CHARACTERIZATION OF A HUMAN ACUTE PHASE PROTEIN FOUND IN ASSOCIATION WITH RUBELLA VIRUS INFECTION*

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We recently described a new precipitating antigen, *rho*, in the blood of patients with rubella infection, and in the fluids from rubella virus-infected cell cultures (1). The antigen was detected by gel diffusion using antisera from rabbits which had been hyperimmunized with fractions of purified rubella virus. Although, on the basis of these and other preliminary experiments it seemed likely that *rho* was a structural protein of the virus, as the sensitivity of the assay was increased it became clear that the antigen was not restricted to rubella. Not only did we find it in sera from normal newborn infants but it was also detected in sera collected from populations living in remote areas where rubella had not penetrated, as shown by a lack of hemagglutination-inhibiting (HAI)[1] antibodies to the virus in all age groups.[2] Furthermore, we found that *rho* titers were elevated in situations and diseases other than rubella. These several pieces of evidence led to the conclusion that *rho* was an antigenic determinant of a normal plasma constituent which, like the acute phase proteins (2), increases in concentration following certain stresses.

The origin of the antigen in rubella virus-infected cultures was less clear, however, since *rho* released into the medium had about the same titer as rubella-specific antigens, and it could not be demonstrated in uninfected cells. A possible explanation seemed to be that rubella virus coded for a determinant which was antigenically indistinguishable from that of a normal serum component. This report compares the properties of *rho* obtained from the two sources (human sera and rubella-infected tissue culture media) and presents other data bearing on the genetic origin of the antigen. Our findings support the hypothesis that *rho* is a newly recognized acute phase protein, inducible both in vivo and in

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[1] *Abbreviations used in this paper*: CRP, C-reactive protein; HAI, hemagglutination inhibiting.

[2] We are grateful to Dr. Francis L. Black, Yale University School of Medicine, for providing samples from his collections of sera from isolated Indian tribes in Brazil.

vitro by rubella virus infection, and by other as yet unidentified factors in pregnancy and rheumatoid arthritis.

Materials and Methods

Rho Antigen.—The standard antigen was a 200-fold Aquacide (Calbiochem, San Diego, Calif.) concentrate of fluids from Vero monkey kidney cells grown in roller bottles and infected with the Therien wild-type strain of rubella virus isolated in our laboratory in 1965 (3). The medium consisted of 5% kaolin-absorbed fetal calf serum in Eagle's minimum essential medium, containing 100 U of penicillin, 100 μg streptomycin, and 10 U of Mycostatin/ml. Details of cell and virus propagation have been described previously (4).

Rho Hyperimmune Rabbit Serum.—A suspension of rubella virus purified according to the method of Liebhaber and Gross (3) was degraded with 0.5% Nonidet P-40 in Tris buffer (0.01 M Tris, pH 7.2, 0.1 M NaCl) containing 0.01 M $MgCl_2$. After 15 min at room temperature, the products of the reaction were separated by equilibrium density gradient centrifugation in 15–65% (wt/wt) sucrose gradients for 24 h at 40,000 rpm in an SW 41 rotor at 4°C. The peak of UV-absorbing material banding at a solution density of 1.25 g/ml was collected and dialyzed overnight against Tris buffer.[3]

Equal volumes of antigen suspension and complete Freund's adjuvant were thoroughly emulsified and injected intramuscularly into rabbits. The animals were thereafter inoculated at monthly intervals in the same manner and were exsanguinated 10 days after the third injection. Sera were absorbed with lyophilized calf serum and with sonicated Vero cells before use. The serum pool contained anti-*iota* and trace amounts of anti-*theta* rubella precipitating antibodies (5) as well as antibodies against *rho*.

Immunodiffusion.—Tests were done on 75- × 25-mm glass slides covered with 2.5 ml of gel consisting of 0.4% agarose in 0.01 M Tris buffer containing 0.1% sodium azide. Reactants were placed in wells 5 mm in diameter and 3 mm apart, and the slides were incubated in moist chambers at room temperature. Results were read after 24 h. In the standard *rho* antigen test, 20-μl aliquots of samples were tested using a 1:4 dilution of the standard rabbit antiserum; the titer was expressed as the reciprocal of the highest antigen dilution giving a line of precipitation which fused with the standard *rho* antigen-antibody precipitate.

Immunoelectrophoresis.—The micromethod described by Scheidegger (6) was used.

Theta and Iota Rubella Antigens (5).—The *rho* antigen standard also was used in immunodiffusion tests for *theta* and *iota* rubella reactants. Identification of the specific precipitates was aided by the use of human anti-*theta* serum and an *iota* standard antigen kindly provided by Dr. George Le Bouvier, Yale University School of Medicine.

Identification and Titration of C-Reactive Protein (CRP) and Other Human Proteins.—Immunodiffusion tests were done in the same manner as those for *rho* antigen. Purified CRP and specific hyperimmune serum were purchased from Difco Laboratories, Detroit, Mich. Purified plasma proteins and other specific antisera, including some prepared in rabbits and goats against whole human serum, were obtained from Hyland Div, Travenol Laboratories, Inc, Costa Mesa, Calif.

Partial Purification of Rho from Infected Tissue Culture Fluid and from Human Serum.—100 ml of medium obtained from rubella infected Vero cells was concentrated 200-fold by forced dialysis with Aquacide before fractionation. Human serum with a high *rho* titer and obtained from a patient with rheumatoid arthritis was first concentrated 10–20-fold by precipitation with 50% saturated ammonium sulfate. Each *rho* concentrate was dialyzed overnight against Tris buffer, reduced to a final volume of 0.5 ml with Aquacide, and layered over

[3] The standard antiserum was prepared by Dr. Harvey Liebhaber in this laboratory in the course of an investigation of rubella virus structure. Further details are to be published.

a 15–50% (wt/wt) sucrose gradient prepared in Tris buffer. The gradient was spun at 35,000 rpm for 16 h at 4°C in an SW 50.1 rotor. Twenty fractions were collected through the bottom of the tube and tested by immunodiffusion for *rho*. The positive fractions were then pooled, dialyzed against Tris buffer, concentrated to 0.5 ml by forced dialysis, and recentrifuged as described above except that a 15–30% sucrose gradient was used. Positive fractions were again pooled, layered over a 5 ml 12–65% sucrose gradient prepared in Tris-buffered D_2O, and centrifuged at 35,000 rpm for 48 h in an SW 50.1 rotor. The *rho*-positive fractions were pooled, dialyzed, and used for the determination of physicochemical properties of the antigen and for immunizing rabbits.

Fractionation of Human Serum.—140 ml of serum obtained from a single donor with juvenile rheumatoid arthritis and an elevated *rho* titer was centrifuged (2,000 rpm, 20 min) and the supernate was dialyzed overnight against 0.0033 M sodium phosphate buffer, pH 5.2. The precipitate was collected by centrifugation, washed twice with the dialyzing buffer, and dissolved in 2 ml of pH 8.5 Tris buffer containing 0.15 M NaCl. The serum supernatant fraction was precipitated in stepwise fashion with increasing concentrations of ammonium sulfate. Each mixture was stirred for 30 min before centrifugation and each precipitate was washed two times with ammonium sulfate at the concentration used for precipitation.

Sedimentation Analyses.—Buoyant density was determined by sedimentation through a preformed CsCl gradient, 20–40% (wt/wt), for 48 h at 5°C. Fractions were collected through the bottom of the tube and the density of each fraction was determined from its refractive index.

To estimate sedimentation coefficients, 0.2 ml of sample was layered on a 15–30% (wt/wt) sucrose gradient in Tris buffer and centrifuged in an SW 50 L rotor for 16 h at 5°C. Fractions collected through the bottom of the tube were assayed for *rho* activity. Heterophile antibody, 19S, and measles convalescent antibody, 7S, were used as sedimentation markers in tubes run in parallel (7).

Rubella Hemagglutination-Inhibiting Antibody.—Tests were done as described by Liebhaber (8) on 19S and 7S antibody fractions prepared by sucrose gradient centrifugation as described previously (9).

Fluorescent Antibody Staining.—Smears of normal or rubella virus infected Vero cells were prepared on glass slides. The cells were fixed in acetone at 20°C for 3 min. Rabbit anti-*rho* antiserum, human convalescent rubella serum, or other test serum described in the text was added and the slides were incubated at 37°C for 45 min in a moist atmosphere. The slides were then thoroughly washed with phosphate-buffered saline (0.01 M PO_4, 0.15 M NaCl pH 7.2). Goat antirabbit IgG or antihuman IgG globulin conjugated with fluoresceinisothiocyanate (Custom Reagent Lab., San Diego, Calif.) was then added for 45 min, 37°C. After a second washing, the slides were examined using a Leitz fluorescence microscope.

Serum Specimens.—The sera tested were obtained from several sources. Stored specimens from previous rubella investigations conducted in our laboratory provided the samples from normal children and adults, from patients in the acute phase of rubella, from virus-positive infants with the rubella syndrome, and from experimentally infected chimpanzees.

Cord sera were obtained from normal infants. Specimens were obtained from pregnant women and from patients with rheumatoid arthritis, degenerative joint diseases, and lupus erythematosus. All the above were patients on the wards or in the clinics of Yale New Haven Hospital and West Haven Veterans Administration Hospital. We appreciate the help of Dr. Eric Knox, Dr. Richard Lee, and Dr. Ernest Kohorn in obtaining these sera. We are indebted to Dr. Alfred Evans and Dr. James Niederman of the World Health Organization Reference Serum Bank, Yale University School of Medicine, for additional normal sera collected from Yale University freshmen. Dr. Eric Nordenfelt of the University of Lund, Sweden, kindly provided specimens from hemophiliacs, and we are grateful to Dr. Charles Alford, University of Alabama, for cord sera from cases of congenital cytomegalovirus infection.

RESULTS

A Comparison of the Properties of Rho Isolated from Human Serum and from Rubella Virus-Infected Tissue Culture Medium.—The properties of *rho* antigen in serum from a patient with rheumatoid arthritis and in fluids from rubella virus infected Vero cells were compared. The antigens had been partially purified by ammonium sulfate precipitation followed by two cycles of velocity sedimentation and one sedimentation to approach density equilibrium before testing, as described above.

Antigenic characteristics: When the antigens derived from the two sources were tested side-by-side with antisera from rabbits immunized with rubella virus-associated antigen the precipitin lines fused, giving a reaction of identity (Fig. 1). However, when the antigens were tested against a rabbit antiserum prepared against *rho* obtained from human serum, a slight spur was noted. This result could indicate that the serum particle carrying the *rho* determinant also contains a determinant not present on the antigenic particle produced in rubella virus-infected monkey tissue culture; it also could indicate the presence of an impurity. The latter might contribute to the higher sedimentation coefficient found for serum *rho* as noted below.

All sera screened for *rho* antigen were also tested for *rho* antibody at the same time, since each serum was reacted with both the standard antiserum and the standard antigen. We did not detect *rho* antibody in any of 306 early and late

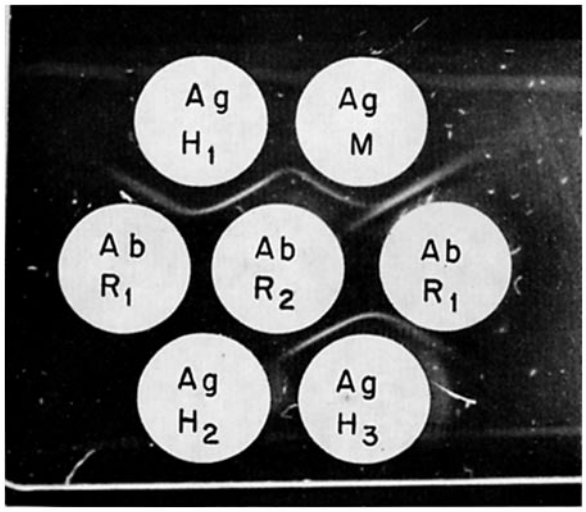

FIG. 1. Precipitin reactions between antigens from human sera or monkey cells and antibodies against monkey cell-derived *rho* antigen. Ab-R_1 and Ab-R_2 are antisera from rabbits immunized with rubella virus grown in monkey cells. Ag-H_1 and Ag-H_3 are *rho*-positive human sera, and Ag-M is concentrated medium from rubella virus-infected monkey cell cultures. Ag-H_2 is a *rho*-negative human serum. The second line seen between Ag-M and Ab-R_1 is a rubella *theta* antigen-antibody reaction.

convalescent rubella sera. However, anti-*rho* antibodies were found in sera from 3 of 18 rubella syndrome babies and also in the sera of 5 of 44 hemophiliacs.

Electrophoretic mobility and relation to recognized plasma proteins: Antigen from both sources showed identical migration patterns in the β-1 region as detected by immunoelectrophoresis. When human serum-derived *rho* was electrophoresed and tested against antisera prepared against homologous antigen and antigen from rubella-infected Vero cells their reaction lines met, showing a slight spur, again suggesting minor antigenic differences or the presence of an impurity (Fig. 2).

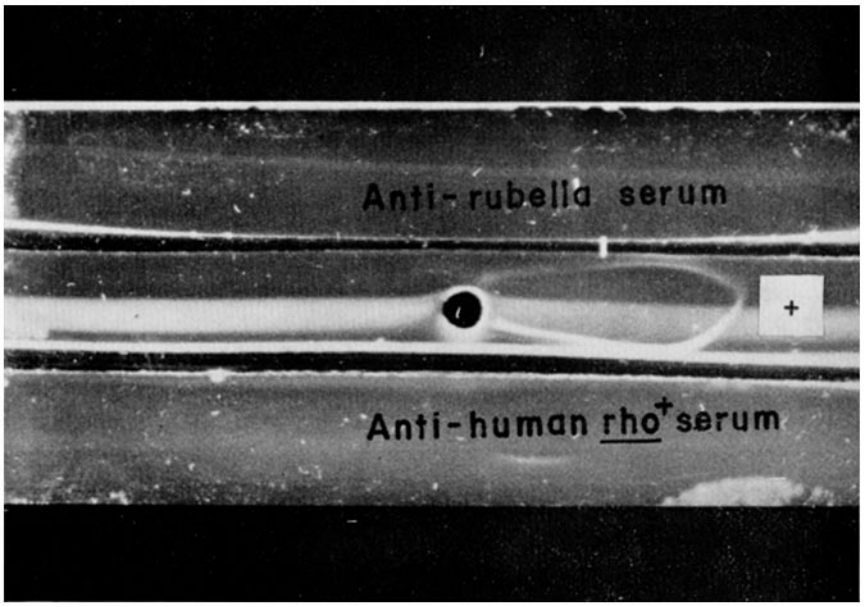

FIG. 2. Immunoelectrophoresis of *rho* antigen. The antigen was concentrated from human serum by dialysis against low ionic strength buffer as described in the text. After electrophoresis, antisera from rabbits immunized with *rho* derived from monkey cells, infected with rubella virus, or from human serum were added to the troughs.

Antisera specific for a number of normal plasma proteins[4] and CRP as well, failed to react with *rho* in either the immunodiffusion or immunoelectrophoresis test. Both goat and rabbit antihuman serum did contain anti-*rho* activity, however. After absorption with *rho*-negative human serum, these hyperimmune sera each gave a single line of precipitation when reacted against electrophoresed concentrated *rho*-positive human serum. It should be emphasized that neither antihuman sera nor the standard rabbit anti-*rho* serum detected *rho* in electrophoresed human sera, unless the latter were of high titer (e.g., rheuma-

[4] Antisera to the following were tested: IgG, IgA, IgM, 3rd and 4th components of complement, transferrin, haptoglobin, β-lipoprotein, fibrinogen, orosomucoid, and α-2-macroglobulin.

toid arthritis sera) and were concentrated three-to-fivefold before use. These results indicating the difficulty in detecting *rho* in human serum help to explain why *rho* has not to our knowledge been described before.

Sedimentation characteristics: Estimates of the sedimentation coefficients were obtained using 7S and 19S antibody markers. *Rho* from human serum appeared heterogeneous, sedimenting between 15 and 21S (Table I). *Rho* obtained from tissue culture seemed more homogeneous and banded between 11 and 14S. The role played by aggregation or disaggregation is not known. When sedimented for 48 h in CsCl gradients, both antigens banded alike at a density of 1.31 g/ml.

Stability: As shown in Table I, antigen from both sources exhibited similar stability characteristics typical of proteins.

TABLE I

A Comparison of the Characteristics of Rho Antigen Derived from Rubella Virus-Infected Vero Cells and from Human Serum

	Human serum	Tissue culture
Buoyant density	1.305 g/ml	1.305 g/ml
Sedimentation coefficient	15–21S	11–14S
Stability		
56°C, 60 min	+	+
Acetone 33%, 4°C, 30 min	+	+
Sodium deoxycholate 0.5%, 37°C, 1 h	+	+
Sodium dodecyl sulfate 1.0%, 37°C, 1 h	−	−
Trypsin 1%, 37°C, 1 h	−	−
2-mercaptoethanol 0.5%, 37°C, 30 min	−	−
pH 2, 22°C, 1 h	−	−

Solubility: When serum was serially fractionated, over 50% of *rho* activity was concentrated by overnight dialysis against low ionic strength buffer (Table II). The balance was recovered in the 33% ammonium-sulfate fraction after stepwise precipitation of serum.

In Vitro Synthesis and Inhibition by Actinomycin D.—

Intracellular localization with fluorescent antibody: Rubella virus-infected and uninfected Vero cells were examined by immunofluorescent antibody staining to detect the presence of *rho*. An antihuman-derived *rho* rabbit serum was used followed by fluorescein-conjugated antirabbit IgG. No fluorescence was seen in control cells at any time; in infected cells it did not appear until 2 days after inoculation. At that time the antigen was revealed as tiny starlike points of fluorescence throughout the cytoplasm (Fig. 3 *B*). The cultures were examined daily for 1 wk postinfection, and the stained granulations gradually increased in size but remained small and discrete. The reaction was not inhibited by pretreatment of the cells with rubella convalescent human serum which was used to demonstrate rubella-specific antigen in cultures infected at

TABLE II

*Percentage of Individual Human Serum Components Recovered after Successive Precipitations with Ammonium Sulfate**

Precipitation step	Percent of recovered activity in each precipitate						
	Rho	IgM	IgA	IgG	C'3	Beta-lipo-protein	Albumin
Dialysis against 0.0033 M Na+	52	64	3	5	3	6	<1
33% saturated $(NH_4)SO_4$	48	10	20	80	43	11	1
40% saturated $(NH_4)SO_4$	0	16	38	9	40	10	1
50% saturated $(NH_4)SO_4$	0	10	38	4	10	20	2
75% saturated $(NH_4)SO_4$	0	0	1	<1	3	53	96

* The serum was obtained from an adult with juvenile rheumatoid arthritis; no rheumatoid factor was detectable by latex agglutination.

the same time (Fig. 3 *C*). The *rho*-specific reaction was inhibited by an anti-human goat serum which had been absorbed with *rho*-negative human serum. This serum, as noted in the section on electrophoretic properties of *rho*, was monospecific for *rho* by immunoelectrophoresis.

Effect of actinomycin D: As a further test of the host or viral origin of *rho* in rubella virus-infected cultures, experiments were set up to see if we could show a selective inhibition of *rho* in comparison with a rubella antigen. Vero cells were grown in roller bottles and infected with rubella virus. 4 days after inoculation, at a time when virus was being maximally produced, the cultures were drained and fresh medium was added. One-half of the cultures received actinomycin D at a concentration of 5.0 μg/ml or 2.5 μg/ml, depending upon the experiment (Table III). 24 h later the cultures were harvested and the levels of *rho* and of the rubella antigen, *iota*, were determined. The results show a greater reduction in *rho* titer than in *iota* titer, providing additional evidence that *rho* may be an inducible host protein.

In another experiment actinomycin was added to cultures 48 h after infection when both *rho* and rubella antigens could be demonstrated intracellularly by immunofluorescence. *Rho* could not be detected after a 24 h exposure to drug, whereas rubella-specific fluorescence appeared the same as in untreated control cultures.

Production in virus-infected cell cultures from different species: Induction of *rho* by rubella virus was not limited to Vero cells (a monkey kidney line), but the virus induced *rho* in pig kidney and BHK 21 hamster cells as well. *Rho* obtained from all these sources gave reactions of identity with the standard antiserum. However, the antigen could not be detected in uninfected cultures even when media were highly concentrated or when cell packs were repeatedly used in an attempt to absorb out *rho* antibodies.

Concentrated medium from cell cultures infected with herpes simplex, measles, influenza, parainfluenza, or SSPE virus did not contain detectable

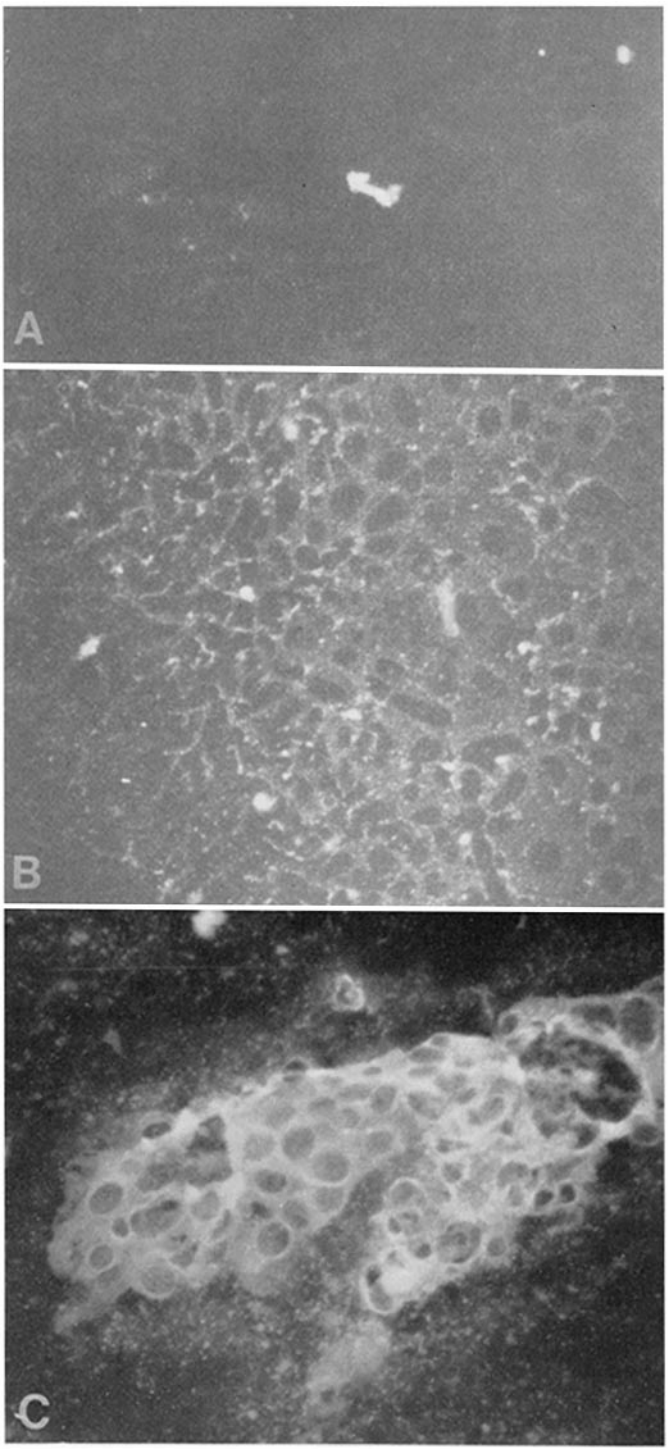

FIG. 3. Intracellular localization of *rho* antigen by fluorescent antibody staining. A. Uninfected Vero cells exposed to anti-*rho* rabbit serum followed by fluorescein-conjugated anti-rabbit IgG. B. Vero cells 48 h after infection with rubella virus and stained as in "A". C. Vero cells, 48 h after infection with rubella virus, exposed to human rubella convalescent serum, followed by fluorescein-conjugated antihuman IgG.

TABLE III

Effect of Actinomycin D on Yields of Rho and Iota Antigens in Rubella Virus-Infected Vero Cells 24 h after Exposure to Drug

	Total Immunodiffusion Units*			
	Expt. 1‡		Expt. 2§	
	Control	5 μg/ml actinomycin	Control	2.5 μg/ml actinomycin
Iota	120	80	800	400
	120	160	800	400
Rho	60	20	3200	200
	60	20	1600	200

* Total units = titer × volume of concentrate × 50. The results of two separate titrations are given.

‡The medium was pooled with a frozen-thawed cell extract; three cultures per group.

§ The medium alone was pooled; 16 cultures per group.

levels of *rho*, but two pools of fluids from Vero cells infected with adenovirus type 5 were shown to contain low levels of activity.

Similarities between Rho and Acute Phase Proteins.—It is a well known observation that in the course of viral or bacterial infections, chronic diseases, pregnancy, or after body injuries, the concentration of several plasma proteins is increased, and that some new proteins, like CRP in man and α-2-globulin in rats, become detectable in the blood. The latter are often referred to as acute phase proteins (2). The following results document the prevalence of *rho* in certain disease and physiologic states, and indicate the patterns of rise and decline of *rho* in comparison with CRP after natural and experimental rubella virus infection.

Rho and CRP in the blood after acute rubella: Serum specimens from 11 young male recruits during 2 wk postrubella were available for testing.[5] Fig. 4 shows that on the day of onset 8 patients had elevated *rho* titers, and CRP was detectable in sera from 10 of the men. The percent with elevated titers of both antigens declined from the 2nd day on, while antibodies to rubella virus increased in titer.

Appearance of Rho and CRP in the blood of experimentally infected chimpanzees: Serum specimens collected from two chimpanzees during an earlier study, in this laboratory, of experimental rubella infection and reinfection (10) were titrated for *rho* antigen and tested for the presence of CRP. The animals had been given live rubella virus by three different routes at intervals of 4–10 mo as shown in Fig. 5. The serologic findings were almost identical for the two animals, but for simplicity the results are shown only for one. After the first exposure, which was by virus feeding, no antibodies were detected and the only

[5] We are grateful to Maj. Donald M. Rosenberg, Medical Corps U. S. Army, for collecting these specimens.

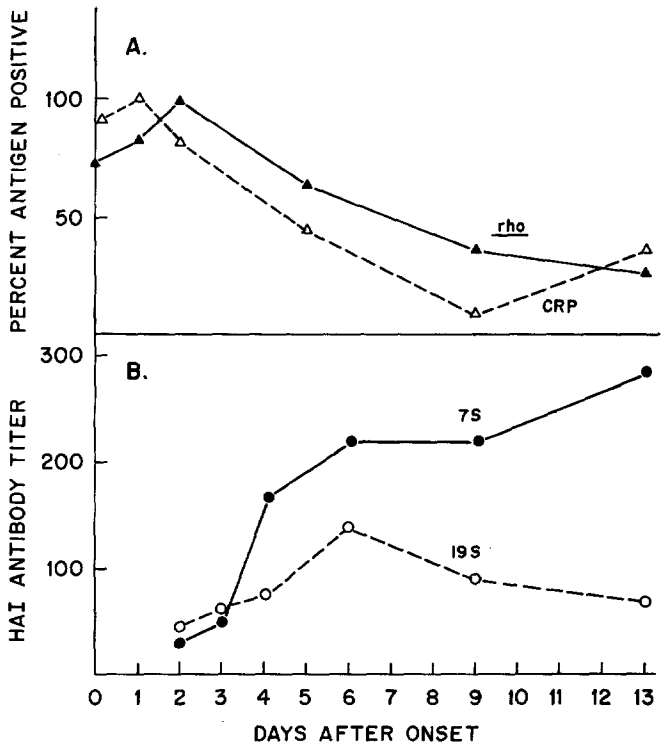

FIG. 4. Comparison of the clearance of *rho* and CRP after acute natural rubella infection: results on 11 patients. A. Percent with elevated titers of *rho* (≥ 2) and CRP by day after onset. B. Geometric mean rubella HAI antibody titers in 19S and 7S serum fractions.

evidence that infection had taken place was provided by the isolation of virus from the throat and from the blood on a single day. When the sera were subsequently tested for *rho* activity, a clear rise and decline was found commencing on the 10th day. No CRP was detectable during the 4 wk postinoculation period. When the animals were given virus by intravenous inoculation several months later, a typical rubella infection ensued with viremia and virus shedding from the throat, followed by the appearance of rubella neutralizing, complement-fixing, and HAI antibodies. *Rho* and CRP were below threshold levels in sera collected on the 6th day after inoculation, but both antigens were elevated in sera collected on day 10, and both were absent in specimens obtained after the 3rd wk. After intramuscular injection of live virus 8 mo later, no virus was isolated but there was a boost in antibody titer and a prompt, possibly accelerated, response to both CRP and *rho*.

Prevalence of rho: Table IV shows the prevalence and geometric mean *rho* titers in a preliminary sampling of sera from normal newborns and adults, pregnant women, and patients with chronic inflammatory disease. *Rho* was

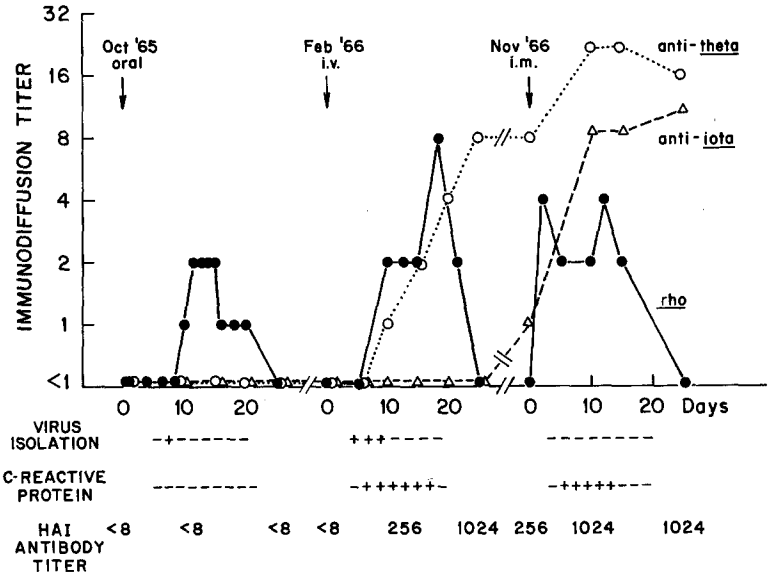

FIG. 5. Appearance of *rho* antigen in the blood after experimental rubella infection and reinfection in a chimpanzee.* The first virus exposure was by ingestion, the second by intravenous inoculation, and the third by intramuscular injection. The indicated virus isolations were from blood specimens.

detectable in 90% of newborn sera and titers were slightly elevated relative to adult controls. Of the groups tested, titers were most enhanced during pregnancy and in rheumatoid arthritis. In these cases, 100% of sera were positive, with geometric mean titers 5–7 times that of controls. The prevalence and titer of CRP is also shown, and while both antigens tend to be elevated in the same conditions, the correlation is not an invariable one.

DISCUSSION

We have presented evidence that *rho* is a protein antigen common to man and many mammals, and that rubella virus infection and a number of other stimuli may induce its increased production. The antigen has been induced in monkey, hamster, and pig cells and it has been demonstrated in sera from infected baboons, chimpanzees, and from pregnant rhesus monkeys. Antigens from all these sources gave reactions of identity in immunodiffusion tests with antiserum to antigen induced in monkey kidney cells. The spur noted when monkey and human-derived antigens were tested with a rabbit antiserum prepared against *rho* from human serum may reflect slight species-specific differences, or it may indicate the presence of impurities. When these experiments

* The serologic picture was duplicated in a second animal. Complete details of inoculation procedures and other serologic results have been described (10).

TABLE IV

Serum Levels of Rho and CRP in Normal Humans and in Certain Disease States

Source	Number tested	Age	*Rho* positive	*Rho*	CRP positive	CRP
			%	*GMT**	%	*GMT*
Normal adults	54	18–45	65	1.2	NT‡	NT
Normal cord	19	Newborn	90	1.5	NT	NT
Pregnancy (2nd and 3rd trimester)	24	14–37	100	5.2	77	3.3
Acute Rubella (15 days postonset)	31	25–30	96	3.7	30	1.0
Acute adenovirus infection (Untyped, 7–14 days postonset)	12	—	92	1.6	NT	NT
Congenital rubella	17	Newborn	94	4.8	NT	NT
Congenital cytomegalovirus infection	24	Newborn	100	3.2	NT	NT
Rheumatoid arthritis-active	12	41–74	100	8.3	90	5.6
inactive	10	44–72	100	3.6	51	4.6
Lupus erythematosus	15	14–35	93	2.0	20	2.6
Other diseases of the joints§	9	22–65	66	1.1	66	2.6
Hemophilia	44	—	88	2.4	43	NT

* Geometric mean titer of positive specimens.

‡ Not tested.

§ Ankylosing spondylitis (1), psoriatic arthritis (2), gout (3), dermatomyositis (1), chondrocalcinosis (1).

were repeated with antigens purified by column chromatography and with different rabbit antisera, no spur formation was seen.[6]

It might be concluded that the goat and rabbit lack normal cross-reactive *rho* antigens, since no antigen was detectable in normal sera from these animals and antibodies were raised after injection of *rho* from other species. However, we do not know the effects of the adjuvant present in the immunizing preparation, nor of hyperimmunization on immune recognition in this case. The presence of *rho* antibodies in sera from multiply transfused persons with hemophilia and in persistently infected rubella syndrome babies indicates that hyperimmunization may induce an immune response even to autologous *rho* antigen.

The possibility that rubella virus also codes for a cross-reacting antigen was considered, since *rho* was produced in normally negative cell cultures in amounts similar to the rubella-specific antigens *theta* and *iota* after virus infection, and it was difficult to dissociate the antigen from the virus during purification. The experiments using actinomycin D coupled with those comparing the virus-induced and rheumatoid arthritis-induced proteins provided evidence that in

[6] Liebhaber, Harvey. Manuscript in preparation. Purified antigen also failed to precipitate when dialyzed against water, indicating that the antigen may not be an euglobulin as was suggested by its insolubility in low ionic strength buffer (Table II).

the in vitro rubella system, too, *rho* is specified by the cell and not by the virus. This conclusion received firm support when it was shown that adenovirus also induced *rho* in Vero cells. Thus, an excellent new model is now available for the study of virus-induced cell genome expression.

When the dynamics of increase and decline of *rho* were compared with that of CRP in the blood of rubella patients and chimpanzees infected with the virus, similar but not identical patterns were found, and both were already elevated in the majority of patients at the time of onset of disease. The sensitivity of *rho* as an indicator of response to an infectious agent was illustrated by its appearance in two chimpanzees given rubella virus orally. There was no other serologic evidence of infection in these animals, but the virus was isolated from the blood and throat of one of them on day 6 postinoculation. Neither animal showed a CRP response nor an antibody response after ingestion of the virus. On subsequent intravenous inoculation of the virus, both chimpanzees developed subclinical infections; they demonstrated viremia, virus shedding from the throat, and developed rubella antibody responses which, in time-course and magnitude, indicated that the animals had not been primed by the first exposure. It may be that following the first exposure, *rho* was an indicator of rubella virus activity too weak even to have stimulated the animals immunologically. After the third exposure to rubella virus, some 10 mo after the second, there was a prompt increase in both *rho* and CRP in the blood. The rapid appearance at this time was most likely a reflection of anamnestic immune response rather than of viral multiplication, since Wood has shown that the level of CRP may reflect the intensity of immune response (11). Both mechanisms may have played a role, however.

We were not able to show a relationship between *rho* and previously described normal human proteins which migrate in the β-1 or α-2 regions during electrophoresis. Despite the fact that *rho* may have a functional relationship to CRP, they are different chemically and physically as well as antigenically (12, 13). *Rho* appeared to have a number of properties in common with another acute phase protein, however, viz., the α-2-globulin of the rat (14). When these two antigens were compared by immunodiffusion, no cross reactivity was found.[7] Whether *rho* has been recognized in man before and given another name is uncertain, but we know of no report of a particular antigenic species like *rho* that is recognized as an acute phase protein. The unusual set of circumstances that provided us with the necessary specific antisera, and the low levels of *rho* in human serum help to explain this gap.

SUMMARY

A precipitating antigen, *rho*, was first detected in the blood of persons with rubella and in rubella virus-infected cell culture fluids (1). Partially purified

[7] We are grateful to Dr. Edward J. Sarcione, Roswell Park Memorial Institute, who did these tests for us.

antigens from both sources were examined and shown to have similar properties, although antigen from serum sedimented more heterogeneously, with estimated coefficients from 15 to 21 S, while that from culture fluids sedimented in the 11–14 S region. In each case, antigen was located in the β-1 zone after electrophoresis in agarose, and at a density of 1.305 g/ml after centrifugation in CsCl. Stability characteristics were typical of protein antigens.

Immunofluorescent microscopy revealed that rubella virus induced the appearance of *rho* antigen scattered throughout the cytoplasm of infected cells. When cells containing antigen were exposed for 24 h to 5 μg/ml actinomycin D *rho* was no longer detectable, indicating the probable cellular origin of the antigen. Also, titers in medium of infected cultures showed a reduction after actinomycin treatment, but levels of the virus-specified antigen, *iota*, were relatively unaffected.

Rho appears to be a protein common to man and many animals. In vitro, it was induced by rubella virus and by adenovirus. In vivo, *rho* titers were shown to be elevated after rubella virus infection and, to a lesser extent, after infection with certain other viruses. High titers were also demonstrated in women late in pregnancy and in patients with rheumatoid arthritis. In man and the chimpanzee, the appearance and decline of *rho* in the blood after rubella virus infection were temporally similar to the patterns of CRP, although *rho* seemed to be a more sensitive indicator of infection.

The data presented indicate that *rho* is a newly recognized acute phase protein inducible by certain virus infections and by other unidentified stimuli present prominently in pregnancy and rheumatoid arthritis.

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