The Effect of Complement Depletion by Cobra Venom Factor on Delayed Hypersensitivity Reactions[1] (36046)

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Although delayed hypersensitivity reactions are clearly cell-mediated immunological events (1), an increasingly large body of evidence indicates that they involve the participation of several humoral factors produced by immune lymphocytes upon interaction with a specific antigen (2). In addition to these factors, the participation of serum complement (C) has been both suggested, (3–5) and denied (6, 7).

Cochrane, *et al.* have isolated a low molecular weight protein component of cobra venom (CoF) which has potent anticomplement activity (6). Since administration of this material *in vivo* causes a profound depression of C levels (6), purified CoF was used in the present study to analyze the role of C in delayed hypersensitivity in actively sensitized guinea pigs.

Materials and Methods. Random-bred female albino guinea pigs weighing 350–500 g were immunized with 0.1 ml of Freund's complete adjuvant (Difco, H37Ra) containing 2×10^{-8} *M* azobenzenearsonate-*N*-acetyltyrosine, distributed among the 4 footpads. Eighteen days later the animals were divided into control and experimental groups. On Day 20, they were shaved, depilated, and skin tested in 2 sites intradermally with 20 μg of azobenzenearsonate-1-glutamylalanyltyrosine (ABA-1-GAT) and 1:100 old tuberculin, (OT) each in 0.1 ml saline. Test sites were observed at 3 hr for evidence of Arthus reactions and measured at 24 hr for induration and erythema. Reactions were graded according to diameter of erythema in millimeters and intensity of induration as follows: — is no induration, + is slight induration, ++ is induration, +++ is central blanch, ++++ is central hemorrhage.

Cobra venom from the cobra *Naja naja* was obtained from the Ross Allen Reptile Institute, Silver Springs, FL. CoF was isolated by DEAE-cellulose and gel filtration chromatography as described by Cochrane *et al.* (6). The low molecular weight factor was sterilized by filtration through a Millipore membrane and stored at 4°. A single band was found after electrophoresis in analytical polyacrylamide gel. The partially purified CoF preparation was prepared in an identical manner except that gel filtration on G-200 Sephadex was not performed.

Beginning on the 18th day after immunization, purified CoF, or sterile saline, was injected intraperitoneally in 4 equally divided doses over the next 24 hr. Each experimental animal received a total of 250 U of CoF/kg during the 24 hr period and 1 booster injection containing 62.5 U the following day (Day 20). The volume of each injection was 1.0 ml.

Serum samples were obtained for determination of C levels prior to the start of treatment on Day 18, and at the time of reading of skin test reactions, by cardiac puncture. Whole C activity was measured by a modification of the method described by Mayer (8).

The effect of purified CoF on the vascular component of delayed hypersensitivity was studied by observing the area of leakage of Evans blue dye given intracardially (ic) 24 hr after skin testing, in a dose of 3 mg/100 g of animal as a 2.5% solution in saline. Readings were made 30 min after administration and the resulting diameter was measured in millimeters (9).

All animals tolerated the above procedures well.

Results. In a preliminary series of experi-

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TABLE I. The Effect of Partially Purified CoF on the Expression of Active Delayed Hypersensitivity.

	Delayed reactions to[a]						
	Old tuberculin 1–100			ABA-1-GAT 20 μg			Posttreatment C activity (CH_{50} units/ml) mean
Treatment	Mean area	0 to ++	+++ to ++++	Mean area	0 to ++	+++ to ++++	
CoF	10	16	0	13	6	0	9
Saline	18	8	7	18	0	7	363

[a] Millimeters of erythema and degree of induration: 0 to ++ none to mild, +++ to ++++ is marked induration with central blanching and hemorrhage.

ments, the effect of partially purified CoF on the expression of active delayed hypersensitivity in guinea pigs was assessed. As noted in Table I, there was some reduction in both the area of reaction (mm erythema), and the degree of induration. It is noteworthy that these changes occurred in conjunction with a marked decrease in C activity.

Since this effect was observed with only partially purified CoF, it was essential to assess the effect of a highly purified factor on these reactions.

To this end, 16 female albino guinea pigs were immunized and studied 20 days later. The data, presented in Table II, fail to show a significant depression in the size or induration of the skin reactions. This lack of an effect occurred in the face of a profound depression of serum C in the treated animals. It is of interest that significant 3 hr-Arthus reactions were observed in the tuberculin skin test sites of all 7 control animals, while only 1 of 9 treated animals showed any evidence of such reactivity.

Changes in vascular permeability have been described in delayed hypersensitivity (9) and were studied here using Evans blue dye as the marker system (10). No significant differences were seen in the size or degree of bluing produced at the skin test sites of C-depleted animals when compared to control animals.

Discussion. Several lines of evidence have suggested that C is involved in reactions of delayed hypersensitivity. Neveu and Biozzi described a parallelism between serum C levels and delayed skin reactivity in rats decomplemented *in vivo* by either antigen-antibody complexes or aggregated gamma globulin (3). Furthermore, contact hypersensitivity in guinea pigs was suppressed by treatment with rabbit anti-guinea pig C (4). Allograft rejection has been claimed to involve serum C activity (5), and both a C dependent and independent pathway may be involved in the latter (5).

However, in an immunofluorescent study, Paronetto *et al.* failed to find evidence of C deposition in delayed hypersensitivity reactions (10), and Cochrane *et al.* failed to suppress contact hypersensitivity in guinea pigs by decomplementation with CoF (6).

In the present study, lesions of delayed hypersensitivity of 2 types were investigated. One—the tuberculin system, is complicated by occurrence of anti-tuberculoprotein antibodies. The other—the azobenzenearsonate system—is a system of "pure" delayed hypersensitivity, in that antibody does not occur in animals immunized with azobenzenearsonate-*N*-acetyltyrosine (11).

Our failure to find inhibition of either of these 2 systems by decomplementation with pure CoF strongly supports the concept that C is not involved in delayed hypersensitivity reactions. The reduction in the area of erythema and induration found in animals treated with partially purified CoF is of interest, and suggests that another, as yet unidentified, factor in cobra venom may affect the expression of delayed hypersensitivity reactions.

As yet unexplained is the pathway by which certain other agents inhibit delayed hypersensitivity. Ellagic acid (12), fumaropimaric

TABLE II. The Effect of Purified CoF on the Expression of Active Delayed Hypersensitivity.

Treatment	Delayed reactions to[a]			
	Arthus reactions O.T.[b]	Old tuberculin 1–100	ABA-1-GAT 20 μg	C activity (CH_{50} units/ml)
CoF	—	7++	15++	<10
	+	7++	16+++	<10
	—	10++	17+++	<10
	—	5+	15++	<10
	—	8++	13++	<10
	±	10++	12++++	<10
	—	5+	13+++	<10
	—	5+	11++	<10
	—	5++	13++	<10
Mean	—	7++	14+++	<10
Saline	++	8++	17+++	450
	+	10++	15++++	500
	+	12++	15++	685
	++	15++	22++++	74
	+	5+	15+++	345
	+	6++	13++++	166
	+	6++	18+++	217
Mean	+	9++	16+++	348

[a] Millimeters of erythema and degree of induration: 0 to ++ none to mild, +++ to ++++ is marked induration with central blanching and hemorrhage.

[b] Arthus reactions at tuberculin skin test sites; + is erythema, ++ is erythema and edema.

acid, (13) heparin and warfarin (14, 15) all inhibit such reactions, and all influence clotting mechanisms. It may be that some event in the development of delayed hypersensitivity reactions either parallels or directly involves a clotting factor (13). Much more work is needed in this area before a clearer understanding may be obtained.

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