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Induction of a Neutralizing Immune Response to Human Respiratory Syncytial Virus with Anti-Idiotypic Antibodies

CONCEPCIÓN PALOMO,[1] JUAN P. ALBAR,[2] BLANCA GARCÍA-BARRENO,[1] AND JOSÉ A. MELERO[1]*

Department of Molecular Biology, Centro Nacional de Microbiología, Majadahonda,[1] and Instituto Llorente-Fides,[2] 28220 Madrid, Spain

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Anti-idiotypic (anti-Id) antibodies were raised in rabbits against monoclonal antibodies that recognized either F glycoprotein 47F or G glycoprotein 63G, 62G, or 74G of the human respiratory syncytial virus Long strain. Anti-Id sera inhibited the virus binding of the immunizing monoclonal antibodies and in some cases the binding of other antibodies reacting with overlapping epitopes. The anti-Id sera also inhibited virus neutralization mediated by the original monoclonal antibodies. Affinity purified anti-Id antibodies were subsequently used to raise a homologous anti-anti-Id response in rabbits. One of the rabbits, inoculated with anti-Id 63G, generated antibodies that reacted with the G protein of respiratory syncytial virus and neutralized the virus to high titers. The antiviral antibodies induced by anti-Id 63G were broadly cross-reactive with strains of the A and B subtypes. However, the specificities of monoclonal antibody 63G and anti-anti-Id 63G were not exactly the same, as indicated by their reaction with escape mutants to antibody 63G. These results demonstrate for the first time the induction of an anti-respiratory syncytial virus response by anti-Id antibodies.

Human respiratory syncytial (RS) virus is the major cause of lower respiratory tract infections in infants and young children (16). Early attempts of vaccination with Formalin-inactivated virus failed to induce protective immunity and, in some children, exacerbated the disease after subsequent natural infections (11). Experimental live vaccines made with attenuated viruses have also failed to induce protection in children (6). Therefore, the design of alternative vaccines requires detailed characterization of RS virus antigenicity and the immune mechanisms operating during the course of infections.

The two external glycoproteins (G and F) of the RS virus particle represent presumptive targets of a protective immune response. The G protein mediates virus binding to the cellular receptor (13), and the F protein mediates the fusion of viral and cellular membranes (32). These steps allow the internalization of viral ribonucleoprotein into the cell cytoplasm and the initiation of a new infectious cycle. The inhibition of either G or F functions should then neutralize virus infectivity. Consistent with this hypothesis, polyclonal antisera raised against a purified G or F protein (31) or sera from animals inoculated with vaccinia virus recombinants expressing G or F antigens (18, 22, 26) neutralized RS virus in tissue culture. Furthermore, the immunized animals were protected against challenge by live RS virus. The neutralizing and protective responses against the G protein were subgroup specific, whereas the anti-F responses were broadly cross-reactive with strains of the two (A and B) viral subgroups (18, 26, 30).

The antigenic characterization of the G and F molecules has been approached with specific monoclonal antibodies (MAbs) (3, 8, 20). The majority of anti-G MAbs had low neutralization indexes, which in some cases were increased by the addition of complement. However, mixtures of some anti-G antibodies show additive effects in the neutralization test (2, 8), explaining the capacity of polyclonal anti-G serum to neutralize the virus. The epitopes of the G protein recognized by MAbs are highly variable, even among strains of the same antigenic subgroup (8).

The MAbs with the highest neutralization index recognize epitopes of the F glycoprotein, which are conserved among strains of the A and B subgroups (8, 28); however, strain variation was recently reported for some neutralizing epitopes of the F protein (5).

The use of anti-idiotypic (anti-Id) antibodies for studies of the RS virus antigenic structure remains largely unexplored in spite of their success in other viral systems (1, 9, 10, 23, 29). Anti-Id antibodies (Ab2) raised against the idiotype of Ab1 may be of three types (12): (i) Ab2α binds through its paratopes to idiotopes associated with the framework of Ab1, (ii) Ab2β bears idiotypic structures that are complementary to the paratope of Ab1 and represent an internal image of the antigenic epitope recognized by Ab1, and (iii) Ab2γ binds to the Ab1 paratope but bears no structural resemblance to antigen. Only Ab2β and Ab2γ should inhibit the binding of Ab1 to the antigen.

Anti-Id antibodies have been used in studies of immunodominance (15), antibody diversity (25), receptor binding (17), and induction of antibody and/or T-cell responses to a given antigen (10, 24). As a first step to performing the same type of studies with RS virus, we raised anti-Id antibodies against one anti-F MAb and three anti-G MAbs. In addition, we investigated the ability of the anti-Id antibodies to reconstruct the epitopes of RS virus glycoproteins. One of the anti-Ids, raised against a G-specific MAb, elicited high titers of virus-neutralizing antibodies after inoculation in rabbits.

MATERIALS AND METHODS

Viruses. The Long strain (subgroup A) of human RS virus, isolated in Baltimore, Md., in 1956, was used as the RS virus prototype throughout this work. Other subgroup A strains were A2 (Melbourne, 1961), RSS-2 (Newcastle-upon-Tyne, 1976), 37271 (Madrid, 1984), 36407 (Madrid, 1984), 37541

* Corresponding author.

TABLE 1. Characteristics of anti-RS virus MAbs

MAb[a]	Antigenic area[b]	Isotype	Neutralization[c]		Strain specificity[d]
			−C′	+C′	
47F	II	IgG1	>+++	>+++	A and B subgroups
49F	II	ND[e]	−	−	A and B subgroups
2F	Ia	IgG1	±	±	A subgroup
63G	II	IgG2a	±	++	Some A and B subgroup strains
62G	VII	IgG1	±	+	Long strain
74G	VII	IgM	±	+	Long strain
25G	I	IgG1	±	−	Long strain
26G	VII	IgG1	+	−	Long and A2 strains

[a] The antibodies in boldface type were used for the preparation of anti-Id antibodies. The viral antigen (F or G proteins) recognized by each antibody is denoted by the letter following the designating number.

[b] The antigenic areas in the F and G proteins were defined by the inhibition of virus binding in competitive ELISAs (8).

[c] Each + sign indicates the reduction of 1 $\log_{10}$ unit in virus titer after incubation with the MAbs either in the absence (−c′) or presence (+c′) of complement. A marginal effect in the microneutralization assay is indicated as ±.

[d] Determined in an ELISA with purified viruses as described previously (8).

[e] ND, Not determined.

(Madrid, 1984), and 35910 (Madrid, 1984). The subgroup B strains were CH 18537 (Washington, 1962), RSN-2 (Newcastle-upon-Tyne, 1972), 41 (Zaragoza, 1986), 25 (Zaragoza, 1986).

The escape mutant viruses RS/63/2/4/1 and RS/63/2/4/8 were independently isolated after serial passage of the Long strain in the presence of antibody 63G and complement (8).

All viruses were grown in HEp-2 cells and purified from culture supernatants as previously described (7).

MAbs. The characteristics of the MAbs used in this study are summarized in Table 1. Each antibody was purified from ascitic fluid by protein A-Sepharose chromatography under conditions that bind all immunoglobulin classes (8). Subsequently, antibodies 47F and 62G (immunoglobulin G1s [IgG1s]) were digested with pepsin (antibody/enzyme mass ratio of 0.05) for 2 h at 37°C in 0.1 M citrate (pH 3.5), and antibody 63G (IgG2a) was digested for 20 h at 37°C in 0.1 M citrate pH 4.2 (21). The $F(ab')_2$ fragments generated after pepsin digestion were separated from smaller peptides by using dialysis membranes with a cutoff pore size of 50 kilodaltons (Spectrapore).

Production and purification of anti-Id (Ab2) and anti-anti-Id (Ab3) antibodies. Outbred New Zealand White rabbits were injected intradermally in multiple sites with 500 μg of purified $F(ab')_2$ fragments from antibody 47F, 62G, or 63G or the same amount of purified 74G antibody with an equal volume of Freund complete adjuvant. Two intramuscular boosts of 100 μg of the same material with incomplete adjuvant were given 4 and 6 weeks later. Sera were collected 7 to 10 days after the last injection and were made idiotype specific by passage over Sepharose columns to which normal mouse immunoglobulin [prepared by precipitation of normal mouse serum with 40% $(NH_4)_2SO_4$] had been coupled. The immunoglobulin fraction of each adsorbed anti-Id serum was precipitated with 40% $(NH_4)_2SO_4$. This material was used for Ab2 antibody characterization.

The anti-Id antibodies were further purified on immunoaffinity columns prepared with the original MAbs bound to CNBr-activated Sepharose (8). The adsorbed material was eluted with 0.1 M glycine hydrochloride (pH 2.5) and dialyzed against phosphate-buffered saline. Purified Ab2 (100 μg) was mixed with Freund complete adjuvant and used to immunize rabbits as above.

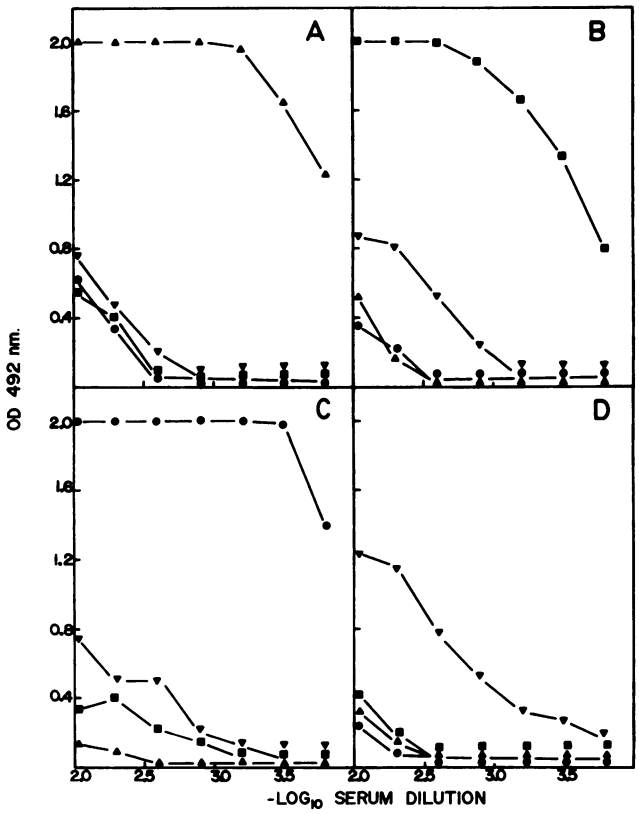

FIG. 1. Detection of anti-Id antibodies in sera. The adsorbed sera of rabbits [$(NH_4SO_4$ precipitated] immunized with 47F (▲), 62G (■), 63G (●), or 74G (▼) MAbs were tested in an ELISA with purified 47F (A), 62G (B), 63G (C), or 74G (D) antibodies on the plates. OD, Optical density.

ELISAs. All enzyme-linked immunosorbent assays (ELISAs) were carried out in 96-well microdilution plates. Wells were coated with 50 to 100 μl of protein in phosphate-buffered saline at 4°C overnight (or 1 h at 37°C) and saturated with 1% bovine serum albumin for 30 min at room temperature. Between subsequent assay steps, the wells were washed with water. Antibodies (diluted in phosphate-buffered saline with 0.1% bovine serum albumin) were incubated for 1 h at 37°C. Then biotin-labeled sheep anti-mouse or donkey anti-rabbit immunoglobulin (Amersham Corp.) was added, and the preparation was incubated for 1 h at 31°C. Finally, streptavidin-peroxidase (Amersham) was added for another hour at 37°C, and the reaction was developed with *o*-phenylenediamine dihydrochloride for 5 to 10 min at room temperature. The A_{492} was determined in a Titertek Multiskan spectrometer (Flow Laboratories, Inc.).

(i) Assay of anti-Id antibody in rabbit antisera. Wells were coated with 0.5 μg of purified Ab1. Serial dilutions of adsorbed anti-Id serum [$(NH_4)_2SO_4$ precipitated] were added, followed by the biotin-streptavidin reagents and the substrate (*o*-phenylenediamine dihydrochloride–hydrogen peroxide).

(ii) Virus binding inhibition assay. MAbs were purified from ascitic fluids as described above and peroxidase labeled by the glutaraldehyde procedure (4). The labeled antibodies were titrated by ELISA with RS virus (0.4 μg)-coated

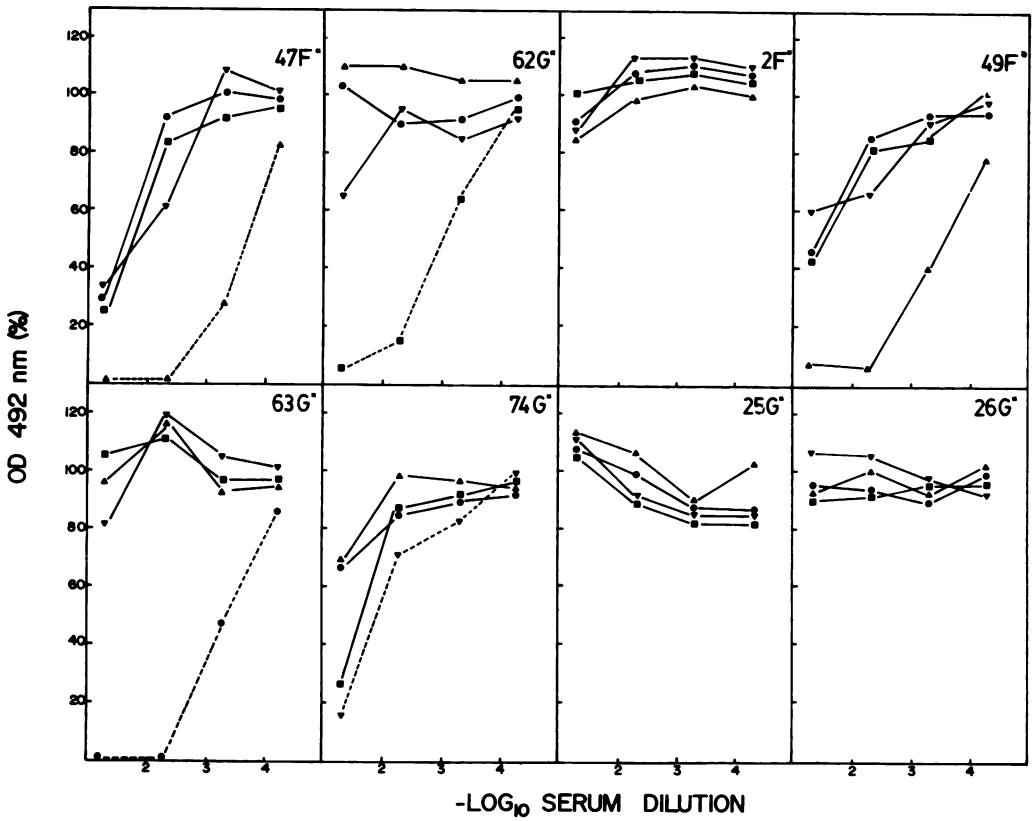

FIG. 2. Inhibition of virus binding by anti-Id antibodies. MAbs (denoted by asterisk) were purified and enzyme labeled as indicated in Materials and Methods. The labeled antibodies were tested for virus binding in an ELISA in the presence of 10-fold serial dilutions of anti-47F (▲), −62G (■), −63G (●), and −74G (▼) sera. The homologous idiotype-anti-Id reactions are shown by broken lines. Results are expressed as percentages of values obtained with each labeled MAb in the absence of anti-Id serum. OD, Optical density.

microdilution plates. The antibody dilution that gave 80% of the maximum value was incubated with different dilutions of the anti-Id sera and retested.

(iii) Virus binding by anti-anti-Id (Ab3) antibodies. Serial dilutions of anti-anti-Id sera were added to wells coated with 0.4 μg of purified RS virus. The amount of bound antibody was determined with biotin-labeled donkey anti-rabbit immunoglobulin, streptavidin-peroxidase, and *o*-phenylenediamine hydrochloride as indicated above.

Virus neutralization. Virus infectivity was determined by limited dilution in duplicate HEp-2 cell monolayers growing in 96-well microdilution plates (8). The highest dilution that caused total monolayer destruction was taken for assaying virus neutralization.

Serial dilutions of either MAbs or heat-inactivated sera were mixed with an equal volume of the predetermined virus dilution and used to infect duplicate monolayers of HEp-2 cells in 96-well plates. The cultures were incubated for 3 days at 37°C, fixed with 10% formaldehyde in phosphate-buffered saline, and stained with crystal violet. The inhibition of the cytopathic effect was evaluated by eye.

Western immunoblotting. Proteins from purified virus were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrotransferred to nitrocellulose paper (8, 27), which was cut into strips and incubated with sera or antibodies. The antigen-antibody complexes were developed by using biotinylated anti-rabbit or anti-mouse immunoglobulin, strepatavidin-peroxidase, and 4-chloro-1-naphthol as recommended by Amersham Corp.

RESULTS

Ab1. The characteristics of the MAbs used in this study are summarized in Table 1. Antibody 47F was chosen to prepare anti-Id antiserum because of its high neutralization index in the absence of complement. This antibody recognizes a F-protein epitope, conserved among subgroup A and B strains, located in the amino-terminal third of the F1 subunit. Viruses resistant to antibody 47F contain amino acid changes at residue 262 or 268 of the F protein; these residues are essential for epitope integrity (14). Antibody 49F competed with 47F for simultaneous binding to the virus and did not recognize the 47F escape mutants. These results indicated overlapping of the two epitopes, although both antibodies differed markedly in their neutralization capacities (Table 1).

The epitope recognized by antibody 63G in the RS virus G protein represents an unique antigenic site, since the virus binding of MAb 63G was not inhibited by 18 other MAbs of the same specificity. In contrast, the virus binding of antibodies 62G and 74G was inhibited by many other anti-G MAbs, which together identified an antigenic site of the G protein containing several overlapping epitopes. The reactivity of antibodies 62G and 74G was restricted to the Long strain used in their preparation, whereas antibody 63G reacted with viruses of the A and B subgroups. Antibodies 63G and 74G recognized the unglycosylated G protein precursor, but antibody 62G required at least the addition of the

O-linked sugar chains for recognition of the G molecule (unpublished data).

All of the antibodies used to generate an antiID response were purified from ascitic fluids by protein A-Sepharose chromatography. To increase the relative concentration of idiotypic determinants in the immunizing material, antibodies 47F (IgG1), 62G (IgG1), and 63G (IgG2a) were digested with pepsin, and their $F(ab')_2$ fragments purified. Antibody 74G was used as purified IgM for the immunizations.

Ab2. Antibodies reacting with normal mouse immunoglobulin were removed from rabbit anti-Id sera by adsorption on normal mouse immunoglobulin-Sepharose columns. Figure 1 shows the binding of each adsorbed anti-Id to MAbs 47F, 62G, 63G, and 74G. Only binding of anti-Id antibodies to the immunizing MAb was observed, indicating that they recognized private idiotypic determinants of the original Ab1s. The serum titer of the rabbit immunized with 74G MAb was lower than the sera of rabbits inoculated with purified $F(ab')_2$ fragments. This might reflect the relative increase of specific idiotypic determinants in the pepsin-treated material with respect to the total repertoire of antigenic determinants present in an immunoglobulin molecule.

Anti-Id sera should inhibit the binding of the original antibodies to antigen if they contain antibody subpopulations that either mimic the viral epitopes recognized by the Ab1 (Ab2β) or bind to Ab1 paratope-associated determinants (Ab2γ). Two different assays were used to test the inhibition of Ab1 binding to the virus.

(i) Inhibition of virus-binding in an ELISA done with enzyme-labeled MAbs. In all cases anti-Id sera contained antibodies that inhibited virus binding of the original Ab1 (Fig. 2). Moreover, anti-Id 47F inhibited the binding of another MAb of the same antigenic site (49F) but not the binding of antibody 2F, which recognizes an epitope of a different site in the F protein.

The anti-Id 74G had the lowest inhibitory titer (as it did in the experiment of Fig. 1). Binding of MAb 74G was also partially inhibited by anti-Id 62G, which may reflect partial overlapping of epitopes 62G and 74G (8). None of the anti-Id antibodies, however, inhibited the binding of 26G or 25G antibodies, even though MAb 26G competed reciprocally with the original MAb 62G for simultaneous binding to virus (8).

(ii) For inhibition of virus neutralization, each Ab1 was tested for its capacity to inhibit virus infectivity in a microneutralization test. In the case of antibodies 62G, 63G, and 74G, rabbit anti-mouse immunoglobulin was included in the assay to increase their neutralization index. The minimal amount of each antibody that inhibited the development of a cytopathic effect was then incubated with $(NH_4)_2SO_4$-precipitated anti-Id serum and tested in the microneutralization assay. In each case the anti-Id antibodies were able to inhibit virus neutralization mediated by the original MAbs (Fig. 3). The addition of other anti-Id antibodies (as shown for 47F and 62G) had no effect on the inhibitory capacity of the Ab1s. Appropriate controls ruled out the possibility that anti-Id sera contained nonspecific neutralizing or cytotoxic antibodies.

If any of the epitopes recognized by the original MAbs were involved in the binding of virus to the cellular receptor, the corresponding Ab2β could interact specifically with the membrane of uninfected cells. This possibility was tested by using the anti-Id sera in an indirect immunofluorescence test with HEp-2 cells. No staining of the cell membranes was observed with any of the antiId sera (data not shown).

Ab3. The results described in the previous paragraph indicated that anti-Id sera inhibited virus binding of the original MAbs. If these inhibitions were mediated by Ab2β antibodies, which mimic virus epitopes, then anti-Id antibodies could be used to generate Ab3s reacting with the original Ab1 epitopes.

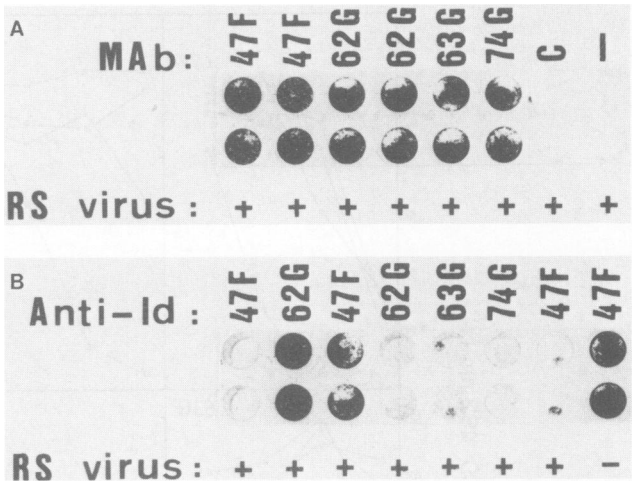

FIG. 3. Neutralization inhibition by anti-Id antibodies. (A) HEp-2 cells growing in 96-well microdilution plates were infected with 100 PFU of the RS virus Long strain (as denoted) preincubated with the same volume of culture supernatants from the indicated hybridomas. In the case of MAbs 62G, 63G, and 74G, a 1:10 dilution of serum containing rabbit anti-mouse immunoglobulin (Behring) was also added. An irrelevant antibody (C) and medium (−) were included as controls. (B) The same amount of virus was preincubated simultaneously with both the hybridomas indicated in the top panel and the anti-Id sera (1:10 dilution, heat inactivated) indicated. After virus adsorption, growth medium was replaced, and the cultures were incubated at 37°C for 3 days. Cells were then fixed and stained with crystal violet (see Materials and Methods).

To increase the concentration of Ab2-specific antibodies in the immunizing material, the anti-Id antibodies were further purified by affinity chromatography with Sepharose-bound Ab1s (data not shown). The presence of antibodies able to react with the viral epitopes in the anti-anti-Id (Ab3) sera was tested by ELISA with plates coated with purified RS virus. Only anti-Id 63G was able to elicit the production of antibodies reacting with RS virus, whereas anti-Id 47F, 62G, and 74G were negative (Fig. 4B). The titer of anti-anti-Id 63G antibodies increased significantly with successive inoculations (Fig. 4A). The presence of antibodies reacting with viral antigens was also tested in Western blots developed with the anti-anti-Id sera. Anti-anti-Id 63 antibodies reacted with a broad band (80 to 90 kilodaltons) that comigrated with the G protein recognized by the original antibody 63G (Fig. 5). The anti-anti-Id 47F, 62G, and 74G antibodies did not react with any specific virus protein in this type of assay (data not shown for 74G).

The Ab3 antibodies were then tested in a virus neutralization test. Again, only anti-anti-Id 63G antibodies neutralized RS virus infectivity (Fig. 6). The serum neutralizing titer increased after the second boost and reached higher levels than the serum titer of a rabbit repeatedly immunized (five times) with purified RS virus. The anti-anti-Id sera raised with MAb 47F (Fig. 6) or MAb 62G or 74G (data not shown) had no effect in the microneutralization test. To evaluate the spectrum of viral strains neutralized by the anti-anti-Id 63G antibodies, these were tested with 11 different viruses pre-

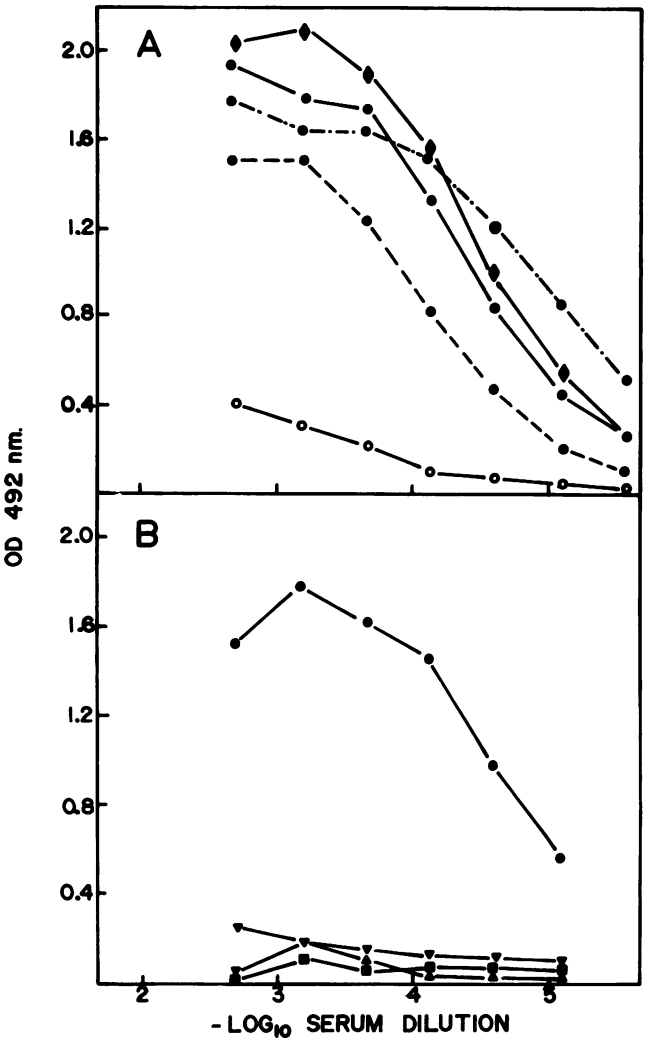

FIG. 4. Reactivity of Ab3 with virus. (A) Threefold serial dilutions of Ab3 63G obtained after the first (●—●), second (●--●), or third (●——●) immunizing dose were tested in an ELISA with purified Long virus on the plates. The results obtained with a hyperimmune rabbit serum against RS virus (◆) and a preimmune serum (○) are also included. (B) The same ELISA test was carried out with the Ab3 47F (▲), 62G (■), 63G (●), and 74G (▼) obtained after the third immunizing dose. OD, Optical density.

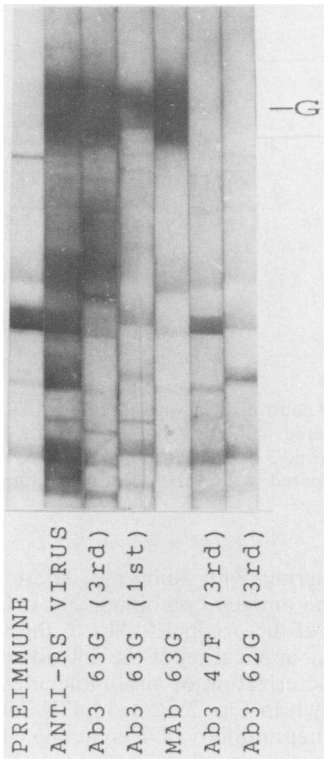

FIG. 5. Western blot of viral proteins with Ab3. Viral proteins from the Long strain were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrotransferred to nitrocellulose paper. The nitrocellulose strips were incubated with the indicated antibodies and then developed by using either anti-rabbit (for sera) or anti-mouse (for MAb 63G) antiserum and the streptavidin-peroxidase system. The reaction with a preimmune serum is also shown. The position of the G protein is indicated in the right margin. Sera were collected after the first and third immunizing doses, as indicated.

viously classified within either the A or B subgroup (8). The same neutralization test was carried out with the original 63G antibody in the presence of rabbit anti-mouse immunoglobulin. MAb 63G neutralized all strains tested to about the same extent (Table 2), even though quantitative differences between strains had been observed in a virus binding assay. Ab3 63G also neutralized all viral strains; however, it is worth mentioning that the Ab3 antibodies neutralized RS virus in the absence of a second antibody or complement.

To further explore the specificities of the Ab1 and Ab3 63G, they were tested by Western blotting against the purified Long virus and two escape mutants selected with antibody 63G. The original MAb 63G reacted with the Long virus G protein but not with the corresponding proteins of the mutants RS/63/2/4/1 and RS/63/2/4/8 (Fig. 7). These two mutants could be distinguished by their reactivities with another MAb (25G), which recognized the G proteins (and some lower band precursors) of both Long and RS/63/2/4/1 viruses but not RS/63/2/4/8. Similarly, the anti-anti-Id 63G antibodies failed to react with the G band of the RS/63/2/4/8 mutant but recognized the protein of both Long and RS/63/2/4/1 viruses. Thus, the specificities of Ab1 and Ab3 63G were distinguishable by their reactions with the escape mutant RS/63/2/4/8 selected with MAb 63G.

DISCUSSION

We report here for the first time the generation of an anti-RS virus response after immunization with anti-Id antibodies. Okamoto et al. (19) had shown that immunization of lactating mothers with an anti-RS virus F protein MAb induced the development of anti-Id antibodies in the suckling mice. The direct administration of Ab2 antibodies in infant mice primed a later immunization with purified F protein. However, the demonstration of Ab3 induction after inoculation with Ab2 was not reported.

The anti-Id antibodies that we have obtained inhibited the virus binding and the neutralizing effect of the original Ab1 in all cases. These results were compatible with the presence in the rabbit sera of Ab2β antibodies, which represented internal images of viral epitopes. However, other anti-Id antibod-

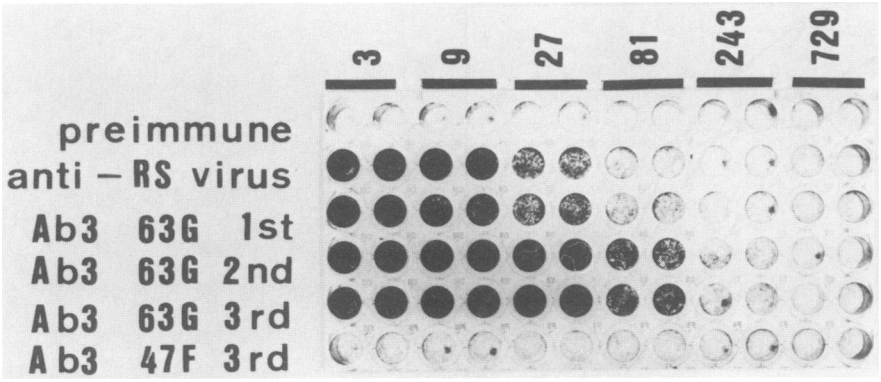

FIG. 6. Virus neutralization mediated by Ab3. The microneutralization test was carried out (see Materials and Methods). The virus (100 PFU) was incubated with 1:3 serial dilutions of the indicated antisera (heat inactivated) and used to infect HEp-2 monolayers. Cytopathic effect was monitored 3 days after infection. The results obtained with Ab3 63G sera collected after the first, second, or third immunizing dose are shown compared with those from preimmune serum, hyperimmune anti-RS virus serum, or Ab3 47F obtained after the third immunization.

ies (Ab2γ) reacting with idiotypic determinants in close proximity to the antigen combining site of Ab1 would also inhibit binding of the original MAbs to the virus (12).

An interesting application of the anti-Id antibodies for the antigenic characterization of viral molecules is indicated by the results shown in Fig. 2. Anti-Id 47F inhibited the virus binding of another antibody (49F) reacting with an epitope of the same antigenic site. In contrast, anti-Id 62G did not inhibit the binding of antibody 26G, which competed with MAb 62G for simultaneous binding to the virus. Thus, anti-Id antibodies may be used either to confirm the overlapping of epitopes in a molecule or to distinguish the epitopes recognized by MAbs that cannot bind simultaneously to the virus. This approach may be particularly fruitful to subdivide the epitopes recognized by the anti-G MAbs that showed extensive partial overlapping (8).

We do not know why anti-Id 47F, 62G, and 74G failed to induce Ab3 that reacted with viral antigens. However, the induction of an anti-anti-Id response has commonly failed in other viral systems (1, 23). In fact, the exact mechanisms that operate in the generation of the idiotype–anti-Id responses are largely unknown, and it is clear that the Ab3 antibodies do not see the antigen epitopes exactly in the same way that the corresponding Ab1s do. For instance, in influenza virus, the induction of anti-anti-Id antibodies with anti-hemagglutinin MAbs leads to the production of Ab3 reacting with strains of different hemagglutinin subtypes, whereas the original Ab1 is subtype restricted (1).

These arguments can also be considered to explain the results obtained with the 63G escape mutant viruses (Fig. 7). The original MAb 63G and the anti-anti-Id 63G antibodies must recognize structurally related epitopes of the G protein,

TABLE 2. Neutralization of RS viruses by MAb 63G and Ab3 63G[a]

Viral strain	Log_3 virus titer reduction with:	
	MAb 63G	Ab3 63G
Long	6	5
A2	4	5
RSS-2	4	5
37271	4	4
36407	4	4
37541	3	5
35910	4	5
CH 18537	3	4
RSN-2	4	5
41Zar	3	4
25Zar	4	5

[a] Dilutions (1:3) of virus seeds were incubated with the same volume of MAb 63G (culture supernatant plus 1:10 dilution of rabbit anti-mouse immunoglobulin) or heat-inactivated Ab3 63G (1:10 dilution) and used in the neutralization test as indicated in Materials and Methods. The reduction of virus titer, compared with that in a preimmune serum, is indicated for each virus.

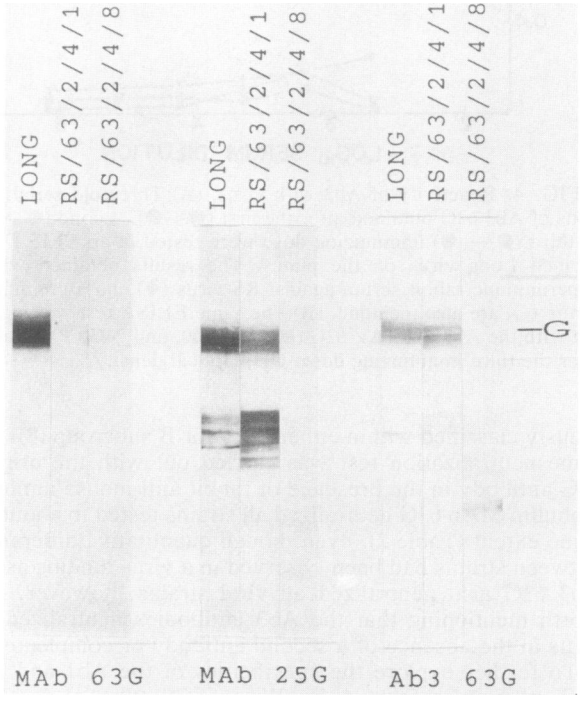

FIG. 7. Reactivity of neutralization escape mutants with Ab3. The proteins from the Long virus or the escape mutants RS/63/2/4/1 or RS/63/2/4/8 were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrotransferred to nitrocellulose paper. The strips were incubated with the antibodies shown at the bottom. The position of the G protein band is indicated on the right.

since both failed to react with mutant RS/63/2/4/8. However, the recognition of mutant RS/63/2/4/1 by the anti-anti-Id 63G indicate that in this case the Ab3 is not seeing exactly the same epitope that the original MAb 63G. This could be explained in terms of fidelity of the internal image. Since most Ab2βs are unlikely to carry a perfect replica of the antigenic epitope, their use as immunogens would be expected to induce not only antibodies with paratopes similar to that of Ab1 but also antibodies with related paratopes that fit the internal image but do not necessarily fit the antigenic epitope (1). Among the latter antibodies may be some that bind to an analog of the 63G epitope that is found in mutant RS/63/2/4/1.

It is interesting that the induction of highly neutralizing Ab3 antibodies was initiated with MAb 63G, which has only a weak neutralizing effect in the absence of complement (8). In a similar manner, Reagan et al. (23) have reported the induction of a neutralizing response against rabies virus starting with a non-neutralizing MAb. Since anti-RS virus neutralizing antibodies might find applications in the vaccine field, their induction by anti-Id antibodies and the characterization of their specificities merit further investigation.

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