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Antibody-Dependent Enhancement of Respiratory Syncytial Virus Infection by Sera from Young Infants

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Respiratory syncytial virus (RSV) convalescent-phase sera and control sera from both infants (<6 months) and older individuals (1.5 to 90 years) were assayed for RSV-specific antibodies by neutralization, in vitro enhancing activity, and immunoprecipitation. Enhancement of RSV infection in U937 cells was demonstrated with convalescent-phase sera and was shown to be dependent on Fc receptors by blocking with human immunoglobulin G ($P < 0.01$). Convalescent-phase sera from infants enhanced infection at concentrations closer to physiological ones (10^{-1} to 10^{-3} dilutions of serum), while convalescent-phase sera from older individuals enhanced infection only at much lower concentrations (10^{-3} to 10^{-6} dilutions of serum; $P < 0.01$). To our knowledge, this is the first report of RSV-enhancing antibody activity in the sera of infants. The observed enhancing activity and the low neutralizing antibody levels are confined mostly to convalescent-phase sera from infants aged 0 to 6 months, suggesting that these factors may contribute to the increased severity of RSV disease frequently encountered in young infants.

Human respiratory syncytial virus (RSV) is the leading cause of lower respiratory tract infection in infancy and early childhood (13, 18, 43). RSV causes severe bronchiolitis and pneumonia during infancy, with peak infection occurring at 2 to 3 months of age. The virus also reinfects children annually, during winter or early spring months in temperate climates, producing epidemics of both upper and lower respiratory tract disease (42).

Infection occurs despite the presence of passively acquired maternal antibody, a pattern that is repeated through life, because antibody-positive adults are also susceptible to repeated RSV infections (8, 15). Prophylactic administration of RSV immune globulin (750 mg/kg of body weight) to high-risk infants has been shown to decrease the incidence and severity of RSV infection (14). Other studies dealing with the apparent risk factors associated with RSV infection in infancy have reported that high levels of neutralizing antibodies, either secretory or serum derived, were important in diminishing the severity of infection but not necessarily the risk of reinfection (12, 13, 18, 28). Even so, studies have shown that severe morbidity in term and preterm infants with RSV infections was not related to deficiency of RSV-specific immunoglobulin (33, 45) but that some other anatomic or immunologic factor was responsible for the increased risk of severe RSV infection.

Antibody-dependent enhancement (ADE) of infection is speculated as a possible mechanism for RSV-induced respiratory disease. ADE of RSV infection has been demonstrated in vitro with human and mouse monocyte-like cell lines in the presence of both RSV-specific monoclonal antibodies and human clinical serum samples (11, 23). ADE may also manifest itself in other ways, whereby viral antigen-antibody complexes stimulate the release of mediators of inflammation which contribute to disease progression (3).

Since infants under 6 months of age are at highest risk of severe RSV disease, we investigated whether sera from these infants are able to enhance RSV infection in vitro. We found that antisera from infants display a unique ability to enhance in vitro RSV infection at concentrations in serum which are close to physiological.

MATERIALS AND METHODS

Virus and cell culture. RSV (Long strain) was grown in HEp-2 cells in minimal essential medium (MEM) supplemented with 5% fetal calf serum and was stored at −70°C. U937 cells, a continuous human macrophage-like cell line, were grown in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, sodium hydrogen carbonate (24 mM), penicillin G (100 U/ml), streptomycin (100 μg/ml), and kanamycin sulfate (100 μg/ml). HEp-2 cells were grown in MEM supplemented with 5% calf supreme (Gibco BRL, Burlington, Ontario, Canada) and antibiotics as above.

Source of human sera. Convalescent-phase serum samples were obtained from routine diagnostic submissions to the Southern Alberta Provincial Laboratory, Calgary, Alberta, Canada. Convalescent-phase sera (at a mean of 2 weeks after initial symptoms) were obtained from RSV-infected infants between 1 and 6 months of age, who were diagnosed by their physicians as having either bronchiolitis or pneumonia on the basis of findings of cough, wheezing, fever, and hypoxemia or tachypnea. RSV infection was confirmed by culturing of nasopharyngeal aspirates (auger suction) to yield positive immunofluorescence for RSV. For comparison, convalescent-phase sera (at a mean of 3 weeks after initial symptoms) were obtained from six persons between the ages of 1.5 and 88 years who were diagnosed with RSV infection by the methods mentioned above. Three of these individuals had an upper respiratory tract infection characterized by cough, fever, and rhinitis. Two older individuals were diagnosed with pneumonia, and a third individual, aged 19 months, was diagnosed with bronchiolitis; the diagnoses were based on the findings mentioned above for the infants. Fourteen convalescent-phase serum samples from non-RSV-infected individuals (used as controls) were also obtained from routine diagnostic submissions. These sera were collected from five newborn infants, for

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routine screening of congenital infections; four children aged 4 to 5 years old, diagnosed with parvovirus infection or respiratory infection due to influenza virus or adenovirus; and five individuals over 70 years old, diagnosed with varicella-zoster virus infection or respiratory infection due to influenza virus. All control individuals were negative for RSV infection at the time of serum collection.

Immunoprecipitation. The 12 convalescent-phase and fourteen control serum samples were used for immunoprecipitation of radioactively labeled RSV proteins. To prepare radiolabeled proteins, HEp-2 cells were infected with RSV at a multiplicity of infection of approximately 0.3 and were incubated with either [^{3}H]glucosamine hydrochloride (35 to 75 Ci/mM; DuPont NEN, Mississauga, Ontario, Canada) at 24 h postinoculation or [^{35}S]methionine (800 Ci/mM; Amersham, Oakville, Ontario, Canada) at 28 h postinoculation (the RSV fusion protein [F] is more efficiently labeled with [^{35}S]methionine, while the RSV attachment protein [G] is better labeled with [^{3}H]glucosamine [7]). Infected cells were harvested at 30 h (at which time the cell monolayer was completely fused) by solubilization in immunoprecipitation buffer (10 mM Na_2HPO_4 [pH 7.2], 0.15 M NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS]). [^{3}H]glucosamine-labeled infected-cell extract was mixed with [^{35}S]methionine-labeled infected-cell extract at a $^3H/^{35}S$ ratio of 3:2.

Cell extracts were preadsorbed for 1 h on ice with protein A-bearing, formalin-fixed whole cells of *Staphylococcus aureus*, after which the *S. aureus* was removed by centrifugation and human serum was added to the supernatant at a final dilution of 1:20. After the supernatant was allowed to stand overnight at 4°C, *S. aureus* was added and allowed to stand at room temperature for 30 min. Immunoprecipitates were recovered by microcentrifugation, washed four times with radioimmunoprecipitation buffer (0.5 M NaCl, 0.1% SDS, 50 mM Tris-HCl [pH 7.4], 1% Triton X-100), resuspended in 30 μl of SDS-polyacrylamide gel electrophoresis (PAGE) dissociation buffer (0.625 M Tris-HCl [pH 6.8], 10% glycerol, 5% 2-mercaptoethanol, 0.08 M SDS, 0.008% bromophenol blue), and stored at −70°C. Immunoprecipitates were analyzed by SDS-PAGE and autoradiography.

Neutralization assays. All sera were examined for neutralizing activity by the plaque reduction technique (38). RSV grown in HEp-2 cells was used at a dilution that produced 20 to 50 PFU per well of 24-well plastic plates (Corning Glass Works, Corning, New York). Serum samples were diluted 10-fold in MEM, RSV was added, and the mixture was incubated for 1 h at 4°C. Mixtures were then inoculated onto monolayers of HEp-2 cells in 24-well plastic plates. Virus was adsorbed with constant rocking at 4°C for 1 h, after which cells were incubated for 72 h at 37°C in MEM supplemented with 5% fetal calf serum and 1% methylcellulose (4,000 cP; Aldrich, Milwaukee, Wis.). Monolayers were fixed with 15% formaldehyde and stained with 1% crystal violet for visualization of plaques.

Enhancement assays. Equal volumes of RSV (approximately 10^5 PFU) were added to serum samples (diluted in MEM), and the mixtures were incubated at 4°C for 1 h to allow the formation of virus-antibody complexes. The controls contained RSV plus MEM instead of antibody. U937 cells (3×10^5 cells) were pelleted in a microcentrifuge tube for each serum dilution and control. Cells were resuspended in the preincubated virus-serum mixtures (100 μl) and so were infected at an approximate multiplicity of infection of 0.3. The cells were incubated with virus-serum mixtures at 4°C for 1 h with constant rocking. Cell suspensions were then diluted fivefold with cell medium and centrifuged, and the supernatant was removed. Cell pellets were washed again, resuspended in 500 μl of fresh cell medium, and plated in 24-well plastic plates. The cells were incubated at 37°C for 48 h, after which culture supernatant was collected from each well and the plaque titer was determined as described above for neutralization plaque titer determination. Infection of U937 cells was allowed to continue for 48 h, because maximum enhancement with human sera occurs at approximately this time (11). Plaque titers for RSV-antibody samples were compared with mean control titers (RSV alone) to produce a fold enhancement value. Enhancement assays were repeated at least three times to allow statistical analysis of data. All mean control titers were standardized to the singlefold enhancement value, and the corresponding sample fold enhancement values were calculated from the sample plaque titers.

Blocking of Fc receptors. U937 cells in microcentrifuge tubes (3×10^5 cells per tube) were treated with human immunoglobulin G (IgG; Sigma, St. Louis, Mo.) to block the cellular Fc receptors prior to incubation with RSV-antibody complexes. U937 cell pellets were resuspended in 50 μl of 10-mg/ml IgG in phosphate-buffered saline (2.7 mM KCl, 1.5 mM KH_2PO_4, 140 mM NaCl, 8 mM Na_2HPO_4 [pH 7.2]) and incubated for 1 h at 4°C with rocking. Following blocking, cells were diluted prior to centrifugation and washed with RPMI medium. They were then immediately used for enhancement assays as described above. Three serum samples from RSV-infected infants were diluted 10^{-1}- to 10^{-3}-fold for use in enhancement assays with IgG-treated U937 cells. Plaque titers were converted to fold enhancement values and standardized to the control as described above.

Statistical analysis. All fold enhancement values were analyzed by a two-tailed Student *t* test for small-sample confidence intervals and are expressed as the mean ± 95% confidence interval. Significance analysis between groups was analyzed by a two-tailed Student *t* test on unpaired data.

RESULTS

Neutralization titers. The clinical diagnoses and ages of all patients involved in the study and neutralization titers for all serum samples are shown in Table 1. Neutralization titers were calculated as the reciprocal serum dilution resulting in 50% reduction of the number of virus plaques. In agreement with previous studies (16, 29, 34), sera from RSV-convalescent infants (under 6 months of age) were found to be poorly neutralizing, with titers ranging from 1:20 to 1:75 (for comparison, antibody levels in serum equal to or greater than 1:200 are considered effective in preventing RSV infection in the lower respiratory tract [14]). Neutralization titers in sera from older RSV-convalescent patients (over 6 months of age) were much higher, with a range of 1:750 to 1:1,500. Control sera from individuals under 6 months old and above 70 years old were for the most part poorly neutralizing, while sera from control patients aged 4 to 5 years neutralized at higher dilutions, probably because of the broadening antibody response to RSV in children who experience repeat infections.

ADE activity in convalescent-phase sera. Culture supernatants from U937 cells infected with RSV-antibody mixtures, or cell medium as a control, were subjected to plaque titer determination, and the calculated fold enhancement values are graphically represented in Fig. 1 and 2. Any value greater than 2 standard deviations (0.54) above the control mean (1.00) was defined as significant for enhancing activity as described elsewhere (21). Fold enhancement values of up to nearly fourfold were observed with sera from RSV-convalescent infants. Sera from five of the six RSV-convalescent infants showed signifi-

TABLE 1. Clinical diagnosis of patients and neutralization titers of patient antisera

Patient	Age	Neutralization titer[a]	Diagnosis[b]
RSV convalescents			
21690	1 mo	1:75	Bronchiolitis
35278	2 mo	1:25	Pneumonia
20085	2 mo	1:70	Bronchiolitis
20088	3.5 mo	1:20	Bronchiolitis
20711	4 mo	1:30	Bronchiolitis
20081	6 mo	1:30	Bronchiolitis
20391	19 mo	1:800	Bronchiolitis
5906	4 yr	1:750	URTI
13483	4 yr	1:800	URTI
11673	4 yr	1:1,250	URTI
12067	71 yr	1:1,500	Pneumonia
5921	88 yr	1:1,250	Pneumonia
Controls			
49277	Newborn	1:75	NA
50488	Newborn	1:60	NA
46156	Newborn	1:40	NA
46157	Newborn	1:30	NA
46895	Newborn	1:50	NA
30827	5 yr	1:100	Parvovirus infection
15470	5 yr	1:200	Influenza virus infection
14659	4 yr	1:600	Influenza virus infection
11293	4 yr	1:200	Adenovirus infection
39252	75 yr	1:20	VZV infection
24092	90 yr	1:25	Influenza virus infection
24098	90 yr	1:40	Influenza virus infection
24095	89 yr	1:150	Influenza virus infection
16564	84 yr	1:10	Influenza virus infection

[a] Neutralization titers are expressed as the reciprocal serum dilution resulting in 50% reduction of virus plaques.

[b] URTI, upper respiratory tract infection; NA, not applicable (diagnosis not made); VZV, varicella-zoster virus.

cant enhancing activity at serum dilutions of 10^{-1} to 10^{-3} (Fig. 1A).

In contrast, enhancing activity was virtually absent from similar dilutions (10^{-1} to 10^{-3}) of serum samples from older RSV-convalescent individuals (Fig. 1B). None of these samples showed enhancement at 10^{-1} or 10^{-2} dilutions, and only one (sample 5906) showed enhancement at a 10^{-3} dilution. Instead, enhancing activity could only be demonstrated at relatively high serum dilutions of 10^{-3} to 10^{-6} (Fig. 1B). Therefore, the most striking difference between the two age groups (Fig. 1A and B) was the dilution of serum at which enhancement was greatest. Most significantly, at low serum dilutions (and therefore close to physiological concentrations), sera from convalescent infants were enhancing while sera from convalescent older individuals were neutralizing for RSV infection ($P < 0.01$ at 10^{-1} and 10^{-2} dilutions of sera).

ADE activity in control sera. Interestingly, control sera from infants under 6 months old were not significantly enhancing (Fig. 2A). At a 10^{-1} dilution, control sera were neutralizing (Fig. 2A), in contrast to the enhancing activity detected in sera from convalescent infants (Fig. 1A). This difference is statistically significant ($P < 0.05$).

For older individuals (>6 months), three control serum samples displayed enhancing activity at low dilutions (Fig. 2B). Two serum samples (15470 and 11293) enhanced RSV infection in U937 cells at a 10^{-2} and 10^{-3} dilution, and one serum sample from an 89-year-old individual (24095) enhanced at a 10^{-1} and 10^{-2} dilution. The remainder of the sera were not significantly enhancing, and most showed neutralization activity at a 10^{-1} dilution.

ADE activity is Fc receptor dependent. Enhancement of RSV infection in U937 cells was blocked by treatment of cells with 10 mg of human IgG per ml (Fig. 3). Fold enhancements for three infant serum samples which enhanced infection at dilutions of 10^{-1} to 10^{-3} were reduced to control values when assayed with cells pretreated with IgG ($P < 0.01$).

RSV F1- and G-specific antibodies in human sera. Antibody responses to the RSV envelope glycoproteins were examined by immunoprecipitation assay to determine whether a correlation existed between infection enhancement and specificity of antibody response. Most sera showed a fairly strong response with RSV F1 protein (Fig. 4). Parenthetically, it should be noted that the F1 and F2 polypeptides of the F protein resolve separately upon 2-mercaptoethanol reduction (43); however, because of poor labeling of the F2 subunit with [^{35}S]methionine (27), only the F1 polypeptide is generally detectable. Control sera from older individuals showed the greatest variability in F1 response, ranging, for example, from strong reactivities (sera 14659, 30827, and 24098) to very weak ones (sera 16564 and 24095).

Considerably more variation was observed in the G glycoprotein-specific immunoprecipitation response (Fig. 4). Convalescent-phase sera from infants were particularly weak in G-specific antibodies when compared with sera from both infant controls and older patients convalescent from RSV infection. In fact, the poor G-specific antibody response observed in infants convalescent from RSV infection was most similar to that seen in the older control group. This observation is consistent with the poor ability of infants to mount an effective antiviral immune response.

DISCUSSION

Fc receptor-dependent antibody enhancement of infection has been demonstrated previously for several viruses, including RSV (11, 23, 40). To our knowledge, this is the first report of enhancing antibodies for RSV in the sera of young infants. The present study also confirms the presence of RSV-enhancing antibodies in fairly high dilutions of sera from older individuals (11, 23).

The fact that sera may show both neutralizing and enhancing activities has been a long-discussed topic in ADE of virus infection. As observed with ADE of dengue virus infection, immune sera can have competing effects, i.e., neutralization or enhancement (21). Cells such as HEp-2, which do not bear Fc receptors, are used as an indicator of virus neutralization. In contrast, Fc receptor-positive U937 cells which permit uptake and infection of virus-antibody complexes provide an indication of enhancing activity. In sera with high neutralizing activity (e.g., from older individuals convalescent from RSV infection in the present study), neutralization predominates at high concentrations in serum even in U937 cells. The balance between neutralization and enhancement is critically a function of the antibody/virus ratio. Complete saturation of virions by antibody will not permit enhancement, as indicated by neutralization of viruses such as dengue virus by high antibody concentrations even in Fc receptor-bearing cells (see, e.g., references 21 and 32). This may indicate a requirement for a certain proportion of viral surface protein epitopes which are necessary for infection, whether antibody is present or not. Obstruction of such epitopes with antibody may block infection even when attachment of the virus-antibody complex to the cell

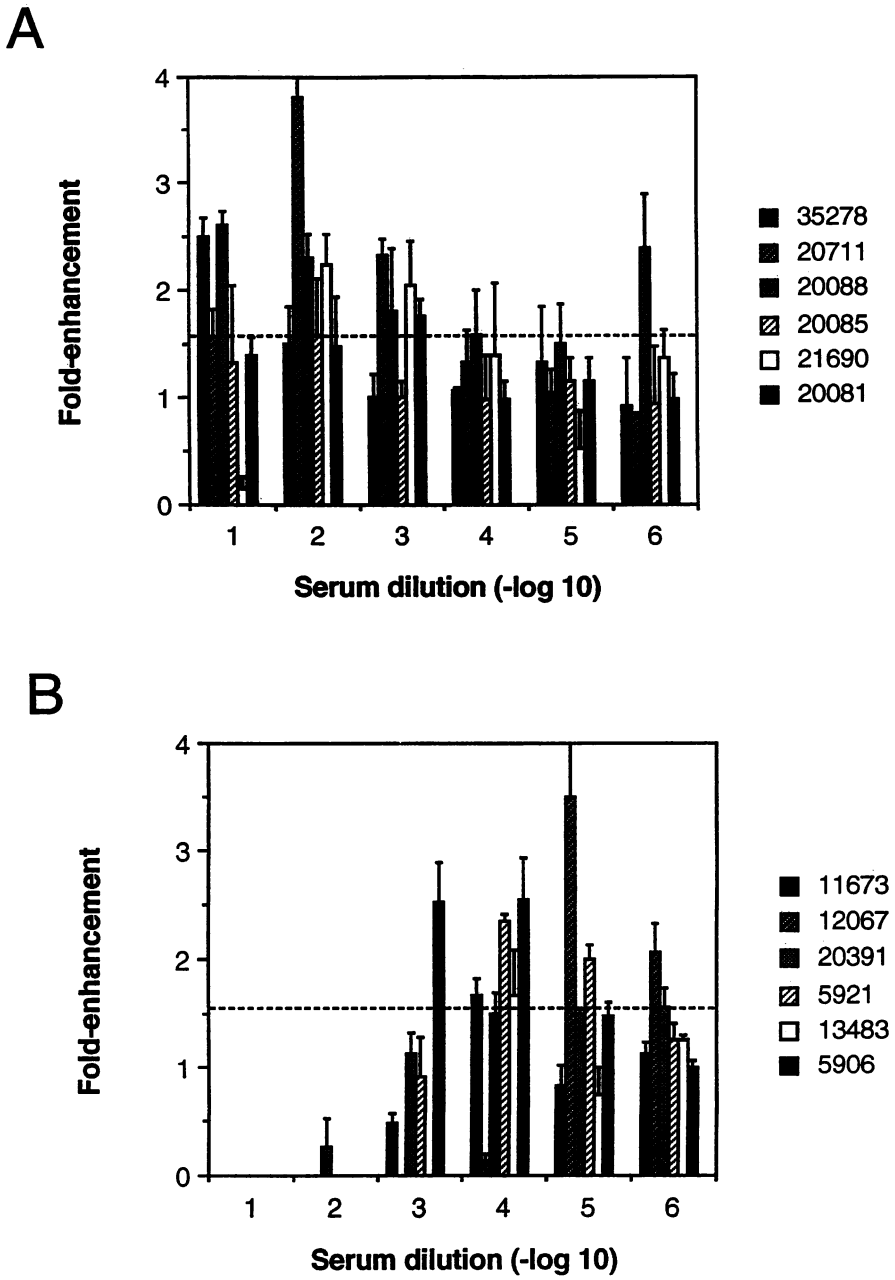

FIG. 1. ADE assays of RSV infection in the presence of convalescent-phase sera. Mean control titers were standardized to equal 1.00, and all sample titers were compared with their mean experimental control to calculate a fold enhancement value. The horizontal line denotes the mean control value plus 2 standard deviations. All values shown are the mean and standard error of the mean (95% confidence interval). Fold enhancement values, as calculated from virus titers from U937 cells infected in the presence of sera, are shown. (A) Sera from infants under 6 months of age convalescent from RSV infection. (B) Sera from patients over 6 months of age convalescent from RSV infection.

is possible via an Fc receptor. Evidence from the field of human immunodeficiency virus research indicates that ADE requires virus interaction through two receptors (i.e., virus receptor as well as the Fc receptor) (44). Future identification and characterization of the RSV receptor should permit the clarification of receptor requirements for ADE of RSV infection.

Immunoprecipitation of individual RSV proteins by patient antisera demonstrated differences in response, particularly to the G protein. Our results correlate with previous immunoprecipitation studies involving RSV-specific antisera (46–48) and agree with results on antibody responses from RSV-infected infants, children, and elderly persons, as measured by enzyme-linked immunosorbent assay, neutralization, and immunoblot assay (2, 10, 16, 29, 39). From these immune-response studies it is observed that the presence of antibodies against proteins other than F appears to be variable among patients, with the response broadening with increased age and exposure to RSV (39, 49). In our study, convalescent infants showed generally lower levels of G-specific antibodies than did controls. It is possible that G-specific antibodies are present but remain undetected by our method (e.g., that IgG3 does not bind

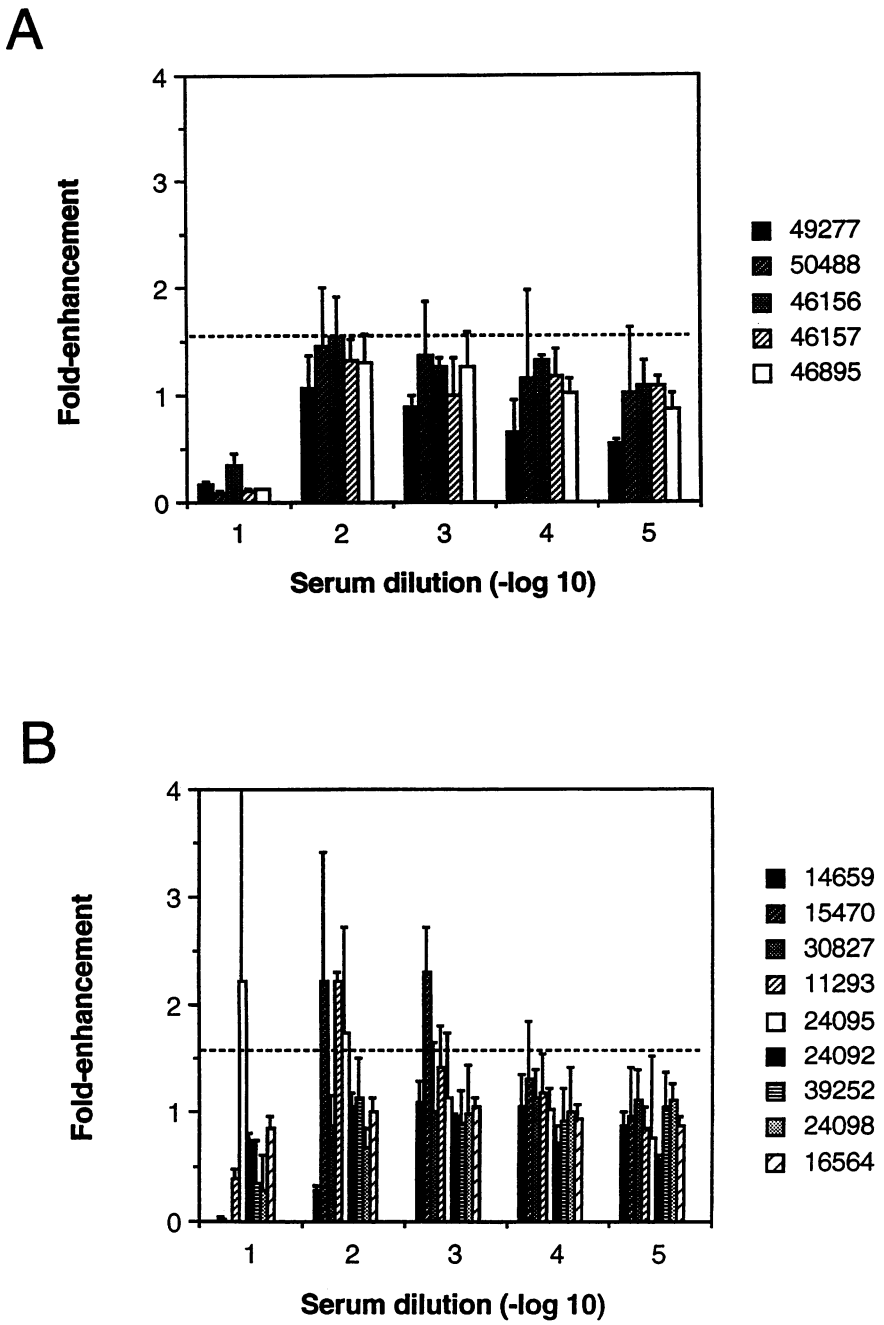

FIG. 2. ADE assays of RSV infection in the presence of control sera. Fold enhancement values, as calculated from virus titers from U937 cells infected in the presence of sera, are shown. (A) Sera from control individuals under 6 months of age. (B) Sera from control individuals over 6 months of age.

protein A [25]). However, we believe that it is likely that the observed low levels of G-specific antibodies reflect the higher propensity for infants with low levels of viral antibodies to be susceptible to RSV infection and suggest a substantial protective effect of anti-G antibodies. This may also be evident in the ADE results obtained with both convalescent-phase infant sera and control sera from older individuals. Samples within both of these groups displayed poor immunoprecipitation of the G protein, suggesting that the lack of G-specific antibodies may have allowed infection enhancement at low antiserum dilutions. Both F- and G-specific antibodies can cause ADE of RSV, as shown by a study with monoclonal antibodies (23). Evaluation of antibody specificity (F versus G), as well as IgG subclass specificity (19), in patient sera and their respective contributions to ADE of RSV deserves further study.

Two previous reports have investigated infant IgG-specific responses to RSV by immune precipitation with protein A (47, 48). Our results are similar to those of Ward et al. (48), who used ^{125}I-labeled RSV proteins for immunoprecipitation, in contrast to the [^{35}S]methionine- and [^{3}H]glucosamine-labeled proteins used in the present study. Vainionpaa et al. (47), whose methods were the most similar to ours, also noted

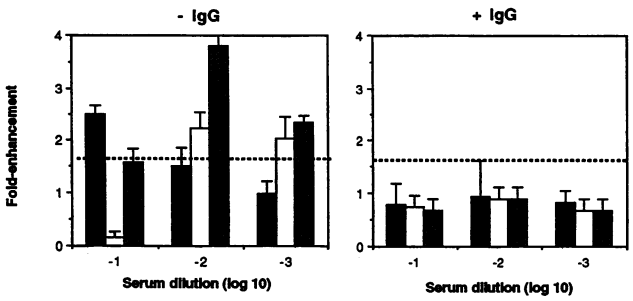

FIG. 3. Blocking of ADE by IgG treatment of U937 cells. Enhancement assays were performed with three RSV-infected infant serum samples at dilutions of 10^{-1} to 10^{-3} with U937 cells either pretreated with IgG (+IgG) or untreated (−IgG). Mean control titers were standardized to 1.00, and all sample titers were compared with their mean experimental control to calculate a fold enhancement value. All values shown are the mean and standard error of the mean (95% confidence interval). Symbols: ■, 35278; □, 21690; ▨, 20711.

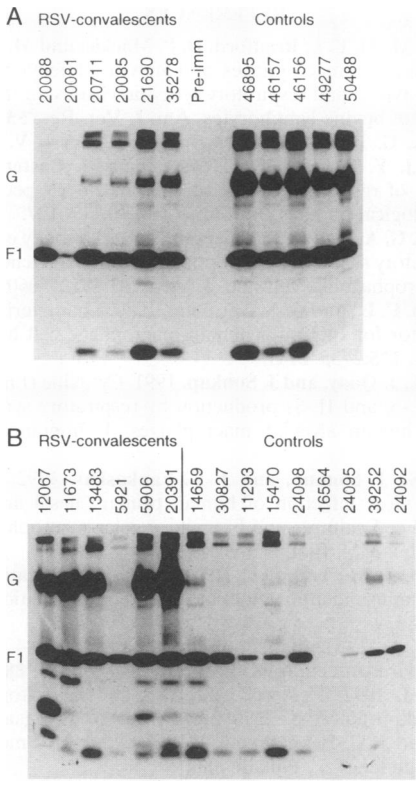

FIG. 4. Immunoprecipitation of radiolabeled RSV proteins with clinical serum samples. (A) Serum samples from RSV-convalescent or control infants under 6 months of age. Radiolabeled RSV protein immunoprecipitated with guinea pig preimmune serum is shown in the lane marked Pre-imm. (B) Serum samples from RSV-convalescent or control individuals over 6 months of age. The G glycoprotein and the F1 polypeptide of the fusion glycoprotein are labeled on the left side of the figure.

relatively poor immunoprecipitation of G by antisera from RSV-infected children.

The involvement of the Fc receptor in ADE of RSV is indicated by our observation that ADE is blocked by pretreating U937 cells with human IgG at 10 mg/ml. Similar concentrations of IgG have been shown to block ADE of dengue virus in U937 cells (22). Normal levels of IgG in serum are within the range 6 to 16 mg/ml (9), suggesting that blockade of ADE of RSV could occur under physiological conditions. Sera from infants will obviously contain a complex mix of IgG specificities, only a portion of which will be RSV specific and therefore capable of participating in ADE. However, as we have shown, unfractionated sera from infants can clearly enhance RSV infection in U937 cells which are not pretreated with exogenous IgG. This suggests that virus-specific antibodies present in serum from infants are able to manifest some degree of ADE even in the presence of physiological levels of irrelevant IgGs, which may potentially block Fc receptors. This is understandable, since cellular Fc receptors generally show higher affinity for polymeric (e.g., virus-bound) IgG than for monomeric IgG, although this varies greatly among different cell types (see, e.g., reference 26).

The Fc receptor-blocking studies mentioned above also rule out a role for other Ig classes contributing to ADE of RSV infection in U937 cells. The IgG receptor on U937 cells does not bind IgM or IgA (4). Inhibition of ADE via blockade of the Fc receptor with IgG clearly implicates serum IgG as the active component in enhancing RSV infection in U937 cells. Because of the young age of the infant group (<6 months of age) and the low neutralization titers of their antisera, it is likely that the major source of IgG in their serum is maternal.

RSV has been shown to productively infect peripheral blood monocytes and alveolar macrophages (6, 24, 31, 37). Alveolar macrophages play a major role in respiratory tract defense against infection and so may be exposed to RSV via the nasopharyngeal tract or by virus replication within the respiratory tract (36). RSV infection and replication in alveolar macrophages may aid in viral dissemination and, more importantly, in alteration of cytokine production in response to RSV infection (1, 5). Enhancement of alveolar macrophage infection in the presence of antibody may further complicate the immune response to RSV and lead to the severe disease experienced by young infants. It will be important to use alveolar macrophages as a vehicle for ADE of RSV infection in an analogous manner to that shown in our present study with U937 cells.

ADE of viral infection has been observed with a number of viruses (40), most notably dengue virus (21, 32). Enhancement of infection is thought to occur by the binding of antibody-virus complexes to antibody-specific receptors on permissive monocytes, macrophages, or lymphocytes (17, 30, 35, 41, 50). Severe RSV disease of young infants may share some characteristics of severe dengue infection (dengue hemorrhagic fever or dengue shock syndrome) in infants younger than 1 year of age (20), in that primary infection may be exacerbated by virus-specific maternal antibodies. ADE of infection may help explain why maternal antibodies are not completely protective and why severe RSV disease occurs most often in infants under 6 months of age. The role of maternal antibodies in severe RSV disease is controversial, but the fact remains that infants are at high risk of developing severe RSV infection during an age when maternal antibodies are present. A better understanding of ADE and its relation to RSV infection may offer useful insights in the search for a practical vaccine for use in infants.

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