Immunoelectrophoretic Patterns of C-Reactive Protein in Serum and In Ascitic Fluid from Patients with Cancer.* (30454)

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The significance of C-reactive protein (CRP) in serum and in certain pathological fluids remains an enigma. Although its prevalence in serum from patients with certain infectious, inflammatory and neoplastic diseases is well recognized, as yet it is not known whether CRP is a protective substance produced by the host to resist disease or is a product of pathologic processes to be eliminated by the host. Even the reported physicochemical properties of CRP are conflicting. Perlman(1) reported that CRP in moving boundary electrophoresis migrated as an α_1-globulin, which was supported by Hornung and Morris(2). The reports, however, were based upon only indirect evidence inasmuch as the CRP content of serum and of other body fluids is extremely small, and specific detection of CRP in the electrophoretic fractions is very difficult. Taylor(3) reported that partially purified CRP migrated as an α-globulin. Immunoelectrophoresis(4,5) and continuous zone electrophoresis(6) have greatly facilitated the electrophoretic study of CRP. Application of these techniques has not settled the problem, however. The reported immunoelectrophoretic and continuous zone electrophoretic mobility of CRP ranges from β_1-globulin(7) and β_2-globulin(8) to γ-globulin(9,10,11,12).

In this study, 3 different immunoelectrophoretic types of CRP in pathologic sera are reported, and the nature of the diversity is discussed based upon results obtained by experiments using partially purified CRP recovered from carcinomatous ascitic fluid.

Materials and methods. One hundred thirty-one sera with CRP determined by quantitative gel-diffusion technique(13) were subjected to immunoelectrophoresis. Eighty-one sera were obtained from patients with cancer, 62 of which had cancer of the lung. The remaining 50 sera were collected from patients with non-neoplastic diseases including rheumatoid disease and other types of acute and chronic inflammatory diseases. Scheidegger's micromodification(5) of immunoelectrophoresis, using veronal buffer, pH 8.2, was employed. Anti-human whole serum produced in the horse (AHW) and anti-CRP serum produced in the rabbit (CRPA) were used. Partially purified CRP was obtained from carcinomatous ascitic fluid by the ammonium sulfate method recommended by Wood, McCarty and Slater(14). It contained traces of albumen and β-lipoprotein. The partially purified CRP was defatted by magnetic stirring in the cold with several volumes of chloroform. The double diffusion precipitin method using a rabbit anti-β-lipoprotein serum was used to detect β-lipoprotein in the water phase. Enzymic digestion studies of the CRP were carried out using the methods employed in the study of antigenic structures of immunoglobulins. These included the method of Hanson and Johansson(15) for tryptic digestion, Porter's technique(16) of crude papain digestion and a modification of the method of Putnam, Lynn and Easley(17) for mercuripapain digestion. Degradation of the partially purified CRP with mercaptoethanol in urea(18) was also studied.

Results. A significant variation in the CRP-CRPA precipitin arc in immunoelectrophoretic agar plates was observed among the 131 sera tested (Fig. 1). In one group of sera the precipitin arc appeared in the mid-gamma zone (γ CRP). In a second group the precipitin arc was located in the beta zone (β CRP), and in a third group of sera, the arc was observed to "dissociate," changing from a single smooth arc into a "two-hump" configuration (2H CRP). These differences did not appear to be due to different electrophoretic conditions, inasmuch as simultane-

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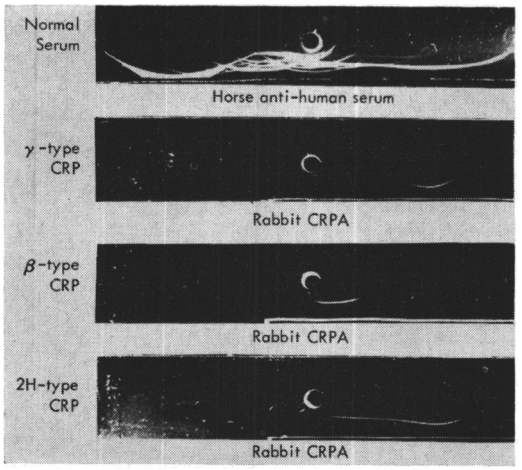

FIG. 1. Types of CRP by immunoelectrophoresis.

ous electrophoresis on the same slide of both "one-hump" and "two-hump" CRP containing sera resulted in their distinctive configurations. The incidences of the 3 types of precipitin arc were quite similar in both the neoplastic and non-neoplastic diseases. Of 81 cancer sera, β CRP was present in 13 or 16.0% of sera, γ in 25 or 30.8%, and 2H CRP in 43 or 53.1%. The 2H CRP was present in 30 or 48.4% of the 62 sera from patients with lung cancer. Among the 50 non-neoplastic sera, 7 or 14.0% contained β CRP, 8 or 16.0% γ CRP, and 35 or 70.0% 2H CRP. Thus, the 2H type was most frequently observed in the 2 groups of sera (Tables I and II). Gel diffusion precipitin analysis of these 3 electrophoretic types of CRP using the conventional 5-well arrangement of the Ouchterlony plate disclosed no evidence of immunologic heterogeneity.

Partially purified CRP was obtained from the ascitic fluid of 10 patients with carcinoma. Although the electrophoretic types of CRP in the ascitic fluid were diverse, being of the 2H type in 4 cases, γ type in 2 cases, β type in one case and undetectable in 3 cases, the partially purified CRP recovered in all instances showed a 2H configuration. However, during the course of isolation with repeated dialysis against $CaCl_2$ followed by centrifugation, the initial precipitates and also the final precipitates often showed either β or γ CRP (Table III). Thus it is possible that the difference in concentration of CRP in the solution was responsible for the diversity of the immunoelectrophoretic types. Effects of dilution of the serum upon the immunoelectrophoretic configuration were then studied. Dilution of CRP antiserum with veronal buffer was most marked in 2H CRP in both the neoplastic and non-neoplastic sera. As the dilution factor increased, the greater was the number of shifts from 2H to β and γ types. Dilution of the β and γ CRP did not change their precipitin arcs to the 2H type. One of the 6 sera with β CRP changed to the γ CRP. None of the 6 remaining C-reactive proteins shifted to either the β or 2H type (Tables IV and V).

Heating the CRP at 56°C for 30 minutes did not change the electrophoretic patterns of either the 2H or γ CRP patterns (Table VI).

TABLE I. Types of CRP in Serum from Patients with Neoplastic and Non-Neoplastic Disease.

	β		γ		2H		Total	
Disease	No.	%	No.	%	No.	%	No.	%
Neoplastic	13	16.0	25	30.8	43	53.1	81	61.8
Non-neoplastic	7	14.0	8	16.0	35	70.0	50	38.2
Total	20	15.2	33	25.2	78	59.5	131	100.0

TABLE II. Types of CRP in Serum from Patients with Lung Cancer and with Other Malignant Tumors.

	β		γ		2H		Total	
Type tumor	No.	%	No.	%	No.	%	No.	%
Lung cancer	11	17.7	21	33.9	30	48.4	62	76.6
Other neoplasms	2	10.5	4	21.0	13	68.5	19	23.4
Total	13	16.0	25	30.8	43	53.1	81	100.0

TABLE III. Comparison of Type of CRP in Serum and in the Ascitic Fluid with That Isolated from the Patients' Ascitic Fluid.

Site of cancer	Type of CRP in Serum	Ascitic fluid	Isolates from ascitic fluid
Ampulla of Vater		—	No. 2, 8, 9: β No. 3 to 7: 2H
Ovary	2H	β	No. 1: γ No. 2 to 10: 2H
Common bile duct		2H	No. 1, 18, 20: γ No. 2 to 17: 2H
Uterus		γ	No. 2 to 10: 2H No. 11 to 14: γ
Uterus		γ	No. 1 to 4: 2H No. 5: γ
Colon		—	No. 6, 8, 11: γ No. 7, 9, 10: 2H
Ovary		2H	No. 2: 2H
Colon	2H	2H	No. 1 to 5: 2H No. 6 to 15: γ
Stomach	β	—	No. 1 to 12: 2H No. 13 to 18: γ
Rectum	2H	2H	No. 1 to 10: 2H No. 11 to 15: γ

Defatting with chloroform failed to change the immunoelectrophoretic pattern of the 2H type of CRP, although this procedure eliminated β-lipoprotein initially present in the preparations (Table VII).

Trypsin digestion of the 2H CRP for 16 hours completely dissociated the protein with 2 precipitin arcs, one in the α_2-globulin zone and the other in the γ-globulin zone. Digestion of 2H CRP with crude papain for 2 hours resulted in incomplete separation into 2 precipitin arcs having a reaction of partial identity with each other. After incubation for 16 hours the antigenicities of both precipitin arcs were completely destroyed. Digestion with mercuripapain for 2 hours resulted in decreased intensity of the β CRP arc, but after digestion for 17 hours only the γ portion of the 2H CRP was demonstrable. Treatment of CRP with mercaptoethanol in urea resulted in almost instantaneous destruction of its antigenicity (Fig. 2).

Discussion. The presence of 3 types of CRP, *i.e.*, β, γ, and 2H, in serum, ascitic fluid, and in partially purified CRP recovered from ascitic fluid obtained from patients with cancer indicates the complexity of this abnormal serum protein. Several investigators have observed 2 or 3 antigenic components in CRP from acute phase serum(19,20). They did not, however, investigate the factors responsible for the variations in transfiguration of the CRP types. In the present study the antigenicity of the different types

TABLE IV. Effect of Dilution of β, γ and 2H CRP in Serum with Veronal Buffer Solution, pH 8.2.

No. serum	Source sera	Type	Converted to type CRP	Dilution 1:1.3	1:2.0	1:2.5	1:3.3	1:5.0
34	Neoplastic	2H	β	1	1	4	5	3
			γ	2	5	4	4	7
			2H	31	28	22	18	11
			Neg	0	0	4	7	13
16	Non-neoplastic	2H	β	0	0	2	2	0
			γ	1	3	4	5	7
			2H	15	13	9	8	4
			Neg	0	0	1	1	5
6	Neoplastic	β	β	5	3	3	2	1
			γ	0	1	1	0	0
			2H	0	0	0	0	0
			Neg	1	2	2	4	5
3	"	γ	β	0	0	0	0	0
			γ	3	2	2	0	0
			2H	0	0	0	0	0
			Neg	0	1	1	3	3
3	Non-neoplastic	γ	β	0	0	0	0	0
			γ	3	3	2	2	2
			2H	0	0	0	0	0
			Neg	0	0	1	1	1

TABLE V. Effect of Dilution of CRP with Veronal Buffer Solution, pH 8.2.

No.	Type CRP	Converted to	Dilution 1:1.3	1:2.0	1:2.5	1:3.3	1:5.0
10	2H	β	0	0	0	0	0
		γ	0	0	0	2	3
		2H	10	10	10	8	7
		Neg	0	0	0	0	0
6	γ	β	0	0	0	0	0
		γ	6	6	5	4	4
		2H	0	0	0	0	0
		Neg	0	0	1	2	2

of CRP was identical regardless of the electrophoretic mobilities. No difference in the incidences of CRP types in neoplastic and non-neoplastic diseases was observed and no significant correlation was observed between the type of CRP and the type of cancer.

Although no opportunity was available to compare CRP types in the serum of families, the influence of genetic factors does not appear probable inasmuch as shifts from one type of CRP to another were observed in serum from the same patient and by dilution of the 2H type CRP sera with the electrophoretic buffer.

TABLE VI. Effect of Heating on CRP at 56°C for 30 Minutes.

No.	Type of CRP	Converted to: Type	No.	%
6	γ	β	0	—
		γ	5	83.4
		2H	0	—
		Neg	1	16.6
10	2H	β	0	—
		γ	1	10.0
		2H	9	90.0

The present data indicate that CRP consists of 2 proteins with different electrophoretic mobilities but with identical antigenic properties. Clausen, Krogsgaard and Quaade(21) observed similar "double hump" configurations of CRP in cerebrospinal fluid of patients with bacterial meningitis. It is postulated that when the two proteins coexist in serum the immunoelectrophoretic precipitin arc shows the 2H configuration. The presence of a single molecular population, however, results in either β or γ CRP. Shifts of 2H CRP into either one of the "single hump" configurations, observed by dilution with a veronal buffer solution, suggests that the "single hump" configuration results when the concentration of either one of the two proteins is at a threshold level not detectable with the immunologic precipitin method. This was supported by the fact that the CRP preparations insofar as they were sufficiently concentrated, uniformly showed 2H configuration regardless of the immunoelectrophoretic type of CRP in the ascitic fluid prior to isolation. Contrary to Taylor's observation(3), defatting with chloroform did not change the electrophoretic mobility. Digestion with trypsin and with papain indicated that β and γ types of CRP possessed different susceptibilities to these proteolytic enzymes. Digestion with trypsin resulted in a change of electrophoretic mobility of β CRP, while the mobility of γ CRP remained unchanged. On the other hand, digestion with crude papain resulted in a partial loss of the antigenic structure of β CRP. Although digestion of 2H CRP serum with mercuripapain re-

TABLE VII. Effect of Defatting with Chloroform on β-Lipoprotein and CRP in Solution During Isolation.

		Before defatting		After defatting for 1 hr		18 hr	
		β-Lp	CRP	β-Lp	CRP	β-Lp	CRP
1.	CRP ("Pa" No. 5)	+	2H	—	2H	—	2H
2.	CRP ("W" No. 1)	+	2H	—	2H	—	2H
3.	CRP ("W" No. 4)	+	2H	Faint	2H	—	2H

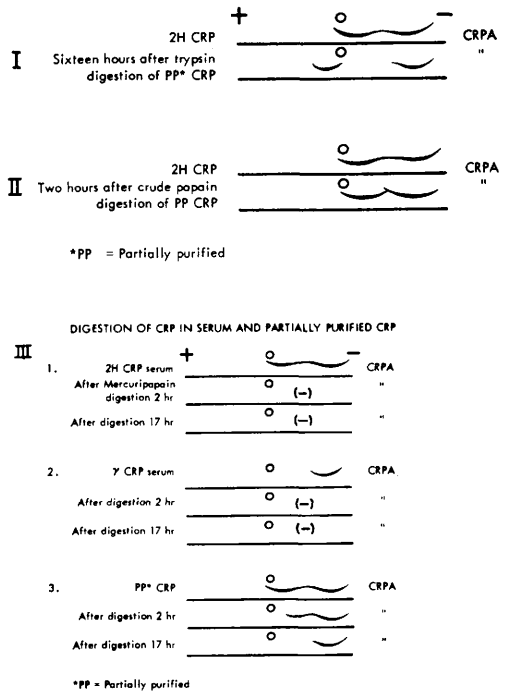

FIG. 2. Digestion of CRP in serum and partially purified CRP.

sulted in a loss of antigenicity of both types of CRP, only γ CRP was present after incubation for 17 hours when a sufficient amount of CRP was used as the substrate in the digestion study. Results of digestion studies and of degradation with mercaptoethanol also suggest that the antigenic structure of γ CRP differs greatly from that of serum γ-globulin, although the immunoelectrophoretic mobilities of the two proteins are similar.

The question of biological significance of the two types of CRP requires further investigation.

Summary. Three immunoelectrophoretic types of C-reactive protein, β, γ, and 2H, have been observed in serum from patients with neoplastic and non-neoplastic diseases. Gel diffusion precipitin analysis disclosed no evidence of antigenic heterogeneity. The nature of the electrophoretic dissimilarity was studied by means of dilution, defatting with chloroform, by heating and by enzymatic digestion of CRP in sera and of partially purified CRP recovered from carcinomatous ascitic fluid. The reported studies indicate that C-reactive protein consists of two proteins with different electrophoretic mobilities but with identical antigenicity. The two proteins differ in their susceptibilities to proteolytic enzymes.

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