Biophysical Studies of Respiratory Syncytial Virus

II. Identification of Two Soluble Complement-Fixing Antigens of Respiratory Syncytial Virus

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Received for publication 18 September 1965

ABSTRACT

FORSYTH, BEN R. (National Institute of Allergy and Infectious Diseases, Bethesda, Md.), HELEN V. COATES, AND ROBERT M. CHANOCK. Biophysical studies of respiratory syncytial virus. II. Identification of two soluble complement-fixing antigens of respiratory syncytial virus. *J. Bacteriol.* **91**:1270-1276. 1966.—Column chromatography of concentrated preparations was used to separate two antigenic components from the "soluble complement-fixing (CF) antigen" of respiratory syncytial (RS) virus. These antigens, designated antigen A and antigen B, stimulated homologous but not heterologous CF antibodies. Only antigen A stimulated the production of neutralizing antibody in guinea pigs. There was a dissociation between the CF and neutralizing antibody responses of guinea pigs immunized with antigen A. The two antigens were shown to have different molecular size, density, and sensitivity to lipolytic agents. Both antigens appeared to be components of the RS viral particle. Serological studies demonstrated that both antigens were formed during RS virus infection of children and adults.

Respiratory syncytial (RS) virus has been shown to be an important cause of respiratory disease in infants and young children (1, 3, 4, 5). Recent epidemiological studies have implicated RS virus as the cause of over one-quarter of the serious respiratory illnesses in the first year of life (Chanock and Parrott, *Pediatrics*, *in press*). Characterization of the antigens of RS virus would aid in the serodiagnosis of infection and the interpretation of antibody changes after immunization. Since conventional inactivated vaccines prepared against this agent have proved to be ineffective, the identification and concentration of the antigen or antigens that stimulate protective antibody would also be of great importance in the development and evaluation of an effective vaccine.

Although RS virus lacks both a demonstrable hemagglutinin and the capacity to propagate in the embryonated hen's egg, it has been classified as a myxovirus on the basis of its size, ether sensitivity, ultrastructure, and nucleic acid type (8, 12). In the case of other myxoviruses, antigens have been separated and purified by use of the hemagglutination reaction. In such studies, both hemagglutinating and nonhemagglutinating antigens have been described, and these antigens

have been related to viral structure (2, 9, 10). Since RS virus does not hemagglutinate, it was necessary to use other approaches to separate and characterize the antigens of this virus. Previous studies have demonstrated that almost all of the complement-fixing (CF) antigen produced when RS virus is propagated in tissue culture cells is soluble and is separable from the virus particle by centrifugation (5). Very little is known about the number or nature of the antigens produced or the relationship of this soluble material to the subunits of the virus. The present study was undertaken to separate and identify components of the RS virus soluble CF antigen by column chromatography, with the expectation that this approach would provide information about the antigenic structure of this virus.

MATERIALS AND METHODS

Virus. The Long strain of RS virus, first isolated from the throat of a patient with bronchopneumonia and since passed six times in tissue cultures of KB cells, twice in Chang liver epithelium, and four or five times in HEP-2 cells, was used in all experiments (5).

Tissue culture. HEP-2 cells were grown in 5-liter Povitsky bottles in Eagle's basal medium with Hanks' balanced salt solution containing 10% inactivated

calf serum (56 C for 30 min). Prior to inoculation, the cells were washed with Hanks' balanced salt solution. Eagle's basal medium containing 5% inactivated agamma chicken serum (Hyland Laboratories, Los Angeles, Calif.) was used to maintain the tissue cultures.

Bovine embryo kidney (BEK) cells grown in 32-oz (ca. 1-liter) prescription bottles were purchased from Flow Laboratories, Rockville, Md., and were washed and maintained in the same way as the HEp-2 cells except for the substitution of agamma calf serum for chicken serum.

All media contained 0.1 mmole of L-glutamine, 100 units of penicillin, 100 μ g of streptomycin and 50 μ g of chlortetracycline per ml, and double the usual concentration of amino acids and vitamins. All tissue cultures were incubated at 33 to 34 C in a stationary position.

Preparation of concentrated antigen pools. HEp-2 or BEK bottles were infected with approximately 10^2 to 10^3 TCID₅₀ of a standard preparation of the Long strain of RS virus. In some experiments, virus was propagated in tissue cultures maintained without serum. Tissue culture fluid was harvested when 75 to 100% of the tissue culture cells showed cytopathic effects. The fluid was then centrifuged at 4 C for 20 min at 2,000 rev/min, and the pellet was discarded.

The clarified tissue culture fluid was concentrated 10 to 20 times by one of the following methods: (i) vacuum dialysis with an LKB ultrafilter, (ii) lyophilization followed by reconstitution with distilled water to one-tenth to one-twentieth of original volume followed by overnight dialysis at 4 C against Hanks' balanced salt solution, or (iii) precipitation of antigen by the addition of 70% (w/v) ammonium sulfate followed by overnight stirring at 4 C. The mixture was filtered, and the precipitate was reconstituted with 0.1 M phosphate buffer (pH 8.0) to one-tenth to one-twentieth of the original volume. The resultant suspension was dialyzed at 4 C against Hanks' balanced salt solution for 24 hr.

Some of the concentrated tissue culture fluids were then centrifuged for 2 hr at $78,000 \times g$ (30,000 rev/min in a Spinco no. 30 rotor), and the upper one-half of the fluid was retained for further study. All of the concentrated tissue culture fluids used in the study contained from 80 to 320 CF antigen units and 100 TCID₅₀ or less of RS virus per ml. All materials were stored at -60 C.

Serology. The technique for the complement-fixation (CF) test and the preparation of the Long CF antigen have previously been described (4, 11). All eluates obtained from chromatographic columns were tested with 4 units of RS human convalescent serum. All immune guinea pig sera were tested with 4 to 8 units of antigen standardized with the same RS human convalescent serum.

Neutralization tests were performed by use of the plaque reduction test which has been described in detail elsewhere (Coates, Alling, and Chanock, Am. J. Epidemiol., in press). Tenfold dilutions of heat-inactivated serum (56 C for 30 min) were incubated at room temperature with an equal volume of diluted virus containing approximately 30 to 130 plaque-

forming units (PFU) per 0.2 ml. An 0.2-ml amount of each mixture was inoculated into three petri dish cultures of HEp-2 cells. After a 2-hr absorption period at 35 C, the cells were overlaid with 1% Eagle's basal medium containing 5% chicken serum and 1% methylcellulose. After 48 to 72 hr, the petri dishes were fixed with 10% formalin for 24 hr and then stained with Giemsa stain. The results in each test were expressed as the per cent reduction of PFU (geometric mean of three plates) of the diluted virus by the given dilution of serum. Fifty per cent or greater plaque reduction was considered as evidence of neutralization.

Chromatographic method. A 7-g amount of Sephadex G-200 was allowed to swell in 500 ml of 0.1 M tris(hydroxymethyl)aminomethane (Tris) buffer (pH 8.0) containing 0.2 M sodium chloride for 72 hr or longer. With this material, columns 2.5 by 40 cm were prepared. Columns were equilibrated by allowing approximately 400 ml of buffer to pass through them. Then 5 ml of the concentrated tissue culture fluid containing 1.5 g of sucrose was layered on the top surface of the column. The same buffer was used for elution of material from the column. Two ml effluent fractions were collected and tested for the presence and titer of CF antigen as described above. Rechromatography was carried out by the same methods.

Immunization of guinea pigs. All antigens used for immunization of guinea pigs were prepared in HEp-2 tissue cultures. The antigens were rechromatographed once, and residual infectious virus was inactivated with ultraviolet light. Animals were immunized by intramuscular injection of 1.0 ml of rechromatographed antigen at 0, 2, 3, and 8 weeks, and were bled at 0, 3, 4, and 9 weeks. None of the animals had neutralizing or CF antibodies for RS virus prior to immunization.

Purification and concentration of RS virus. Partially purified and concentrated virus was obtained from infected BEK tissue culture harvests by the following method. A 25-ml amount of unconcentrated tissue culture fluid, clarified by centrifugation at 2,000 rev/min for 20 min at 4 C was carefully layered over 7 ml of 50% sucrose. This material was centrifuged for 2 hr at $63,000 \times g$ in a Spinco SW25 swinging-bucket rotor. After centrifugation, a visible band was present at the top of the sucrose layer. The bands were removed and resuspended to original volume in Eagle's basal medium without serum. The centrifugation procedure was then repeated. The visible bands were removed in a volume of fluid which was one-tenth of the starting volume. Infectivity titrations demonstrated a 10-fold concentration of virus in the band.

Density gradient centrifugation. The technique for isopycnic density gradient centrifugation of RS virus and antigens has been described in detail elsewhere (6). Tissue culture fluid was concentrated 20-fold by vacuum dialysis. This concentrated tissue culture fluid was mixed with cesium chloride and centrifuged in a SW39 rotor for 72 hr at $115,000 \times g$. Fractions of 0.5 ml were collected from the bottom of the tube, and CF antigen activity was determined by use of specific immune guinea pig sera and an RS human convalescent serum.

RESULTS

Separation of components of RS CF antigen by Sephadex chromatography. Concentrated tissue culture fluids were subjected to chromatographic separation on Sephadex G-200 columns. Fractions of 2 ml were collected, and all fractions were monitored for ultraviolet absorbance at 280 $m\mu$ and tested for CF reactivity against 4 units of RS human convalescent serum. Figure 1 illustrates the characteristic elution pattern which was reproducible from experiment to experiment. Three ultraviolet absorbance peaks were observed. Fractions collected from the first and second peaks contained RS CF antigen, whereas those collected from the third peak lacked antigen. CF activity could not be detected when all of the fractions from the third peak were pooled and concentrated 20 times by vacuum dialysis. When material from the first or second elution peak was concentrated by vacuum dialysis and rechromatographed under the conditions used for the initial separation, the elution pattern of these materials remained the same.

The two components of the RS CF antigen were designated according to their elution pattern from the Sephadex columns. The first antigen eluted from the column was designated antigen A, the second antigen B. For each experiment, the number of CF units in the starting material and in each of the fractions was calculated. Table 1 illustrates the results of 10 consecutive experiments with antigens prepared from infected HEp-2 or BEK tissue culture. Experiment 1 in Table 1 is the experiment illustrated in Fig. 1. In each of the 10 experiments, approximately four to six times more antigen B than A was recovered. Total recovery of CF antigen activity ranged between 34 and 57% with 45% of the CF activity in the starting material usually being recovered. Antigen preparations derived from HEp-2 or BEK tissue cultures did not differ significantly in either the percentage of

TABLE 1. Recovery of antigen A and antigen B after chromatography of "RS soluble CF antigen"

Expt no.	Tissue source	No. indicated CF antigen units recovered*		Ratio of CF units recovered: Antigen B/Antigen A	Per cent of total CF antigen recovered
		Antigen A	Antigen B		
1	HEp-2	16	72	4.5	44
2		22	112	5.1	34
3		28	144	5.1	42
4		22	112	5.1	34
5		40	164	4.1	51
6	BEK	24	144	6.0	42
7		34	146	4.3	45
8		14	96	6.9	55
9		18	94	5.2	56
10		18	96	5.3	57

* Chromatographic fractions were tested with a RS convalescent serum at a dilution which contained 4 antibody units for antigens A and B.

total CF antigen activity recovered or in the antigen B to antigen A ratio.

The elution pattern of the two antigens did not appear to be influenced by the presence of serum in the medium used to maintain the tissue cultures or by the method used to concentrate the tissue culture fluid. The elution pattern, ratio of antigen B to antigen A and the total amount of CF antigen activity recovered were the same, whether or not the tissue culture fluid concentrate was subjected to high-speed centrifugation ($78,000 \times g$ for 2 hr).

The chromatographic methods employed did not effectively separate tissue culture and media components from specific viral antigens. Complement-fixation tests indicated that all of the fractions tested contained at least 10 times more host cell antigens than specific viral antigens.

Prior to chromatography, none of the tissue culture fluid concentrates tested contained greater than 100 TCID₅₀ of infectious virus per ml. Under the conditions of chromatography utilized in the study, only trace amounts of infectious virus were recovered, and these were found irregularly in the first 5 to 10 fractions.

Preparation of specific antisera. Guinea pigs were inoculated with rechromatographed antigen A or B (prepared in HEp-2 tissue culture) which had been ultraviolet-irradiated to inactivate residual infectivity. The CF antigens employed to test the sera from the inoculated guinea pigs were prepared in BEK tissue culture to prevent cross-

FIG. 1. Chromatography of RS soluble CF antigens on Sephadex G-200.

reactions due to tissue host cell antigens. None of the guinea pig antisera reacted at a 1:10 serum dilution in the complement-fixation test with concentrated uninfected BEK material which had been prepared in the same manner as the test antigens. These sera were also tested for the presence of homologous neutralizing antibody by the plaque reduction technique.

The results of these immunization experiments are shown in Table 2. None of the animals had CF or neutralizing antibody prior to immunization. By 3 weeks, all of the animals immunized with antigen A developed a CF antibody response to antigen A, but these animals failed to develop CF antibody for antigen B. In addition, the serum of two of the three animals contained neutralizing antibody at 3 weeks. In one instance, this antibody could only be detected at a serum dilution of 1:10, whereas in the other instance neutralization was also detected at a 1:100 serum dilution. At 4 weeks, the CF antibody titer decreased and subsequently CF antibody could not be detected at 9 weeks despite the administration of a booster inoculation at the 8th week. Although CF antibody could not be detected at 9 weeks, all of the animals possessed significant neutralizing antibody at a 1:10 serum dilution, and two of the three animals also had neutralizing antibody in the 1:100 serum dilution.

The animals inoculated with antigen B exhibited a different serological response. By 3 weeks, two of three animals developed low levels of CF antibody to antigen B. However, by the 4th week this antibody was no longer detectable despite repeated booster inoculations of the

antigen. None of the animals developed CF antibody for antigen A or neutralizing antibody measurable by the plaque reduction technique.

In summary, the guinea pigs immunized with rechromatographed antigens developed specific CF antibody which was transient and which disappeared despite repeated injections of antigen. Only the animals immunized with antigen A developed neutralizing antibody, and the neutralizing antibody response was dissociated from the CF antibody response.

Results of chromatography of ether-Tween treated purified RS virus. An attempt was made to determine whether soluble CF antigens A and B were components of the virus particle. Partially purified and concentrated RS virus was prepared from infected BEK tissue culture material as described in Materials and Methods. This partially purified virus was then shaken with 1.25 mg/ml of polysorbate 80 (Tween 80) and an equal volume of ethyl ether for 2 hr at room temperature. The ethyl ether was then evaporated by passing 100% nitrogen gas through the mixture. After this procedure, no infectious virus was detected. This material was then chromatographed on Sephadex G-200.

The results of this experiment are presented in Table 3. Using the specific immune guinea pig sera, antigen A was demonstrated in the virus concentrate before and after ether-Tween treatment. Antigen B could not be detected. In an attempt to determine whether any antigen B had been present in the ether-Tween virus concentrate, all of the effluent fractions from absorbance peaks one and two were combined and concen-

TABLE 2. *Preparation of specific guinea pig antisera for antigens A and B*

Guinea pig immunized* with	Animal no.	Reciprocal of complement-fixing antibody titer†								Percentage plaque reduction‡				
		Test antigen: antigen A				Test antigen: antigen B				1:10 serum dilution			1:100 serum dilution	
		Pre	3 weeks	4 weeks	9 weeks	Pre	3 weeks	4 weeks	9 weeks	Pre	3 weeks	9 weeks	3 weeks	9 weeks
Antigen A	1	<10	80	40	<10	<10	<10	<10	<10	(39)	70	61	33	61
	2	<10	80	40	<10	<10	<10	<10	<10	(24)	39	93	(14)	59
	3	<10	80	40	<10	<10	<10	<10	<10	0	71	84	71	14
Antigen B	4	<10	<10	<10	<10	<10	40	<10	<10	(30)	(7)	(3)	NT	NT
	5	<10	<10	<10	<10	<10	10	<10	<10	(11)	11	8	8	8
	6	<10	<10	<10	<10	<10	<10	<10	<10	(9)	(24)	11	NT	11

* All animals were immunized intramuscularly at 0, 2, 3, and 8 weeks with 1.0 ml of the indicated antigen obtained from HEp-2 tissue culture-grown material.

† All sera were tested with antigens obtained from bovine embryo kidney (BEK) tissue culture-grown material and did not react at a 1:10 dilution with BEK control antigen.

‡ Values represent per cent plaque reduction obtained in tests in which 36 or 122 plaque-forming units were used. Values in parentheses represent per cent plaque enhancement. NT = not tested.

TABLE 3. Evidence for presence of antigen A and antigen B in partially purified RS virus

Material tested	Reciprocal of CF antigen titer with 4 units of indicated serum		
	Antigen A, immune guinea pig	Antigen B, immune guinea pig	RS human convalescent
Virus concentrate* (10X).....	2	<1	8
Ether-Tween treated virus concentrate* (10X).....	2	<1	8
Pooled concentrated (100X) chromatographic fractions† of ether-Tween treated virus concentrate.....	4	2	8 or >
RS antigen A.....	8	<1	8 or >
RS antigen B.....	<1	4	8 or >
Bovine embryo kidney control antigen.....	<1	<1	<1

* Virus concentrated by centrifugation at 63,000 $\times$ g for 2 hr onto a 50% sucrose cushion.

† Chromatographic fractions concentrated by vacuum dialysis.

trated by vacuum dialysis to a final concentration 10 times that of the original ether-Tween treated virus concentrate. Small quantities of both antigens A and B were demonstrated in this preparation.

The following experiment was undertaken in an attempt to explain the difficulty in recovering antigen B from the ether-Tween treated virus. Antigens A and B prepared by chromatography of tissue culture fluid concentrates, were treated separately with ether-Tween by the method described previously. The CF antigen titer of each preparation was determined before and after ether treatment (Table 4). The potency of antigen B was markedly reduced by the action of ether-Tween, whereas antigen A was more stable under the same conditions. Antigen B treated with ether alone lost all CF activity; however, this CF antigen was stable when treated with Tween alone at a concentration of 1.25 mg/ml. The lability of antigen B during ether-Tween

treatment helps explain the difficulty in detecting this antigen in the ether-Tween treated virus preparation.

Reaction of antigens with human antibody. Antigen A, antigen B, and the standard RS virus CF antigen were used to study the serological response of 12 adults who were naturally or experimentally infected with RS virus. Four antigen units of each preparation, as determined by use of a serum from an adult convalescent from RS virus infection, were used. The results are shown in Table 5. Five individuals showed a fourfold or greater rise in antibody when tested with antigen A, and four of these five men exhibited a response when tested with antigen B.

Seven of eight paired sera obtained from children under 18 months of age with RS infection exhibited a fourfold or greater rise in CF antibody when tests were performed with 16 units of the standard RS CF antigen. Previous studies have demonstrated that this quantity of CF antigen

TABLE 4. Effect of ether-tween treatment on antigens A and B

Material tested		Reciprocal of CF antigen titer against 4 units of RS human convalescent serum
Antigen	Treatment	
A	None*	8
	Ether-Tween †	4
B	None*	8
	Ether-Tween †	<2

* Sample shaken for 2 hr at room temperature with no additives.

† Sample shaken for 2 hr at room temperature with equal amount of ethyl ether and 1.25 mg/ml of Tween 80. Ether removed by evaporation with 100% anhydrous purified nitrogen gas.

TABLE 5. Development of antibody for antigen A and antigen B after infection with RS Virus

Group	No. tested	Number with fourfold or greater CF antibody rise with indicated RS antigen		
		Long*	Antigen A†	Antigen B†
Adult natural infection.....	6	4	2	2
Adult experimental infection....	6	3	3	2
Children.....	8	7	3	1

* Four CF antigen units used four testing adult sera and 16 CF antigen units used for testing children's sera.

† Four CF antigen units used in all tests.

TABLE 6. *Separation of antigen A and antigen B by density gradient centrifugation*

Fraction no.	Density	Per cent of CF antigen activity in each fraction when tested with indicated serum		
		Immune guinea pig antigen A	Immune guinea pig antigen B	RS human convalescent
1	1.386	0	0	0
2	1.373	0	27	0
3	1.325	13	27	10
4	1.298	51	14	19
5	1.276	25	14	19
6	1.255	6	14	10
7	1.234	2	3	19
8	1.216	2	1	19
9	1.296	1	0	4
10	1.168	0	0	0

is required for optimal demonstration of RS CF antibody in the sera of young infants (4). Because of the low potency of antigens A and B, only 4 units of each antigen could be used for testing these sera. Three of the eight children exhibited a fourfold or greater rise in antibody to antigen A, whereas only one had a similar response to antigen B.

Separation of antigens by density gradient centrifugation. An attempt was made to determine the density of the CF antigens of RS virus. Fractions were collected after isopycnic density gradient centrifugation in cesium chloride of a tissue culture fluid concentrate. CF antigen activity of each fraction was measured by use of the specific immune guinea pig sera and a human convalescent serum. Most of antigen A banded at an average buoyant density of 1.30 g/ml, and antigen B banded at an average buoyant density of 1.34 g/ml. Forty-two per cent of the CF antigen activity measured by the RS human convalescent serum was found in those fractions with a buoyant density of 1.20 to 1.23 g/ml. Other studies have demonstrated that infectious RS virus bands at a buoyant density of 1.23 g/ml in cesium chloride. Although intact viral particles might account for some of the CF antigen activity detected by the RS human convalescent serum in these fractions, the titers found were higher than that obtained with purified concentrated virus alone (6). These results suggest that there may be another antigen(s) that is measured by the RS human convalescent serum but not by the specific antigen A and B immune guinea pig sera.

DISCUSSION

The present study demonstrates that at least two antigenically distinct soluble complement-

fixing (CF) antigens were produced during the course of infection of tissue culture cells with RS virus. These two antigens were separable on the basis of molecular size by chromatography of tissue culture fluid concentrates on Sephadex G200 columns. Both antigens were produced in HEP-2 and BEK tissue cultures infected with RS virus.

Guinea pigs immunized with antigen A produced homologous CF antibody. CF antibody for antigen B did not develop. In addition, the animals immunized with antigen A developed neutralizing antibody. There was a clear dissociation of the neutralizing and CF antibody responses. The CF antibody titer was highest at 3 weeks. This antibody was transient and could not be detected at 9 weeks despite repeated administration of the immunizing antigen. In contrast, neutralizing antibody was detected in all animals at 9 weeks, and the level of neutralizing antibody at the 9th week appeared to be greater than that at the 3rd week.

Antigen B was much less antigenic for guinea pigs than antigen A. Only some of the animals immunized with antigen B developed homologous CF antibody, and the CF antibody produced was more evanescent than that stimulated by antigen A. None of the animals immunized with antigen B developed neutralizing antibody or CF antibody for antigen A.

In addition to the serological differences between the two antigens, there were biophysical differences other than molecular size. Antigen B was very sensitive to ether-Tween treatment, whereas antigen A was not. Preliminary data with other detergents confirms the fact that antigen B is more sensitive to lipolytic agents than is antigen A. The two antigens banded at different densities in cesium chloride during equilibrium centrifugation. Antigen A banded at an average buoyant density of 1.30 g/ml, whereas antigen B had an average buoyant density of 1.34 g/ml.

The results of the analysis of the antigenic composition of the ether-Tween purified virus suggests that both antigens are present in the viral particle. This is in agreement with data for other myxoviruses (2, 9, 10). Since antigen A stimulated neutralizing antibody, it is likely that this antigen is present on the surface of the viral particle. The failure of antigen B to stimulate neutralizing antibody indicates that the antigen is present either as an internal viral component or as a surface structure that is not involved in the neutralization of the virus by antibody. The poor antigenicity of this antigen suggests that it is either an internal nucleoprotein or an external lipoprotein. A buoyant density of 1.34 g/ml in

cesium chloride would support the former hypothesis, whereas sensitivity of this antigen to lipolytic agents favors the latter explanation.

The development of CF antibody to antigen A and antigen B after RS infection of certain adults and children indicates that both antigens are produced during the course of natural RS virus infection. Following such infection, some individuals developed CF antibody when tested with the standard tissue culture RS virus suspension, whereas antibody did not develop for the purified A or B antigens. These findings suggest that the RS virus tissue culture suspension contains antigens in addition to antigens A and B. The observation that 42% of the CF antigen activity recovered during density gradient centrifugation experiments could be measured by a serum from an individual convalescent from RS virus infection but not by specific guinea pig antisera for antigens A and B supports this hypothesis. The possibility exists that this additional antigen may be similar to the "T" antigens described for the adenoviruses and papovaviruses (7). "T" antigens are not viral subunits but instead represent cellular antigens induced by viral infection. However, in the case of adenoviruses, antibody to "T" antigens has not been demonstrated after acute infection.

The chromatographic methods utilized in this study separated but did not purify antigens A and B. Each antigen was contaminated with at least 10 times more tissue culture host cell CF antigenic material than viral CF antigen. Additional biophysical and biochemical purification procedures will be required to free antigens A and B from contaminating host cell antigens.

Thus far, the inactivated RS virus vaccines tested have not stimulated an appreciable antibody response in young infants. It is probable that large amounts of protective antigen will be required early in life to stimulate adequate levels of neutralizing antibody. With this requirement for large antigenic mass, it is clear that an effective vaccine must consist largely of protective antigen and be relatively free from nonprotective and host cell antigens. Information obtained from the present study may be helpful in assaying various vaccine preparations in various stages of purification and concentration for content of protective antigen.

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