Serum Complement in Acute Bronchiolitis[1] (34821)

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Respiratory syncytial (RS) virus is a major cause of acute bronchiolitis in infancy, and this most serious form of RS illness characteristically occurs in infants who have a moderately high level of maternally transmitted neutralizing antibody. A milder form of disease usually accompanies infection or reinfection in older children (1–6, 8).

The pathogenesis of RS virus disease, especially the production of severe respiratory tract disease in young infants, has not been fully elucidated; however, a virus–antibody interaction in the lung may be a major factor. This hypothesis is based on the following observations:

1. Moderately high levels of specific neutralizing antibody in serum do not protect against RS virus lower respiratory tract disease in early infancy (7).

2. Serious RS virus lower respiratory tract disease most commonly occurs in young infants during the period when they possess the highest levels of passively transferred serum neutralizing antibody (8, 9).

3. An antigenic inactivated RS virus vaccine administered parenterally to infants failed to provide protection against subsequent natural RS virus disease. Also, when vaccinees at a later date underwent natural infection, they developed serious lower respiratory tract disease of the type characteristically seen during the first few months of life (7–9).

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A decrease in serum complement has been demonstrated in several diseases believed to result from the toxic effect of antigen–antibody complexes [Type III immune reaction as classified by Gell and Coombs (10–13)]. This is thought to reflect fixation and utilization of complement by antigen–antibody complexes. In the present study, we have investigated serum complement levels in infants with RS virus bronchiolitis in an attempt to obtain evidence for an extensive antigen–antibody interaction in the affected lungs of infants with RS virus disease.

Materials and Methods. A period of RS virus prevalence occurred in the Washington, D. C. area during November 1967 through April 1968. This study was conducted during the peak months of RS virus prevalence, February and March 1968. Twenty infants hospitalized with clinical acute bronchiolitis, and seven infants admitted for elective surgery to Children's Hospital, Washington, D. C. were selected for study.

Serum. Serum specimens were obtained from the infants at the time of admission to the hospital and 2–4 weeks later. Blood was collected by antecubital venipuncture, allowed to clot at ice-water temperature, and serum was separated by centrifugation at 2000 rpm in the cold. The serum was then stored at −70° until tested for complement activity. Special care was exercised to avoid hemolysis.

Virus isolation. Oropharyngeal and anal swab specimens for virus isolation were obtained from the infants shortly after their admission to the hospital. The swabs were agitated in 5 ml of veal-infusion broth which contained 0.5% bovine albumin, 500 units penicillin, 500 μg streptomycin, and 10 μg am-

photericin/ml. Two-tenths milliliter of the resultant suspension was inoculated into two tubes each of rhesus monkey kidney (MK), human embryonic kidney (HEK), and HEp-2 cell cultures. The cultures were incubated at 34° for 3 weeks and examined twice weekly for the development of syncytia, other cytopathic effects (CPE), or hemadsorbing activity. To identify RS virus, the isolates were tested against ferret RS hyperimmune serum for complement-fixing (CF) activity.

Complement-fixation tests. Complement-fixation tests (CF) for RS antibody utilized a micromethod, 1.7 units of complement, 16 units of antigen prepared from the Long strain of RS virus grown in HEp-2 cell cultures, and overnight fixation at 4° (2, 14).

Total complement and beta$_{1C/1A}$-globulin determinations. Total complement and $\beta_{1C/1A}$-globulin levels were evaluated in a series of five tests. Each test included acute and convalescent sera from infants with bronchiolitis as well as pre-and postsurgical sera from infants without respiratory tract disease. Total complement activity was measured by the method described by Hook and Muschel (15). Titers were expressed as the reciprocal of the amount of test serum (ml) required to lyse 50% of 1.5×10^8 optimally sensitized sheep erythrocytes, in a reaction volume of 1.5 ml, after 30 min of incubation at 37°. As a control, a reference serum of known titer was tested with each day's titration. $\beta_{1C/1A}$-globulin levels were determined by the single radial diffusion method, utilizing immunoplates from Hyland Laboratories. Three standard reference sera, also obtained from Hyland Laboratories, were used. The inherent variability of the method was about 5%, as demonstrated by triplicate determination.

Results. Virus recovery. RS virus was recovered from oropharyngeal swabs of 8 (40%) of the 20 infants with acute bronchiolitis (Table I). Adenovirus type 3 was isolated from the anal swab of one patient (No. 18) and adenovirus type 1 from the throat swab of another patient (No. 10), and neither of these patients yielded RS virus. RS virus was not recovered from the seven infants without respiratory tract disease. From anal swabs, two (No. 21 and No. 26) of these infants yielded poliovirus and the third infant (No. 25) yielded adenovirus type 13.

Antibody response. A 4-fold or greater rise of RS CF antibody was detected in 7 (35%) of 20 infants with acute bronchiolitis. Those who developed a significant CF antibody response included 5 of 8 infants who yielded RS virus and 2 of 12 infants who did not. On the basis of virus isolation and/or serologic response, 50% of the 20 infants with acute bronchiolitis were considered to have been infected with RS virus. The seven infants without respiratory tract illness did not develop an RS CF antibody response.

Total hemolytic complement and beta$_{1C/1A}$-globulin. Table I shows the total hemolytic complement titers and $\beta_{1C/1A}$-globulin levels in 20 infants with bronchiolitis and in 7 infants with no respiratory tract illness; Table II shows the corresponding mean titers.

Whether RS virus infection was detected or not, the level of total complement or complement component β_{1C} was not depressed during the acute phase of bronchiolitis. In fact, the levels of complement and $\beta_{1C/1A}$-globulin tended to be higher during the acute phase of illness than during convalescence ($p = .025$ for bronchiolitis patients with RS infection). In addition, the levels of complement and $\beta_{1C/1A}$ - globulin were higher in infants in the acute phase of bronchiolitis than in infants without respiratory tract disease (again $p = .025$ for bronchiolitis patients with RS infection). Finally, complement and $\beta_{1C/1A}$-globulin were higher during the acute phase of RS-associated bronchiolitis than in clinically similar illness in which RS virus infection was not detected. The lowest hemolytic complement titer was noted in a 1-month-old premature infant (No. 16, Table I). Anticomplementary activity was not detected in those serum specimens with low complement titers.

The serum levels of total hemolytic complement and $\beta_{1C/1A}$ - globulin among the 20 infants with bronchiolitis and 7 infants without respiratory tract illness generally appeared to parallel each other. These findings are in agreement with those of Klemperer *et al.* (16), and West *et al.* (13). The levels of

TABLE I. Total Serum Complement Titers and β 1C/1A-Globulin Levels in Acute Bronchiolitis Patients With and Without Evidence of RS Virus Infection, and in Infants Without Respiratory Tract Disease.

Group	Virus recovery	CF antibody rise	Patient	Age (months)	Days after onset of illness		Total C′ titer[a]		β 1C/1A-globulin[b]	
					Acute	Conval.	Acute	Conval.	Acute	Conval.
Bronchiolitis with evidence of RS virus infection	+	+	1	4	2	17	167	147	120	120
	+	+	2	5	8	23	187	143	160	110
	+	0	3	4	2	17	240	172	150	90
	+	+	4	5	8	25	181	146	110	120
	+	+	5	4	3	25	222	210	220	270
	+	0	6	2	5	24	186	103	125	100
	+	0	7	2	8	39	176	143	180	150
	+	+	8	14	3	39	214	146	210	170
	0	+	9	4	5	27	158	139	92	120
	0	+	10	4	6	29	120	166	110	160
Bronchiolitis without evidence of RS virus infection	0	0	11	9	2	17	200	214	185	170
	0	0	12	6	4	19	137	177	95	130
	0	0	13	7	4	19	171	136	140	110
	0	0	14	2	8	22	200	130	140	110
	0	0	15	2	6	22	127	125	110	75
	0	0	16	1	4	15	88	60	125	80
	0	0	17	1	8	52	181	150	125	125
	0	0	18	1	10	48	176	146	105	74
	0	0	19	4	6	23	142	141	115	110
	0	0	20	4	3	27	222	181	190	160
No respiratory tract disease	0	0	21	4	*c*	16[d]	154	171	130	150
	0	0	22	11	*c*	17[d]	166	153	170	125
	0	0	23	4	*c*	17[d]	130	146	100	120
	0	0	24	1½	*c*	17[d]	111	107	100	72
	0	0	25	13	*c*	19[d]	81	140	110	160
	0	0	26	10	*c*	16[d]	173	128	160	98
	0	0	27	8	*c*	16[d]	176	200	160	220

[a] Titer in 50% hemolytic units of C′ per ml of serum ($C'H_{50}$/ml).
[b] Expressed as milligrams per 100 ml.
[c] Preoperative specimens taken on admission to the hospital.
[d] Postoperative specimens.

TABLE II. Mean Serum Total Complement Titers and β 1C/1A-Globulin Levels in Acute Bronchiolitis Patients With and Without Evidence of RS Virus Infection, and in Infants Without Respiratory Tract Disease.

Group	No. of infants	Total complement[a]		β 1C/1A-globulin[b]	
		Acute	Convalescent	Acute	Convalescent
Bronchiolitis with evidence of RS virus infection	10	185[a]	152[d]	148[g]	141
Bronchiolitis without evidence of RS virus infection	10	164[b]	146[e]	133[h]	114[i]
No respiratory tract disease	7	142[c]	149[f]	133	135

[a] Titer in 50% hemolytic units of C′ per ml of serum ($C'H_5O$/ml).

[b] Expressed as milligrams per 100 ml.

Note: Arithmetic means calculated because of symmetric distribution of levels. Significance of differences: a vs b $p > .2$; a vs c $p = .025$; a vs d $p = .025$; b vs c $p > .2$; b vs e $p > .2$; g vs h $p = .4$; h vs i $p = .2$.

serum $\beta_{1C/1A}$ - globulin were higher than those previously reported for the same age group; however, the wide range of variability is similar (17, 18).

Discussion. The results of the present study suggest that complement does not participate in the mediation of lung injury in infants with acute bronchiolitis associated with RS virus infection. This view is further supported by the failure to demonstrate β_{1C} in the lung tissue sections of three children who died of RS disease using the immunofluorescent technique (Ward and Bellanti, unpublished studies).

Our failure to detect a reduction of whole serum complement and/or component C′3 in the infants with bronchiolitis, does not negate the possibility that an immunological reaction in the lungs represents an important component in RS lower respiratory tract disease of early infancy. It is possible that a Type III (Arthus-type) immune reaction may occur, without complement being fixed; alternatively, complement may be fixed but the quantity bound may be insufficient to effect an overall reduction in total complement.

The elevated complement titer and $\beta_{1C/1A}$-globulin level in the acute-phase sera of patients with bronchiolitis may be what generally occurs in the "acute phase" of certain disease processes as a result of an inflammatory reaction (19–23). The mechanism for this observed elevation is not known. Since the serum level of complement components reflects the balance between rates of synthesis and catabolism, the elevated levels seen in the acute phase in this study could represent a nonspecific increase in one of the limiting components. Such an increase might actually mask a state of depression. However, interpretation of this point is difficult since individual components were not measured.

Summary. The levels of total complement and $\beta_{1C/1A}$-globulin were tested in 20 infants with acute bronchiolitis and in 7 infants without respiratory tract disease. The level of total complement or complement component β_{1C} was not depressed in the acute phase of acute bronchiolitis whether RS virus-associated or not. This does not negate the possibility of an immunological pathogenesis of RS virus disease.

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