PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 149, 782–784 (1975)

Effect of Leukocytic Endogenous Mediator on C-Reactive Protein in Rabbits (38898)

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C-reactive protein (C-RP) has been shown to be an acute-phase protein which appears in the serum during infection and various other pathological conditions (1). It can also be induced in a variety of animals by injecting endotoxin, turpentine, croton oil, or paratyphoid–typhoid vaccine (2). The incorporation of amino acids by liver slices *in vitro* (2), as well as the time course of appearance of C-RP in serum and synovial fluid (3), indicates that C-RP synthesis occurs in the liver.

The mechanisms mediating the appearance of C-RP are unknown. Several recent studies have shown that leukocytic endogenous mediator (LEM) when injected in rats will cause the elevation of a number of the acute-phase serum proteins (4–7). LEM has also been shown to mediate a number of other acute phase changes in the rat, such as decreased plasma iron and zinc concentration and increased numbers of peripheral blood neutrophils (9, 10). Previous studies on LEM injections in rabbits showed that it produced fever (11), lowering of plasma zinc, and an increased flux of amino acids to the liver (12). There has been no study on the effects of LEM on acute-phase proteins in the rabbit. This report investigates additional responses of the rabbit to LEM, especially its possible role as a mediator of C-RP.

Materials and Methods. Animals. New Zealand white rabbits weighing 2–4 kg were purchased locally. All animals appeared healthy, and routine checks for C-RP were negative. Injection and blood sampling were from the marginal vein of the ear and each rabbit was used only once for C-RP determination.

Preparation of LEM. LEM was prepared from rabbit peritoneal granulocytes (13) and filtered through a membrane which retained molecules of molecular weight >100,000 daltons (Amicon XM-100). This crude LEM was used in the initial studies (Fig. 1). A similar number of peripheral blood leukocytes were isolated and homogenized for the control preparation (14).

The LEM was partially purified by the butanol–methanol method of Rafter *et al.* (15) for the studies shown in Table I. The dose of LEM injected was expressed in terms of the number of leukocytes in the original preparation.

Determination of C-RP. Serum or plasma levels of C-RP were quantitated by radial immunodiffusion as described earlier for α_2-macrofetoprotein (6). Rabbit serum, prepared 24 hr after injection of 2 ml of oil of turpentine into the *rectus femoris*, served as a standard source of C-RP and was arbitrarily assigned a concentration of 100 units/ml. Appropriate dilutions of the same standard serum were assayed on every plate and gave consistent ring sizes throughout. Goat antiserum to C-RP was purchased from Miles–Yeda Ltd., Rehovoth, Israel.

Measurement of other biological activities of LEM. Total blood neutrophils were determined by diluting blood with Turks' diluting fluid and counting total leukocytes in a hemocytometer, followed by a 100-cell differential count of a smear stained with Wright's stain (10).

Plasma iron and plasma zinc were determined on deproteinized samples by atomic absorption spectroscopy on a Model 403 Perkin–Elmer. Fibrinogen was determined by heat turbidity (16).

Results. The C-RP levels in the plasma of rabbits at various times after injecting LEM prepared from 3×10^8 peritoneal granulocytes or the homogenate from the same number of blood leukocytes are shown in Fig. 1. The level of C-RP was significantly increased ($P < 0.05$) at 8 hr after injection and continued to increase for 24 hr. After

48 hr, C-RP had declined to 2.4 units and at 72 hr could not be detected in four of the five rabbits tested. The normal level of C-RP was given as zero, since it could not be detected in 23 of 25 rabbits tested.

The effect of this same dose of LEM on fibrinogen, another acute phase protein, is shown in Fig. 2. Fibrinogen was significantly elevated at 12 hr, and the highest concentration was found at 24 hr after injection. Fibrinogen levels, in contrast to C-RP, remained significantly elevated 48 hr after injection.

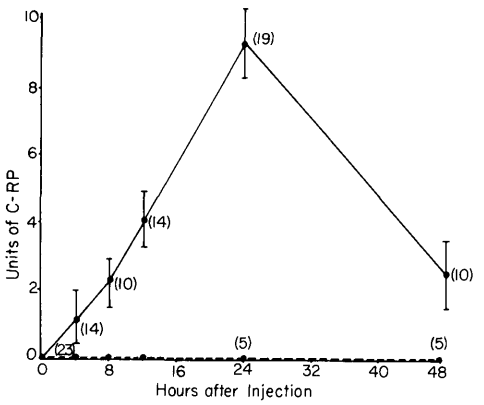

FIG. 1. Plasma C-RP response, expressed in arbitrary units, after iv injection of either 3×10^8 LEM (solid line) or leukocyte homogenate from the same number of cells (dotted line). The bars give the mean ± SE for the number of animals shown in parentheses.

After partial purification, the LEM preparation from 1×10^8 granulocytes contained 170 μg of protein. The effect of varying doses of this LEM preparation on several acute phase changes, including C-RP, is shown in Table I. There was a dose-dependent increase in body temperature which reached a maximum at about 45 min. Dose-dependent changes were also found in the number of peripheral blood neutrophils and the decreased concentration of plasma iron and zinc. Plasma iron in the rabbit was unusually sensitive showing a significant decrease after the injection of LEM from 1×10^6 granulocytes. C-RP was determined separately 24 hr after LEM injection. Only one dose (1×10^8 LEM) was used, but the level of 4.7 units corresponded well with the 9.3 units found 24 hr after injection of 3×10^8 crude LEM (Fig. 1).

Discussion. Previous studies in the rat showed that LEM induced increases in fibrinogen, haptoglobin (4), ceruloplasmin (5), the glycoprotein fraction (7), and α_2-macrofetoprotein (6). All of the previous studies on these acute-phase proteins used LEM prepared in rabbits with the increase occurring after injection into rats. It was, therefore, of interest that the fibrinogen increase in rabbits (Fig. 2) was similar to that previously reported for the rat (4), especially since some degree of species specificity for LEM has been demonstrated (12).

The time course of appearance of C-RP

TABLE I. CHANGES AFTER INJECTION OF LEM INTO RABBITS.

	Injection			
	None	10^6 LEM	10^7 LEM	10^8 LEM
Degree fever after 45 min	0	0.17 ± 0.08[a]	0.59 ± 0.10[b]	0.90 ± 0.05[b]
Blood neutrophils after 8 hr (per $mm^3 \times 10^2$)	16 ± 1	25 ± 7	37 ± 4[b]	71 ± 5[b]
Plasma iron after 8 hr (μg/100 ml)	211 ± 8	134 ± 12[b]	130 ± 12[b]	88 ± 8[b]
Plasma zinc after 8 hr (μg/100 ml)	222 ± 7	244 ± 15	164 ± 10[b]	180 ± 8[b]
Units of C-RP after 24 hr	0	ND[c]	ND[c]	4.7 ± 1.0[b,d]
Number of animals	36	6	10	27

[a] Mean ± SE.
[b] Significantly different from control ($P \leq 0.05$).
[c] ND = Not determined.
[d] Number of animals = 11.

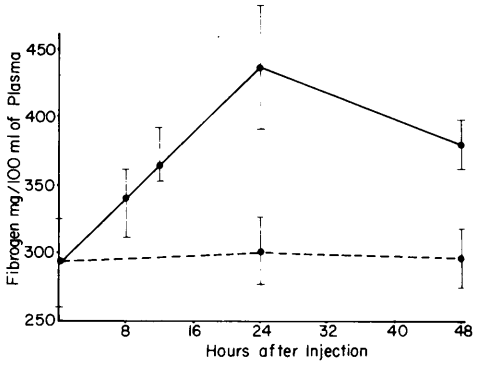

FIG. 2. Plasma fibrinogen response after iv injection of either 3×10^8 LEM (solid line) or leukocyte homogenate from the same number of cells (dotted line). The bars give the mean ± SE for the five rabbits at each point.

after LEM was similar to reports of other acute phase proteins. All of them attain their highest plasma levels approximately 24 hr after injecting LEM obtained from 1×10^8 granulocytes. C-RP is either absent or present at very low levels in the serum of normal rabbits. Measurable amounts of C-RP were found in 24 of 29 rabbits by Yen-Watson and Kushner (17).

This report adds C-RP to the list of acute phase proteins which are increased by LEM. It also shows that LEM may be the mediator for stimulating synthesis of these proteins in the rabbit as well as the rat.

Summary. Elevations in the plasma levels of the acute phase proteins—C-reactive protein (C-RP) and fibrinogen—were found after injection of leukocytic endogenous mediator (LEM) into rabbits. C-RP in the plasma was elevated 8 hr after injection of LEM, and maximum elevation occurred 24 hr after injection. Injection of LEM into rabbits also produced alterations in body temperature, in levels of plasma iron and zinc, and in the number of neutrophils in the peripheral blood.

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Received Feb. 3, 1975. P.S.E.B.M., 1975, Vol. 149.