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Rabbit CRP Production After Inulin Injection[1] (40423)

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The synthetic rate of C-Reactive Protein (CRP), which under normal conditions is a trace serum protein, increases by as much as 1000-fold during inflammation and tissue necrosis (1).

Although, several *in vitro* studies have indicated that CRP may play a role in host defense, the biological function of this protein is not yet clear. CRP in complex with an appropriate ligand can activate the classical complement pathway (2, 3). It has also been shown to enhance phagocytosis (4, 5), bind to certain subpopulations of T cells interferring with some of their functions (6), and inhibit platelet aggregation and mediator release (7).

It has been established that CRP is synthesized by the liver cells (8). However, the mechanisms which mediate CRP production are unknown. A recent study provided evidence that the signal for enhanced CRP production is blood borne (9).

In two experimental animal models associated with *in vivo* complement activation, intravenous endotoxin administration (10) and one shot serum sickness (11), increased CRP synthesis has been demonstrated. In addition, in turpentine induced inflammation, a classical model for CRP production, evidence has been presented suggesting that there is consumption of C3 (12). These observations prompted us to investigate the possibility that complement activation per se might result in increased CRP synthesis. *In vivo* activation of the classical pathway by immune complexes or aggregated γ globulin is associated with tissue and inflammatory changes which by themselves can be the stimulus for CRP production. For this reason activation of the alternative pathway by particulate inulin was studied.

Materials and methods. New Zealand white rabbits, weighing 2.5–3.5 kg were used in this study. Sterile particulate inulin (Armor-Stone Labs) was prepared according to Veroust *et al.* (13). The suspension of inulin was heated for 5 min at 56°, then centrifuged at 300*g* for 30 min resulting in very fine inulin particles. Three-hundred to six-hundred milligrams of this preparation of inulin, suspended in 3–6 ml of pyrogen-free, sterile saline were injected into the marginal ear vein. Blood was drawn by venipuncture before the injection and 4 hr and 24 hr later for CRP and C3 determination. These procedures were carried out very carefully in order to minimize stimulation of CRP synthesis by needle trauma. Each rabbit was used as his own control, 10 days before or after the injection of inulin. For a control experiment, each animal was injected with the same volume of sterile saline and blood was drawn as described above.

Serum CRP concentration was determined in duplicate by single radial immunodiffusion (14), using an antibody concentration of 1%. The CRP concentration of a standard rabbit acute phase serum used throughout this work was kindly determined by Dr. A. Osmand (Department of Immunology, Rush Medical College, Chicago, IL) using single radial immuno-diffusion. C3 concentration was determined according to Propp *et al.* (15) using the rocket electrophoresis method of Laurell (16). Six percent anti-rabbit C3 serum kindly provided by Dr. R. Propp (Department of Medicine, Albany Medical Center Hospital, Albany, New York) was used. The standard was a pool of normal rabbit serum.

The monospecific anti-rabbit CRP used in these experiments was raised in a goat using highly purified rabbit CRP as antigen. The specificity of the anti serum was checked by immunoelectrophoresis against acute phase serum, normal rabbit serum and purified rabbit CRP.

Acute phase rabbit serum containing CRP

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was obtained 24–72 hr following injection of 15 ml, 1% croton oil in corn oil in multiple sites (17).

Rabbit CRP was purified by affinity chromatography according to the method of Volanakis *et al.* (18), based on the Ca^{2+}-dependent affinity of CRP for phosphorylcholine. In brief, four successive chromatographic steps were used including Sepharose-4B-phosphorylcholine affinity chromatography, ion exchange chromatography on DE-52 cellulose, Sepharose-4B-phosphorylcholine chromatography and gel filtration using Sephadex G-200.

This preparation of rabbit CRP was pure as judged by: S.D.S. polyacrylamide gel electrophoresis (19) and by immunoelectrophoresis against anti serum to normal rabbit serum (Fig. 1).

Results. Results are summarized in Tables I and II. There were no significant differences in serum CRP and C3 levels between control and inulin treated animals, at 0 time. However, 24 hr after inulin injection, there was a significant increase in CRP levels in treated animals versus the control ($P < 0.018$), with a mean change of 33.0 ± 33.3 μg/ml and 3.7 ± 8.6 μg/ml in the inulin treated and saline treated groups respectively. There was a significant decrease in C3 levels 4 hr after inulin injection compared with the pretreatment levels and controls; ie there was a mean reduction of 35.1% ± 27.0 in the inulin-treated animals and no change in the control animals. Although, there was no direct linear correlation between the magnitude in fall in C3 levels and the rise in CRP, every animal which demonstrated increase in CRP level showed also reduction in C3 levels.

Discussion. Our results demonstrate that injection of small doses of fine particles of inulin to rabbits is followed by a measurable increase in CRP levels and decrease in C3 levels in the blood.

A previous report indicated that a single iv injection of similarly prepared finely particulate inulin did not cause any destructive or inflammatory tissue changes (13). On the other hand, it is well established that particulate inulin is an efficient activator of the alternative complement pathway both *in vivo* and *in vitro* (13). Our results, showing consumption of C3, also indicated that complement was activated following inulin injection. Most of the known biological activities of the complement system are generated during activation of its late acting components C3–C9. These late components can be activated by

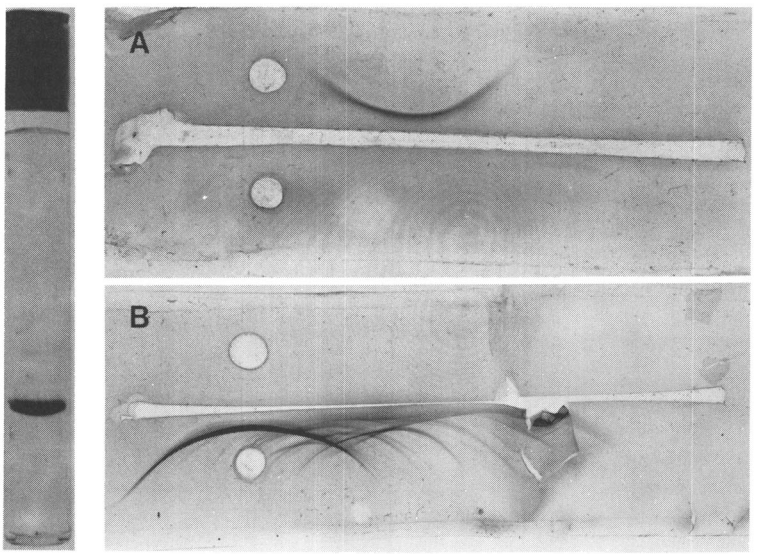

FIG. 1. Left: S.D.S. polyacrylamide gel electrophoresis of purified rabbit CRP; a single protein band is evident. Right: A. Immunoelectrophoresis against anti rabbit CRP (trough). Upper Well: Purified rabbit CRP. Lower Well: Normal rabbit serum. B. Immunoelectrophoresis against anti normal rabbit serum (trough). Upper Well: Purified rabbit CRP. Lower Well: Normal rabbit serum.

TABLE I. CRP RESPONSE TO INTRAVENOUS INULIN AND SALINE.

Rabbit No.	Inulin injection CRP μg/ml 0	24 hr	Δ CRP[a] μg/ml	Saline injection CRP μg/ml 0	24 hr	Δ CRP[a] μg/ml
1	5.8	12.0	6.3	8.3	8.3	0
2	8.7	27.7	19	5.3	5.3	0
3	7.9	24.8	16.8	9.9	10.6	0.7
4	8.9	26.4	17.5	5.3	8.6	3.3
5	15.5	35.0	19.5	5.9	15.5	9.6
6	5.6	28.7	23.1	8.3	19.5	10.9
7	7.8	55.0	47.2	5.3	13.2	7.9
8	13.5	26.4	12.9	5.3	8.6	3.3
9	6.9	46.2	39.3	28.4	28.4	0
10	5.9	118.8	112.9	21.8	12.5	−9.2
11	6.9	99	92.1	8.3	7.9	−0.3
12	10.9	15.2	4.3	6.6	34.7	28.1
13	7.3	25.1	17.8	5.0	5.0	0
mean ± SD	8.6 ± 3.0	41.6 ± 32.2	33.0 ± 33.3	9.5 ± 7.2	13.2 ± 8.9	3.7 ± 8.6
p[b]	N.S.[c]	<0.01[c]	<0.018[c]			

[a] CRP concentration difference in μg/ml between 0 time and 24 hr.
[b] Student paired t test.
[c] Versus saline injected animals.

TABLE II. C_3 RESPONSE TO INTRAVENOUS INULIN AND SALINE.

Rabbit No.	Inulin injection C_3 %[a] 0	4 hr	Δ C_3[b] %	Saline injection C_3 %[a] 0	4 hr	Δ C_3[b] %
1	68	74	+6	108	122	+14
2	108	52	−56	74	60	−14
3	62	32	−30	51.2	62	+10.8
4	114	90	−24	124	130	+6
5	200	102	−98	138	142	+4
6	106	53.6	−52.4	62	84	+22
7	68	48	−20	86	73.2	−12.8
8	142	88	−54	114	110	−4
9	176	148	−28	128	138	+10
10	124	106	−18	126.8	111	−15.8
11	80.8	67.2	−13.6	124	110	−14
12	114	100	−14	100	90	−10
13	108	53.2	−54.8	56	66	+10
mean ± SD	113.1 ± 41.1	78 ± 31.7	−35.1 ± 27.0	99.4 ± 30.3	99.8 ± 29.2	+0.46 ± 12.8
p[c]	N.S.[d]	<0.0016[d]	<0.0016[d]			

[a] % C_3 of pooled normal rabbit sera.
[b] C_3 difference between 0 time and 4 hr.
[c] Student paired t test.
[d] Versus saline injected animals.

either the classical or alternative pathway (23).

Except for complement activation, inulin has no other known biological effects. It is thus tempting to speculate that the increase in CRP levels observed in these experiments is related to complement activation by inulin, ie that complement activation products might act as one of the signals for CRP synthesis. Indeed, generation of complement activation

products has been demonstrated in many of the disease states commonly associated with increased CRP synthesis, e.g. acute infection, rheumatoid arthritis, etc. (20–22). However, it should be pointed out that the results represent an association not necessarily causal, and that the rise in CRP and fall in C3 may be only indirectly related. One alternative possibility is that the phagocytosis of inulin particles by the reticulo–endothelial system, perhaps Kupffer cells, stimulates synthesis of CRP. It has been shown previously that inulin particles are phagocytosed by the reticulo–endothelial system (13).

The mechanism by which complement products may stimulate CRP production by the liver cells is unknown. However, it is known that Kupffer cells have surface receptors for C3b (24) and a previous report has shown that Kupffer cells can regulate the activity of certain parenchymal liver enzymes (25). It is possible that C3b, alone or bound to inulin particles can trigger Kupffer cells to turn on CRP synthesis by the liver cells.

The lack of direct correlation between the decrease in serum C3 and the increase in CRP levels, after inulin injection, does not necessarily exclude a cause and effect relationship. It is well known that changes in serum complement levels do not necessarily reflect the true amount of complement activation products, generated by complement activation. This has been shown in various inflammatory states where there were normal or high complement levels but increased amounts of cleavage products (21–23).

Summary. The effect of intravenous injection of particulate inulin on CRP synthesis and serum C3 levels was studied in 13 rabbits. The rabbits showed a significant increase in serum CRP levels 24 hr after inulin injection, when compared to pretreatment levels and controls. There was a significant reduction in serum C3 levels in the treated animals at 4 hr.

The possible role of complement activation in Rabbit CRP production was discussed.

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