response to any stimulus used in

¹ Thorotrast (Batch 07991) Testagar Company, Inc., Detroit.

this laboratory. The amount of Cx-reactive protein in the blood increased progressively to reach maximum values between the 28th and 32nd hour after the first injection of thorotrast and then decreased rapidly to become negative between the 72nd and 96th hours. The successive injections of thorotrast resulted in decreasing Cx-reactive protein responses until finally the last injection elicited little or no response. This phenomenon is illustrated in Fig. 1 where the precipitin reactions obtained between antiserum to Cx-reactive protein and sera from successive bleedings on three rabbits following three serial intravenous injections of thorotrast are shown.

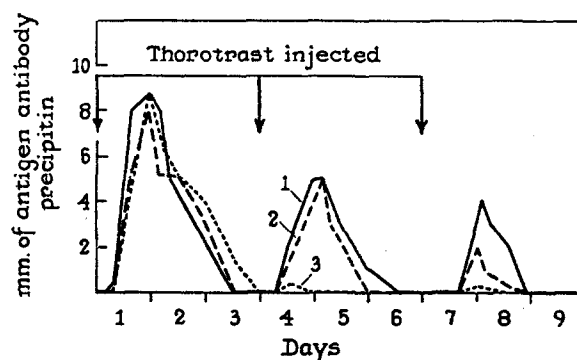


FIG. 1. The precipitin reactions obtained between antiserum to Cx-reactive protein and sera from successive bleedings on three rabbits following three serial injections of thorotrast.

TABLE I
Maximal Antigen-Antibody Precipitin Reactions for Cx-Reactive Protein (Measured in Millimeters) Following Successive Injections of Thorotrast

Rabbit No.	1	2	3	4	5	6
First injection	8	24	5	24	8	12
Second injection	7	15	5	8	6	10
Third injection	6	13	4	6	5	9
Fourth injection	3	4	3	5	5	Tr
Fifth injection	2	3	—	4	2	Tr

The maximal antigen antibody precipitin readings obtained with sera from six rabbits following each of five successive injections of thorotrast are presented in Table I.

This pattern of decreasing Cx-reactive protein response may be the expression of a progressive loading of the reticulo-endothelial system, since it has been shown that the greater the stimulating action of a blocking agent on the reticulo-endothelial system immediately following its administration, the higher is its capacity to block the system (6).

Findings with Injection of Cx-Reactive Protein in Adjuvant in Thorotrast-Treated Rabbits.—Following five successive injections of thorotrast the reticulo-

endothelial system was thought to be almost completely blocked and rabbits were challenged with two different stimuli to the production of Cx-reactive protein, (a) Cx-reactive protein incorporated in adjuvant and, (b) dermal infection with pneumococcus Type I. Experiments reported in the preceding study showed that intracutaneous injection of Cx-reactive protein in adjuvant is followed by typical cutaneous inflammatory lesions in the recipient rabbits, and by the appearance of large amounts of Cx-reactive protein in the blood (4).

Three rabbits whose reticulo-endothelial systems were loaded with thorotrast each received 1 cc. of Cx-reactive protein in adjuvant intracutaneously 72 hours following the final injection of thorotrast. The results obtained were compared with those obtained with the same rabbits following a similar injection administered 7 days before the first injection of thorotrast was given. Following the second injection of Cx-reactive protein in adjuvant marked differences were noted in all three animals both in the cutaneous response and in the blood Cx-reactive protein response. None of the "blocked" rabbits developed the typical inflammatory lesion seen in the control experiment. The nodules produced by the second injection of Cx-reactive protein in adjuvant did not change in size following injection and at 24 hours their diameters had not increased. Only a very slight inflammatory change occurred, consisting of a faint erythematous halo about the site of injection; one of the rabbits exhibited no erythema whatever.

In response to this second injection of Cx-reactive protein in adjuvant all of the rabbits produced a much smaller amount of Cx-reactive protein than in the control experiment and it was detectable in the blood for a shorter period of time. The maximal precipitin readings for Cx-reactive protein in the sera of these rabbits following the second Cx-reactive protein in adjuvant injection as compared with the maximal readings obtained in the control experiment were reduced by 78 per cent in that rabbit which gave a trace amount of Cx-reactive protein following the last injection of thorotrast and by 52.9 per cent and 50 per cent in the other two rabbits.

Findings with Pneumococcal Infection of the Skin in Thorotrast-Treated Rabbits.—Rabbits are known to respond to experimental pneumococcal infection of the skin by producing large amounts of Cx-reactive protein (1).

In this experiment three normal control rabbits and three thorotrast-treated rabbits were injected intracutaneously with 0.15 cc. of an undiluted 12 hour blood broth culture of pneumococcus, Type I, strain SV1. Within 18 hours Cx-reactive protein was detectable in the blood of both the control and the experimental animals. It increased sharply in amount in the control animals to reach its highest value in the 32nd to 36th hour following infection. At the site of injection an area of erythema developed which at its height (36 to 48 hours) covered an area of approximately 6 x 9 cm.

The three rabbits which had previously been treated with 41 ml. of thorotrast exhibited a striking difference in their response to dermal infection with pneumococcus as compared with the control group. This difference was particularly notable in regard to the elaboration of Cx-reactive protein. One of the rabbits which had failed

to produce Cx-reactive protein following the final injection of thorotrast produced very small amounts of the substance in response to the pneumococcal infection. The remaining two rabbits produced relatively small amounts of Cx-reactive protein. Comparison of the Cx-reactive protein responses of these two groups of three rabbits, the control group and the thorotrast-treated group, are shown in Figure 2. At the injection sites the area of erythema was definitely less marked in the thorotrast-treated rabbits than in the control animals, covering an area of approximately five times five cms. as compared with six by nine cms. in the control group. One of the thorotrast-treated rabbits had only a faint erythematous area three cms. in diameter.

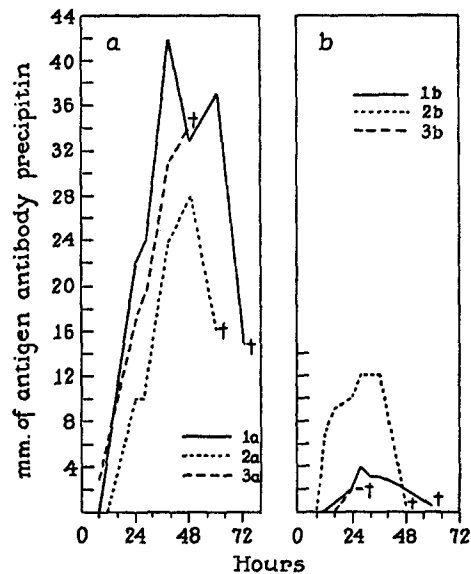


FIG. 2. Comparison of the Cx-reactive protein responses of two groups of three rabbits infected dermally with pneumococcus Type I. The animals in group *a* were normal, untreated rabbits. Those in group *b* had received successive injections of thorotrast.

DISCUSSION

The data obtained in this study suggest that blockade of the reticulo-endothelial system may markedly inhibit the production of Cx-reactive protein. The production of relatively small amounts of this substance after the administration of large amounts of thorotrast may be explained by the residual functioning elements of the reticulo-endothelial system which had not been reached by the blocking agent. Following the administration of Cx-reactive protein in adjuvant, thorotrast-treated rabbits responded with the production of less circulating Cx-reactive protein than did the control animals. More marked differences were noted when a more potent stimulus such as dermal infection with a Type I pneumococcus was used. In this group of animals the thorotrast

injections almost completely inhibited the production of Cx-reactive protein. It is probable that this inhibition was caused by blockade of the reticulo-endothelial system, but a direct toxic effect of thorotrast on cells other than those of the reticulo-endothelial system cannot be ruled out.

These experiments further suggest that Cx-reactive protein may be involved in the mechanism of local inflammatory lesions, since inflammation at the site of injection of Cx-reactive protein in adjuvant was reduced when Cx-reactive protein production was inhibited following successive thorotrast injections. Further experiments on a possible protective effect of injected Cx-reactive protein on rabbits subjected to chemical injury and to experimental infection are indicated. The inhibition of endogenous Cx-reactive protein production by thorotrast provides a method which renders such experiments possible.

SUMMARY

It has been found that normal rabbits respond to the intravenous administration of thorotrast by producing Cx-reactive protein. The amount of Cx-reactive protein produced in response to successive injections of thorotrast progressively diminishes until finally little or no further Cx-reactive protein production can be elicited. The reticulo-endothelial system is believed to be effectively blocked at this point. When such "blocked" rabbits are injected intracutaneously with Cx-reactive protein incorporated in adjuvant they produce significantly less Cx-reactive protein than the amounts elicited by the same stimulus prior to thorotrast treatment. They also fail to develop the characteristic inflammatory reaction seen in the control experiments. In addition, rabbits whose reticulo-endothelial systems are loaded with thorotrast respond to dermal infection with Type I pneumococcus by producing only very small amounts of Cx-reactive protein in comparison with the control animals.

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