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Serum Immunoglobulin G Antibody Subclass Response to Respiratory Syncytial Virus F and G Glycoproteins after First, Second, and Third Infections

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Serum samples from 31 children who experienced two or three infections with respiratory syncytial virus (RSV) in the first four years of life were tested in an enzyme-linked immunosorbent assay to examine the immunoglobulin G (IgG) subclass responses to the RSV F and G surface glycoproteins associated with primary infection and reinfection. We sought to determine whether the greater degree of glycosylation of the G glycoprotein was reflected in an IgG subclass immune response more like that to a polysaccharide antigen than to a protein antigen. We found that the IgG1/IgG2 ratio of postinfection antibody titers to F was fourfold higher than that to the G glycoprotein after RSV infections 1, 2, and 3. The IgG2 response to the heavily glycosylated G glycoprotein differed from that to a polysaccharide antigen in that the IgG1/IgG2 ratio remained constant with age, whereas the response to a polysaccharide antigen decreased as the IgG2 response increased with age. We also noted that antibody responses to both surface glycoproteins in the IgG1 and IgG2 subclasses reached their maximum levels after RSV infection 2.

Respiratory syncytial virus (RSV) is a significant cause of lower respiratory tract illness in infants and children, and reinfections are common (4, 7). The major protective antigens of RSV are the two surface glycoproteins (11). The fusion (F) glycoprotein has the structure of a typical paramyxovirus F glycoprotein with an estimated molecular weight of 70,000 (2). The G glycoprotein, however, has a unique structure with over 50% of its molecular weight estimated to be carbohydrate, the majority of which is O-linked sugars (19). We previously characterized the immunoglobulin G (IgG) subclass antibody responses of infants and children undergoing primary RSV infection and of adults undergoing experimental RSV infection (16, 17). In those studies, the pattern of IgG subclass antibody response to the two RSV glycoproteins differed. In IgG1/IgG2 ratio of postinfection antibody titers was higher to the F than to the G glycoprotein. We suggested that the IgG1/IgG2 ratio of titers to the heavily glycosylated G glycoprotein was more characteristic of that to a polysaccharide than that to a protein antigen (17). In the previous studies (16, 17), only the RSV subgroup A G glycoprotein was used as an antigen in the enzyme-linked immunosorbent assay (ELISA) to detect subclass responses. Since that time, the G glycoproteins of the subgroup A and B RSV strains have been shown to be only 5% related antigenically (5, 8) and 53% related by amino acid sequence analysis (9). The previous results could have underestimated the frequency and magnitude of the IgG subclass antibody responses to the glycoprotein since the infants and children studied (16) could have been infected with either an A or B strain of RSV.

In the present study, it was possible to more fully characterize the IgG subclass antibody response to the RSV G glycoprotein after infection by testing each serum sample against both subgroup A and subgroup B G glycoproteins. By using these glycoproteins as well as the F glycoprotein of the subgroup A virus, which is highly related antigenically to that of the RSV B subgroup (8), we sought to further characterize the IgG subclass antibody response to the RSV glycoproteins after infections 1, 2, and 3. We examined the postinfection IgG1/IgG2 G glycoprotein antibody titer ratio to determine if this ratio changed as the child matured as it does for polysaccharide antigens (3, 13). We also determined when children achieved levels of IgG subclass antibodies found in adults.

Serum samples were obtained at intervals of approximately twice a year from 31 children attending the Frank Porter Graham Child Development Center from 1978 to 1986, usually in November and June. Each child was monitored for an average of approximately 4 years. Children were monitored clinically, and RSV respiratory infection was documented by virus isolation or by a rise in the neutralizing antibody or ELISA titer by methods previously described (4, 10). The average interval between the illness and collection of serum was approximately 5 months. RSV isolates were obtained from 19 children with infection 1, and these were assigned to RSV subgroup A or B by using reactivity with a panel of RSV subgroup A- and B-specific monoclonal antibodies (1). The probable strain causing infection 1 was assigned in eight cases by epidemiologic association; in four children, the probable strain causing infection 1 could not be established. The ELISA for detection of subclass antibody

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responses to the purified F and G glycoproteins was performed as previously described (16), except that alkaline phosphatase-conjugated goat anti-fluorescein isothiocyanate (Tago, Inc., Burlingame, Calif.) was used instead of alkaline phosphatase-conjugated rabbit anti-fluorescein isothiocyanate. The ELISA for G and F glycoprotein responses was performed by using G glycoprotein obtained from the subgroup A and B strains of RSV, Long and 18537, respectively, and the F glycoprotein from the Long strain. Since we previously demonstrated that infants and children infected with RSV had poor IgG4 antibody responses to RSV glycoprotein antigens (16), IgG4 responses were not tested in this study.

The IgG subclass responses of the infants and children to the RSV G glycoproteins with primary RSV infection are presented in Table 1. The response to the RSV G glycoproteins was predominantly virus subgroup specific as indicated by a higher frequency and greater magnitude of antibody titer rise to the homologous glycoprotein. These findings are compatible with recent observations that infants and children infected with RSV subgroup A or B virus produced antibodies reactive predominantly with the homologous G glycoprotein (5). Importantly, the patterns of IgG subclass antibody response of infants and children undergoing primary infection with either subgroup A or B RSV were similar with respect to the homologous G glycoprotein. Therefore, to simplify and facilitate subsequent analyses, we report the higher titer achieved to either subgroup G glycoprotein as G-max (Table 2). The IgG subclass responses of the infants and children to the RSV F and G (G-max) after infection 1, 2, or 3 are presented in Table 2. The highest frequency of IgG subclass antibody response in each infection was in the IgG1 subclass. The frequency of IgG2 antibody response was only slightly greater for the G glycoproteins than for the F glycoprotein after each infection. The frequency of IgG3 response was greatest for each antigen after infection 1. There was a 50% drop in frequency of IgG3 response to the F glycoprotein after infection 2.

The level of IgG1 and IgG2 antibodies achieved after each infection was greater for the F glycoprotein than for the G glycoprotein, which is consistent with previous observations indicating the greater immunogenicity of the former glycoprotein (11). Importantly, substantial titers of IgG2 antibodies to both F and G glycoproteins are generated after infection 1. The postinfection IgG1 and IgG2 titers to the F and G glycoproteins reach near maximum levels after infection 2 with little further increase after infection 3. Finally, little increase in the magnitude of postinfection titer of IgG3 antibody to either glycoprotein is seen in response to infection 2 or 3.

The postinfection IgG1/IgG2 antibody titer ratios to the F glycoprotein for infections 1, 2, and 3 were 5.2, 3.5, and 5.6, respectively. The same comparison of postinfection G glycoprotein titer ratios after infections 1, 2, and 3 (1.1, 0.9, and 1.4, respectively) indicates that the relative magnitudes of the IgG subclass-specific responses did not change markedly during infections 2 and 3.

The patterns of IgG subclass antibody response to the two RSV glycoproteins clearly differ, with the IgG1/IgG2 ratio of postinfection antibody titer to the F glycoprotein being higher than that to the G glycoprotein. This difference primarily reflects a higher response of IgG1 antibody to the F glycoprotein after each of three sequential infections. Thus, the F glycoprotein is a more potent IgG1 immunogen than the G glycoprotein. We had expected to observe a maturational effect of the IgG2 subclass antibody response after repeated RSV infection as is seen with polysaccharide

TABLE 1. IgG subclass antibody response to the RSV subgroup A or B G glycoprotein after infection 1

Subgroup of RSV[a] (no. of children infected)	ELISA of indicated antibody to G glycoprotein from:															
	A (Long)								B (18537)							
	IgG1			IgG2			IgG3		IgG1			IgG2			IgG3	
	Mean log_2 titer (reciprocal ± SE)		% With ≥four-fold rise	Mean log_2 titer (reciprocal ± SE)		% With ≥four-fold rise	Mean log_2 titer (reciprocal ± SE)		% With ≥four-fold rise	Mean log_2 titer (reciprocal ± SE)		% With ≥four-fold rise	Mean log_2 titer (reciprocal ± SE)		% With ≥four-fold rise	Mean log_2 titer (reciprocal ± SE)		% With ≥four-fold rise
	Pre[b]	Post[c]		Pre	Post		Pre	Post		Pre	Post		Pre	Post		Pre	Post	
A (18)	3.6 ± 0.2	6.3 ± 0.3	94	5.0 ± 0.3	6.3 ± 0.4	61	4.3 ± 0.4	6.4 ± 0.4	72	3.7 ± 0.3	4.0 ± 0.3	17	4.3 ± 0.3	4.4 ± 0.4	17	3.7 ± 0.3	4.6 ± 0.4	38
B (9)	4.6 ± 0.5	4.2 ± 0.4	22	6.2 ± 0.6	5.3 ± 0.8	11	4.0 ± 0.3	4.2 ± 0.4	22	3.3 ± 0.0	7.9 ± 0.6	100	4.6 ± 0.7	6.8 ± 0.9	56	4.2 ± 0.5	6.8 ± 0.6	78

[a] The assignment of virus causing the infection was determined by characterization of the isolate or by the year in which the strain was isolated. In the day-care setting, only one RSV subgroup was isolated in the majority of the years tested. In this way, 27 of 31 of the primary RSV infections could be unequivocally subgrouped.
[b] Pre, Preinfection.
[c] Post, Postinfection.

antigens, in which a shift occurs from a predominantly IgG1 response in infancy and childhood to a predominantly IgG2 response (or an equal IgG1 and IgG2 response) in adolescence and adulthood (3, 13). However, a maturational effect of the IgG1 and IgG2 responses to the RSV G glycoprotein was not observed since the IgG1/IgG2 ratio remained similar after each infection and was comparable to that previously observed in adults (17). Thus, the IgG2 response to the RSV G glycoprotein differs from that to a polysaccharide antigen, and therefore we cannot conclude that the IgG subclass response to the RSV G glycoprotein is like that to a polysaccharide antigen as we previously suggested (17). However, the IgG1/IgG2 ratio of approximately 1 that we have observed for the G glycoprotein is unique for a viral glycoprotein (14) and clearly reflects a different response of the immune system to the two RSV glycoproteins.

Antibody responses in the IgG1 and IgG2 subclasses reached their maximum levels after infection 2, and these titers were similar to those observed previously in adults after experimental RSV infection (17). This is consistent with the observation that a considerable reduction of serious illness is seen with infection 3 and subsequent infections (4).

In contrast to our previous study of primary RSV infections, in this study we found a higher response for IgG2 and a lower response for IgG3 subclass (16). Both differences are probably due to the timing of collection of serum samples and the use of G glycoproteins from both RSV subgroups to measure the immune response to infection. Obtaining serum samples later after infection would probably have allowed us to detect the development of higher IgG2 titers. This suggestion is supported by the fact that infants injected with polysaccharide antigen required a longer interval (2 to 3 months) to reach maximum antibody concentrations (12). IgG2 antibody catabolism is more rapid than that of the other IgG subclasses (15); thus, the pattern of IgG3 antibody response to infection is more like that of the IgM response, and lower IgG3 titers would be expected in specimens collected in late convalesence. In fact, previous studies in which IgG3 antibodies were measured after RSV infections showed rapid postinfection decline in IgG3, but not in IgG1 and IgG2 titers (6, 18).

The relative contribution of the different subclass antibodies to resistance to viral infection is not known. The IgG subclasses that have the greatest antiviral effect may differ for different virus glycoproteins. It is possible that the IgG2 antibodies to the heavily glycosylated G glycoprotein might possess the greater antiviral effect. Therefore, it remains to be determined whether the different pattern of IgG subclass antibody response to the two RSV glycoproteins reflects a differing role in immunity to this virus.

TABLE 2. IgG subclass antibody responses to RSV F and G glycoproteins in infants and children with several RSV infections

Infection no.	No. of patients (avg age in mo)	RSV glycoproteins	Subclass response of:								
			IgG1			IgG2			IgG3		
			Titer[a]		% Patients with ≥fourfold rise in antibody titer[b]	Titer[a]		% Patients with ≥fourfold rise in antibody titer[b]	Titer[a]		% Patients with ≥fourfold rise in antibody titer[b]
			Preinfection	Postinfection		Preinfection	Postinfection		Preinfection	Postinfection	
1	31 (10.6)	F	4.9 ± 0.3	9.4 ± 0.4	90	5.7 ± 0.4	7.0 ± 0.4	45	4.8 ± 0.4	8.3 ± 0.5	79
		G-max[c]	3.7 ± 0.1	6.7 ± 0.4	93	4.8 ± 0.4	6.5 ± 0.5	62	4.2 ± 0.3	6.6 ± 0.3	72
2	30 (26.2)	F	9.3 ± 0.4	11.2 ± 0.4	62	8.4 ± 0.5	9.4 ± 0.5	48	6.8 ± 0.5	6.5 ± 0.4	24
		G-max	6.0 ± 0.4	8.1 ± 0.4	59	6.8 ± 0.4	8.2 ± 0.4	55	5.1 ± 0.5	6.5 ± 0.6	55
3	13 (35.8)	F	9.6 ± 0.4	10.7 ± 1.0	62	7.8 ± 0.8	8.2 ± 1.0	31	6.2 ± 0.6	6.4 ± 0.8	23
		G-max	6.7 ± 0.6	8.4 ± 0.7	77	6.9 ± 0.7	7.9 ± 1.0	46	6.7 ± 0.9	7.2 ± 1.0	38

IgG1/IgG2 titer ratio of postinfection sera
5.2
1.1
3.5
0.9
5.6
1.4

[a] Reciprocal mean $\log_2$ ELISA titer ± standard error of the mean.
[b] A rise in titer to the RSV A or B subgroup G glycoprotein was used.
[c] G-max is the higher of serum antibody titers with the G glycoprotein of the Long or 18537 strains of RSV as ELISA antigen.

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