CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, May 1996, p. 280–286 Vol. 3, No. 3
1071-412X/96/$04.00+0

Neutralizing and Enhancing Activities of Human Respiratory Syncytial Virus-Specific Antibodies

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Received 14 August 1995/Returned for modification 12 December 1995/Accepted 30 January 1996

The neutralizing and enhancing activities of respiratory syncytial virus (RSV)-specific antibodies were examined. These two biological activities were measured for a panel of six monoclonal antibodies (MAbs) specific to the RSV surface F and G glycoproteins. Four MAbs specific for the F protein possessed both neutralizing and enhancing activities. One MAb (11-2-D2), specific to the G protein, enhanced RSV infection of U937 cells, a human macrophage cell line, but did not neutralize virus infectivity. One MAb (11-3-A3), specific to the F protein, efficiently neutralized virus infectivity but did not enhance RSV infection of U937 cells. MAb 11-3-A3 neutralized representative strains of the two antigenic subtypes of RSV. Assays performed with mixtures of MAbs showed that high concentrations of MAb 11-3-A3 masked the enhancing activity of MAb 11-2-D2. The assay of mixtures of two MAbs possessing only enhancing activities demonstrated that this response was synergistic. The role of neutralizing and enhancing antibodies in determining the outcome of RSV infection was examined for infants from whom cord blood serum samples were collected at birth. There was no significant difference in the magnitude of the serum-enhancing activities between infants who were hospitalized with RSV infections and a group of age- and sex-matched control infants with no reported respiratory illness requiring hospitalization. However, the results indicated a possible correlation between RSV infection of the infants and the occurrence of in vitro antibody-dependent enhancement of the cord blood sera at a serum dilution of 10^{-2}. A significant inverse correlation was found between the plaque-neutralizing and enhancing activities of the cord blood sera from infants, irrespective of subsequent RSV infection. These data are discussed in relation to the possible contribution of antibody-dependent enhancement to the normal course of RSV pathology in vivo.

Human respiratory syncytial virus (RSV) is a major cause of severe respiratory infections in infants, with a peak incidence among 2-month-old babies. The pathogenic mechanisms involved in RSV infection are poorly understood. Epithelial cells of the respiratory tract are the primary target of virus infection, as shown by the detection of RSV-specific antigen and RNA in these cells during infection of humans and cotton rats (25, 27). In addition, the interaction between RSV and cells of the monocyte lineage might also play a role in virus pathogenesis. The susceptibility of monocytes and macrophages to RSV infection has been examined. Human alveolar macrophages as well as peripheral and cord blood monocytes are susceptible to RSV infection in vitro (3, 23). Moreover, RSV-infected alveolar macrophages can be detected in bronchial-lavage washes from adult transplant patients suffering from RSV infection (31).

In general, high levels of RSV-specific neutralizing antibodies in serum reduce the risk of severe virus infection but do not provide complete protection against reinfection (15). The reason for this lack of protection from virus infection is unknown. This report discusses the involvement of antibody-dependent enhancement (ADE) in the normal pathogenesis of RSV. In vitro ADE has been described for several viruses, including human immunodeficiency virus type 1 and influenza A virus (18, 28). A role for ADE has been proposed for both dengue virus and rabies virus infections in vivo (33). Kliks et al. (19) reported that the presence of maternally acquired dengue virus-specific antibodies either protects infants from or increases their risk of severe dengue virus infection, depending on the ADE activity of the serum. RSV-specific monoclonal antibodies and human sera from children and adults with low titers of RSV-specific antibodies can enhance RSV infection of human monocyte cells in vitro (13, 20). Osiowy et al. (29) have also demonstrated ADE for convalescent-phase sera collected from young infants (<6 months) and adults who had had recent RSV infections. We propose that the infection of monocyte cells by RSV in the presence of antibody during in vivo RSV infection could contribute towards the normal virus pathology either by increasing virus infection (i.e., ADE) or by modifying the role of the monocyte as a control point in the host's immune response; these effects may not be exclusive. Recently, it was reported that BALB/c mice inoculated with high doses of RSV produced high levels of inflammatory cytokines (tumor necrosis factor alpha and interleukin 6) known to be expressed by macrophages (17). A reduction in the microbicidal activity of RSV-infected murine alveolar macrophages was also reported by Frankeullmann et al. (10). Moreover, RSV infection of U937 cells, a monocyte cell line, causes a stimulation of leukotriene C_4, a mediator of bronchial inflammation (1). In the present study, we investigated the relationship between the neutralizing activity of RSV-specific antibodies and their ability to enhance virus infection of human monocytes in vitro. The possible role of ADE in RSV pathogenesis is discussed.

MATERIALS AND METHODS

Source of virus and cells. The origin of the RS-A2 strain of RSV has been detailed elsewhere (11). The RSF-44 and RSF-20 strains of RSV were isolated from infants admitted with respiratory infections to the Royal Aberdeen Children's Hospital in 1984. The RS-A2 and RSF-44 virus strains were classified within antigenic subgroup A and RSF-20 was classified within antigenic subgroup

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B by immunoblotting with monoclonal antibody (MAb) 4-14, which is specific for the virus phosphoprotein and distinguishes the virus subgroups (12). During the current study, the isolation of RSV from nasopharyngeal secretions was performed as described by Gimenez et al. (12). The HEp-2, BS-C-1, and human monocyte (U937) cell lines were maintained as described previously (12, 13).

Sources of MAbs and human cord blood sera. RSF-44-specific MAbs were produced in our laboratory by following the procedure described by Gimenez et al. (11). For the preparation of these MAbs, the virus was concentrated by ultracentrifugation prior to the inoculation of mice. The protein specificities of the MAbs were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting against virus proteins electrophoresed under reducing and nonreducing conditions (12). The immunoglobulin subtypes were determined with a commercial isotyping kit (Amersham, Ltd.). The concentrations of immunoglobulins in the ascites fluids were determined by radial immunodiffusion (The Binding Site, Birmingham, United Kingdom). MAb 30 (immunoglobulin subtype G1 [IgG1]) was kindly supplied by G. Taylor, Institute for Research on Animal Diseases, Compton, United Kingdom.

Cord blood sera were collected from babies born at Aberdeen Maternity Hospital between August 1991 and August 1992. The blood was allowed to clot, and the serum was collected after centrifugation for 10 min at 2,000 rpm (Centaur 2 centrifuge; MSE). The sera were stored at −20°C until required. The diagnosis of RSV infection was made by both virus isolation in HEp-2 cells and immunofluorescence staining of nasopharyngeal cells collected on a catheter tip; the latter was carried out at the Virus Laboratory, Department of Medical Microbiology, University of Aberdeen.

Determination of serum antibody activities. (i) Neutralization assay. Neutralizing-antibody titers were determined essentially as described by Pringle and Cross (34) with minor modifications. Briefly, approximately 2×10^3 PFU of RSV was mixed with an equal volume of either cord blood serum or MAb at an appropriate dilution; cord blood sera were assayed at 2-fold serial dilutions, and MAbs were assayed at 10-fold serial dilutions. The sera and MAbs were heat inactivated for 30 min at 56°C prior to the neutralization assay. After the appropriate incubation period (see below), the residual infectious-virus titer was determined by plaque assay on BS-C-1 cell monolayers (12). The control mixture for the neutralization assay consisted of Glasgow Minimum Essential Medium containing 1% (vol/vol) fetal calf serum in place of the serum or MAb. The neutralization assays of the cord blood sera were carried out by incubating the serum and virus mixtures for 18 h at 4°C. The virus-MAb mixtures were incubated for 30 min at 37°C before the residual infectious virus was determined. The neutralization and enhancing assays for the MAbs were performed simultaneously. The 90% plaque reduction neutralization titer ($PRNT_{90}$) was the reciprocal of the dilution of serum or MAb causing a 90% reduction in the number of virus plaques compared with the control.

(ii) ADE assay. The ADE assay was carried out as described by Gimenez et al. (13) with minor modifications. Serial 10-fold dilutions of the antibody preparations (i.e., MAb or serum) were prepared, and 100 μl of each dilution was mixed with 100 μl of RSV (containing approximately 7.5×10^4 PFU). Control mixtures were prepared with 100 μl of RPMI 1640 containing 5% (vol/vol) fetal calf serum (RPMI-5) in place of the antibody dilution. The virus-antibody and control mixtures were incubated for 30 min at 37°C before the addition of 100 μl of a U937 cell suspension with a concentration of 2×10^6 cells per ml (the final concentration of U937 cells was 7×10^5/ml). Following a 60-min incubation at 37°C, the cells were washed three times with RPMI-5, resuspended in fresh RPMI-5, and incubated for a further 24 h at 37°C. The complete cell suspension was frozen in liquid nitrogen, and infectious-virus titers were determined by plaque assay in BS-C-1 cell monolayers. Previously, only the titer of the virus released from RSV-infected U937 cells was determined (13). In the present study, both the cell-associated virus and the cell-released virus were titrated to improve the accuracy of determining virus replication. The infectious-virus yields in the presence and absence of antibody were considered significantly different ($P \leq 0.05$), as determined with the following equation (8): $(x_1 - x_0)/\sqrt{x_1 + x_0} \geq 1.96$, where x_1 is the number of plaques obtained from U937 cells infected in the presence of antibodies and x_0 is the number of plaques obtained from U937 cells infected in the absence of antibodies. The values of x_1 and x_0 were used to calculate the enhancement index (EI) (i.e., $EI = x_1/x_0$). Virus enhancement was scored as positive when the EI was ≥2. For each MAb (or serum) the EI was calculated at dilutions between $10^{-0.9}$ and 10^{-4}. From these data, the maximum value of the EI was defined as the peak EI.

RESULTS

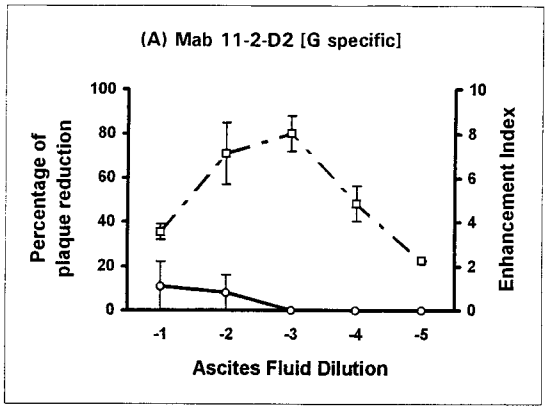
Characterization of MAbs specific to the RSV F and G glycoproteins. Previous studies have shown that MAbs specific for the F and G surface glycoproteins of RSV enhance virus infection of macrophage cells in vitro (13, 20). To determine if specific epitopes on the glycoproteins induce neutralizing antibodies without enhancing activity, and vice versa, six MAbs specific for the F and G proteins were screened for both activities. Two measurements were scored in the ADE assay: the EI, which indicated the magnitude of the enhancing activity of the antibody (MAb or serum), and the antibody dilution at which the EI was maximal (i.e., the peak EI). The MAbs had different patterns of neutralizing and enhancing activities (Table 1). MAb 11-2-D2, which is specific for the G protein, did not neutralize RSV but enhanced virus infection of U937 cells. Four of the MAbs specific for the F protein (11-6-F9, 11-2-C9, 11-2-F3, and 11-3-A3) neutralized RSV to different extents. Three of the MAbs specific to the F protein (11-5-G9, 11-6-F9, and 11-2-C9) enhanced virus infection of U937 cells. MAb 11-2-F3 enhanced RSV infection to only a limited extent (mean EI = 2.3 [four determinations]). MAb 11-3-A3 neutralized RSV but showed no evidence of enhancing virus infection in U937 cells.

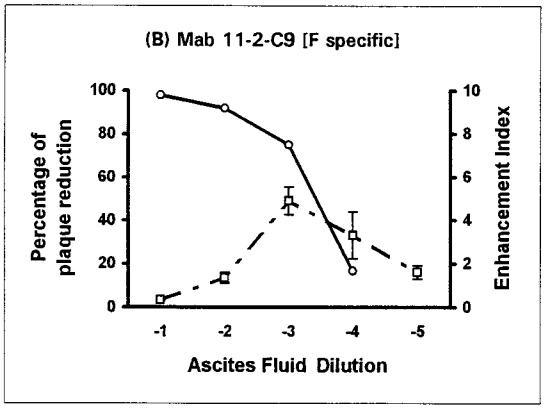
TABLE 1. Characteristics of RSV-specific MAbs

MAb	RSV protein specificity	Antibody subtype	Immunoglobulin concn (mg/ml)	$PRNT_{90}$	Maximum EI (range)
11-2-D2	G	IgG2a	5.5	<10	4.1–23.7
11-5-G9	F	IgG1	9.5	<10	3–4
11-6-F9	F	IgG1	2.8	$10^{1.3}$	2.5–5.3
11-2-C9	F	IgG1	5.1	10^2	4.3–5.6
11-2-F3	F (F1)	IgG1	7.3	$10^{2.2}$	1.4–3.3
11-3-A3	F (F1)	IgG1	11.2	10^3	1.1–1.3

Figure 1 shows representative data for three MAbs, illustrating the main patterns of neutralizing and enhancing activities MAb 11-2-D2 did not neutralize RSV and had a peak EI at an antibody dilution of 10^{-3} (Fig. 1A). Ascites fluid specimens prepared from different mice inoculated with MAb 11-2-D2 hybridoma cells gave similar patterns of neutralization and enhancement (data not shown). Moreover, when assayed with either RSF-44 (subtype A) or RSF-20 (subtype B), this MAb had an EI of ≥3.6 (data not shown). MAb 11-2-D2, at a dilution of 10^{-3}, was used during the present study as a positive control for the determination of the enhancing activities of the cord blood sera and other MAbs. This internal control ensured that the assay conditions for ADE were optimal, particularly in cases in which the test antibody or serum showed little or no enhancing activity. MAb 11-2-C9 neutralized and enhanced RSV infection, although the maximum activities for virus neutralization and enhancement occurred at different antibody dilutions (i.e., antibody dilutions of 10^{-1} and 10^{-3}, respectively) (Fig. 1B). MAb 11-3-A3 efficiently neutralized RSV ($PRNT_{90} = 10^3$) but failed to enhance virus infection at antibody dilutions between 10^{-1} and 10^{-5} (Fig. 1C). The lack of virus enhancement was not due to a nonspecific toxic effect of the ascites fluid, since the same pattern was obtained with independent ascites fluid specimens prepared from two mice. ADE was also determined for MAb 11-3-A3 against RSV isolates from antigenic subgroups A (RS-A2 and RSF-44) and B (RSF-20). Similar patterns of virus neutralization and enhancement were obtained irrespective of the antigenic type of the virus used in the assay (data not shown).

The identification of MAbs possessing either neutralizing or enhancing activities alone (MAbs 11-3-A3 and 11-2-D2, respectively) allowed the investigation of the interaction between these two opposing activities. When MAbs 11-3-A3 and 11-2-D2 were mixed at a milligram-per-milliliter ratio of 2:1, the peak EI occurred at a MAb 11-2-D2 dilution of 10^{-4} (i.e., 0.55 μg/ml) (Fig. 2A), compared with a dilution of 10^{-3} (i.e., 5.5 μg/ml) when MAb 11-2-D2 was assayed alone (Fig. 1A). With a 20-fold excess of MAb 11-3-A3 compared with MAb 11-2-D2, the peak EI was at a MAb 11-2-D2 dilution of 10^{-5} (i.e.,





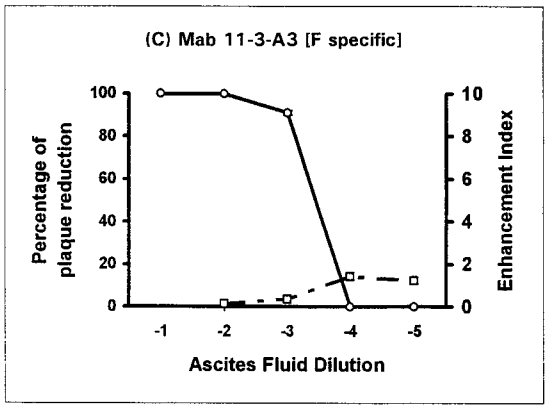
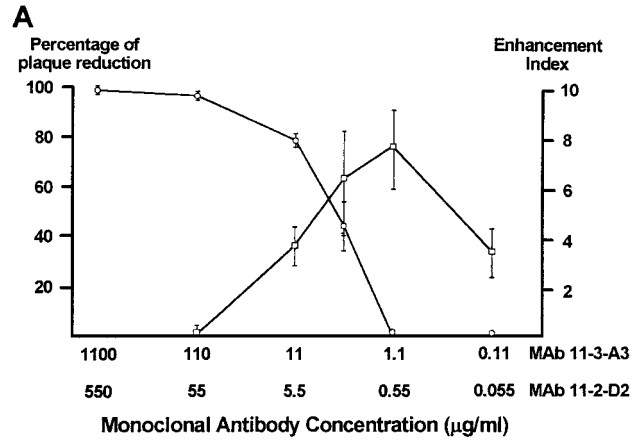


FIG. 1. Determination of the neutralizing and enhancing activities of RSV-specific MAbs. Neutralization and ADE assays were carried out as described in Materials and Methods. Both activities were determined with the RS-A2 strain of RSV. ○, percent plaque reduction; □, EI. The bars indicate the ranges for duplicate assays. Ascites fluid dilutions are 10^{-1} to 10^{-5}.

0.055 μg/ml) (Fig. 2B). The $PRNT_{90}$s of both MAb mixtures were $10^{2.5}$ (Fig. 2), while that of 11-3-A3 alone was 10^{3} (Fig. 1C). These results demonstrated that both the neutralizing and enhancing activities were modified when the two MAbs were mixed. The extent of masking of the MAb 11-2-D2-induced enhancement by the neutralizing activity of MAb 11-3-A3 depended on the concentration of the latter. High concentrations of MAb 11-3-A3 (110 to 1,100 μg/ml) masked MAb 11-2-D2 enhancement, but low concentrations of MAb 11-3-A3 (0.11 to 11 μg/ml) did not mask the enhancing activity of MAb 11-2-D2 (Fig. 2).

The possibility that the enhancing activities of two different



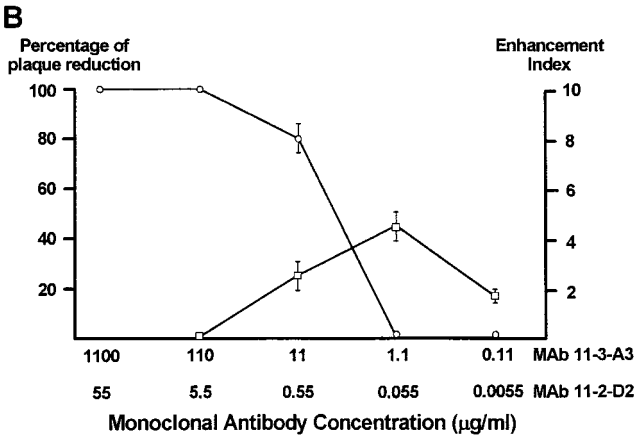


FIG. 2. Determination of the neutralizing and enhancing activities of MAbs 11-2-D2 and 11-3-A3, assayed as mixed preparations. The two MAbs were mixed in the indicated proportions, and the neutralizing and ADE assays were performed as described in Materials and Methods. ○, percent plaque reduction; □, EI. The bars indicate the ranges for duplicate assays.

enhancing MAbs were synergistic was investigated. MAb 30 exhibited virus neutralization and enhancement activities similar to those for MAb 11-2-D2, shown in Fig. 1A. The enhancing activities of MAbs 11-2-D2 and 30 individually and as a 1:1 mixture were determined (Table 2). The EI of the antibody mixture was higher than that obtained for either of the two

TABLE 2. Determination of enhancing activities of mixtures of enhancing MAbs[a]

Antibody prepn	Volume added (μl)			EI
	MAb 11-2-D2	MAb 30	RPMI-5	
1	100		100	29
2	200			20
3	100	100		51
4		200		24
5		100	100	29
6			200	1

[a] The concentrations of MAbs 11-2-D2 and 30 in the ascites fluid were 5.5 and 6.6 mg/ml, respectively. The ascites fluid specimens, diluted 10^{-3}, were assayed independently (preparations 1, 2, 4, and 5) or as a mixture (preparation 3). Preparation 6 was a control.

MAbs alone (compare the EI value of preparation 3 with the EI values of preparations 1, 2, 4, and 5 [Table 2]). The synergetic response of these two MAbs was a consistent phenomenon in replicate experiments.

Analysis of RSV-specific antibodies present in human cord blood sera. In order to determine the role of ADE in the pathogenesis of RSV, enhancing and neutralizing activities were determined for cord blood sera from infants who had succumbed to RSV infection by 9 weeks of age. Cord blood sera were collected for all babies born at Aberdeen Maternity Hospital between August 1991 and August 1992. Neutralizing and enhancing activities were determined for 13 infants from this group who were later hospitalized with RSV infection (group I). A second group of 13 infants (group II) were selected as age- and sex-matched controls for the infants in group I. Infants within group II had no reported respiratory illness requiring hospitalization. Group II would likely include infants who either were not exposed to RSV or were protected against severe virus infection. RSV antibody levels were determined in cord blood sera rather than in infants' preinfection sera for ease of sample collection and for ethical reasons. The maternal RSV-specific antibodies present in cord blood serum are passively acquired by the infant, and the levels decrease after birth, with an antibody half-life of approximately 35 days (7). The rate of decay was assumed to be equivalent for all sera tested, irrespective of whether the infant was infected with RSV.

Cord blood sera from the 26 infants in groups I and II were assayed for RSV-neutralizing antibody and ADE as described in Materials and Methods. No clearly defined peak EI was found for two cord blood serum specimens, which were excluded from the subsequent data analysis. Cord blood serum specimen 1242 (group II) had EI values of <1, 1.9, 1.4, and 1.1 at the dilutions tested and a $PRNT_{90}$ of 64. The demonstration that this serum neutralized RSV suggested that the low enhancing activity (EI < 2) was not due to a lack of RSV-specific antibodies but was likely due to the masking effect of the neutralizing antibodies on a low level of enhancing antibodies. Cord blood serum sample 1694 (group I) showed maximum enhancing activity (EI = 3.4) at dilutions of 10^{-2} and 10^{-3}. For the remaining 24 cord blood serum specimens, the numbers of sera with positive enhancing activity (EI ≥ 2), with no enhancing activity (EI < 2), and with maximum enhancing activity (peak EI) at each serum dilution were noted, and the data are shown in Table 3. The majority of the cord blood sera, irrespective of the group category, had EIs of ≥2 at serum dilutions of 10^{-2} and 10^{-3} (Table 3). The peak EIs of the group II sera were evenly distributed at dilutions of 10^{-2} and 10^{-3}. In contrast, the majority of the sera in group I showed peak EIs at 10^{-2} serum dilutions. At serum dilutions of 10^{-2} and 10^{-3} there was a significant difference ($P < 0.05$) (9) in the distribution of the sera in groups I and II.

The neutralization assay was carried out on 18 of the cord blood serum samples from groups I and II at serum dilutions between 1/16 and 1/128. From these data, the percent plaque reduction at a serum dilution of 10^{-2} was estimated and compared with the value of the EI at the same serum dilution. This serum dilution was selected since the majority of the sera (83%) showed EIs of ≥2 (Table 3). The sera were ranked according to decreasing percent plaque reduction at a serum dilution of 10^{-2}, and the results are shown in Fig. 3. There was a significant inverse correlation between the percent plaque reduction and the EI of each serum specimen (Kendall rank correlation coefficient [K] = −0.673; statistical significance of K, $Z = 4.4858$ and $P \leq 1\%$).

TABLE 3. Classification of cord blood sera according to enhancing activity at various serum dilutions

Cord blood serum dilution	Infant group	No. of serum samples			
		With an EI of:		With peak EI	Total tested
		<2.0	≥2.0		
$10^{-0.9}$	I	9	2	0	11
	II	11	0	0	11
	Total	20	2	0	22
10^{-2}	I	1	11	11	12
	II	3	9	6	12
	Total	4	20	17	24
10^{-3}	I	5	7	1	12
	II	1	11	6	12
	Total	6	18	7	24
10^{-4}	I	9	2	0	11
	II	8	4	0	12
	Total	17	6	0	23

Determination of the neutralizing activities of cord blood sera against the RSV responsible for infection. To determine the significance of the neutralization titers obtained for the cord blood sera, each serum sample was assayed against the RSV isolate responsible for the infant's infection and the prototype RS-A2 strain of RSV. The recent RSV isolates were passaged a minimum number of times in vitro in order to maintain the virus as close to the original as possible. Eighteen RSV isolates were obtained from hospitalized infants between August 1991 and August 1992. Cord blood sera from five of these infants were among those collected at birth. Three of the five RSV isolates were of sufficient titer for the neutralization assays (the virus isolate designations are in parentheses): samples 639 (RSF-65), 1194 (RSF-75), and 1303 (RSF-87). The antigenic subgroup of each RSV isolate was determined as described by Gimenez et al. (12); all three virus isolates belonged to antigenic subgroup A. Neutralization assays were performed on the recent virus isolate and RS-A2 virus in parallel with similar amounts of virus. Figure 4 compares representative data for RSF-75 and RS-A2. For this experiment, 4×10^3 PFU of each virus was used in the neutralization assay. Sixfold more serum was required to neutralize ≥90% of the RSF-75 than was needed for the RS-A2 virus. Similarly, three-

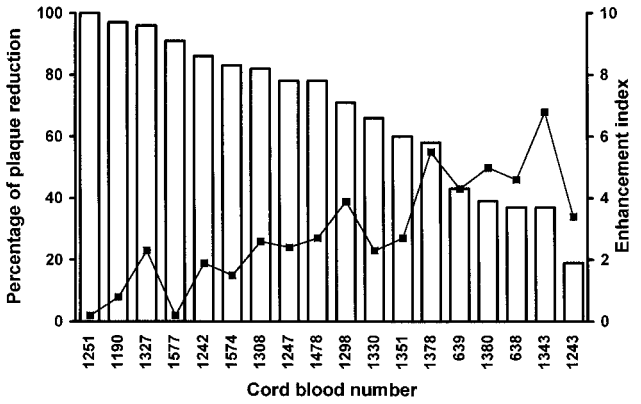


FIG. 3. Neutralization and enhancing activities of cord blood sera. The neutralizing (bars) and enhancing (■) activities of 18 cord blood serum samples were calculated for a serum dilution of 10^{-2} as described in the text.

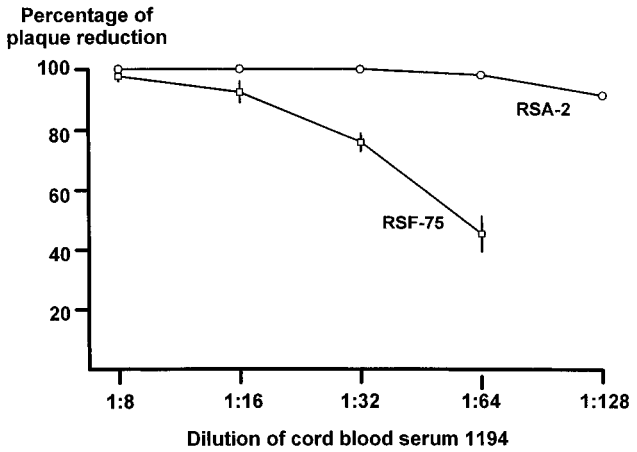


FIG. 4. Neutralizing activity of cord blood serum specimen 1194 against RSF-75 and RS-A2. The neutralization assays were performed as described in Materials and Methods. ○, percent plaque reduction determined against RS-A2 virus; □, percent plaque reduction determined against RSF-75 virus. The bars indicate the ranges for duplicate assays.

fold-higher concentrations of cord blood serum specimens 639 and 1303 were required to neutralize the corresponding virus isolates than were needed to neutralize the prototype RS-A2 strain (data not shown). Thus, in each case the serum was less neutralizing for the virus actually responsible for the infection than for RS-A2. Glezen et al. (14) showed that the severity of RSV infection correlates with low neutralizing-antibody titers in the acute-phase serum. However, patients infected with RSV may still possess neutralizing antibodies in their acute-phase sera (5, 32). In the latter studies, the serum neutralizing-antibody titers were determined with either a prototype RSV strain or a virus isolate from the outbreak. We have shown that three- to sixfold more cord blood serum was required to neutralize the RSV causing the infection than was needed to neutralize the prototype RS-A2 virus of the same antigenic subgroup. This difference could have been due to naturally occurring variations among the viruses or to alterations arising during the extensive in vitro passaging of RS-A2 compared with the more limited passage history of the recent virus isolate. Thus, acute-phase-serum neutralizing-antibody titers determined with a prototype RSV strain might be artificially high. This might explain, in part, why patients with relatively high serum neutralizing-antibody levels are still susceptible to RSV infection.

DISCUSSION

The data presented above showed that five MAbs to the virion surface proteins (F and G) enhanced RSV infection of monocytes in vitro and that one MAb to the F protein (MAb 11-3-A3) had no enhancing activity. These data, together with those in previous reports (13, 20), demonstrated that RSV-specific MAbs can be classified into the following three groups: (i) MAbs possessing only neutralizing activity, (ii) MAbs possessing only enhancing activity, and (iii) MAbs possessing both neutralizing and enhancing activities. A similar classification of MAbs into these three categories was described for dengue virus (21). The relative amounts of each category of antibody induced during natural RSV infection are unknown and might differ among infected individuals. We propose that the net activities of sera specific to RSV are determined by a balance between the neutralizing and enhancing activities. We have shown that the enhancing activity of MAb 11-2-D2 was masked in the presence of neutralizing MAb 11-3-A3 (Fig. 2). On the basis of these observations, the data in Table 3 can be interpreted as follows. The absence of enhancing activity observed for the majority of the sera at a dilution of $10^{-0.9}$ was due to a masking effect by the serum neutralizing antibodies at that dilution. When the sera were diluted to 10^{-2} and 10^{-3}, the masking was less effective and enhancement was detected. At high serum dilutions ($\geq 10^{-4}$) both activities were low. The effect of the neutralizing activity on the enhancing activity was also apparent when the cord blood sera, diluted 10^{-2}, were compared with each other (Fig. 3). Under these conditions, it was observed that sera with high neutralization activities had low enhancing activities and vice versa.

ADE can be described in terms of either the magnitude of the EI or the dilution at which the EI was greater than 2. The EI values for 25 cord blood serum samples analyzed in the current study were between 2.2 and 9.1. There was no significant difference in the magnitude of the EI between the two groups of infants (data not shown). Thus, there was no evidence for a correlation between the magnitude of the EI and the occurrence of RSV infection. However, there was a significantly greater proportion of sera showing peak EIs at a dilution of 10^{-2} in group I than in group II (Table 3). These data suggested a positive correlation between RSV infection of the infants and the occurrence of in vitro ADE by the cord blood sera at a dilution of 10^{-2}.

In vitro ADE was observed in preinfection sera and in convalescent-phase sera (references 13 and 29 and data presented above); therefore, antibodies responsible for ADE appear to be a normal part of the immune response to RSV infection. Preinfection nasal samples from infants have lower RSV-specific antibody titers than their sera (22, 24). Thus, it is possible that infants with low serum neutralizing-antibody titers could have respiratory-tract antibodies at levels at which ADE can occur. If ADE does occur during infection, it might contribute to the normal pathology of RSV either by increasing the yield of infectious virus or by causing a virus-induced modification of the monocyte immune response. A combination of these two mechanisms is also possible. Formalin-inactivated RSV has been evaluated in a vaccine trial (6). However, the results obtained were unsatisfactory, since some vaccinees either were unprotected or were susceptible to a more severe RSV infection than nonvaccinated individuals. Increased lymphocyte infiltration in the lungs might have caused the exacerbation of the disease, but this would not explain the high rates of virus isolation from the vaccinated children (6) and the increased levels of nonneutralizing antibodies present in the sera of vaccinated children (26). Additional factors might have contributed to the exacerbation of the RSV disease. Halstead (16) suggested that the enhanced RSV disease in the vaccinees could be due to ADE. It is possible that formalin treatment of RSV modified the epitopes of the virus surface proteins, altering those which stimulate neutralizing antibodies, as proposed by Murphy et al. (26). We suggest that this could have caused an imbalance in the normal production of antibodies possessing either neutralizing or enhancing activities (or both), thereby changing the ratio of these two activities. A reduction in the titers of neutralizing antibodies in the vaccinees might have limited the normal masking of the serum-enhancing activity, resulting in an enhanced virus infection. ADE might also account for the increase in influenza virus titers in the lungs of mice immunized with inactivated influenza A virus vaccine and challenged with homologous influenza A virus at 11 to 26 weeks postvaccination; at this time the mice had very low levels of influenza virus-specific antibodies (36).

The actual mechanism for ADE of RSV in U937 cells is currently unknown, although a likely explanation is that the exposed Fc regions of the antibodies that are bound to virus particles bind to the Fc receptors on the surfaces of the U937 cells, thereby increasing the internalization of the virus. This mechanism is supported by the observation that blocking the Fc segment of the monoclonal antibody or the Fc receptors on the macrophage cells reduces ADE (20, 29). We observed that high antibody concentrations lead to increased neutralization, with a parallel reduction of ADE (Fig. 3). It is possible that high antibody concentrations lead to the saturation of the cellular Fc receptors by monomeric IgG, causing reduced binding of immune-complexed RSV to U937 cells. Alternatively, high antibody concentrations may promote the aggregation of the virus particles, reducing the ability of immune-complexed virus to bind to Fc receptors on the cell membrane as well as increasing the neutralization of infectious virus. Virus aggregation by antibody has been proposed as one mechanism for the neutralization of influenza virus (30).

RSV-infected human alveolar macrophages produced lower yields of virus than bronchial epithelial cells (3). However, these virus infections were carried out in the absence of antibody, and it would be of interest to determine the influence of ADE on RSV infection of the alveolar macrophages. Increased RSV infection of macrophages through ADE could favor the helper lymphocyte subset Th2 response and in turn cause enhanced disease. The association of the Th2 response and RSV pathogenesis has been reviewed by Anderson and Heilman (2). Increased infection of macrophages by RSV could also modify the level of virus-specific cytotoxic T cells, which play a role in recovery and possibly in RSV pathogenesis (4). Enhanced RSV infection of macrophages through ADE could result in increased expression of tumor necrosis factor alpha and interleukin 6; this occurs following RSV infection of BALB/c mice (17) and murine alveolar macrophages (10). Moreover, ADE might lead to a reduction in the microbicidal activity of the macrophages, thereby accounting for the increase in bacterial infections associated with RSV (10, 35). There are thus a number of stages in RSV pathogenesis at which ADE could play a role. There is now a need to determine the occurrence of ADE in vivo, and the data presented in this report will contribute to these future studies.

ACKNOWLEDGMENTS

We thank the staffs of the Aberdeen Maternity Hospital and the Royal Aberdeen Children's Hospital for collecting the cord blood samples and the nasopharyngeal secretions from infants. We also thank the Virology Laboratory, Department of Medical Microbiology, for providing information on the diagnosis of viral infections from nasopharyngeal secretions, to G. Taylor for supplying MAb 30, and to Irene Wood for her help in tissue culture.

This work was funded in part by grants from the British Lung Foundation and the Cunningham Trust.

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