Neutralizing Human Fab Fragments against Measles Virus Recovered by Phage Display

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Five human recombinant Fab fragments (Fabs) specific for measles virus (MV) proteins were isolated from three antibody phage display libraries generated from RNAs derived from bone marrow or splenic lymphocytes from three MV-immune individuals. All Fabs reacted in an enzyme-linked immunosorbent assay with MV antigens. In radioimmunoprecipitation assays two of the Fabs, MV12 and MT14, precipitated an ~80-kDa protein band corresponding to the hemagglutinin (H) protein from MV-infected Vero cell cultures, while two other Fabs, MT64 and GL29, precipitated an ~60-kDa protein corresponding the nucleocapsid (N) protein. In competition studies with MV fusion, H- and N-protein-specific monoclonal antibodies (MAbs), the H-specific Fabs predominantly blocked the binding of H-specific MAbs, while the N-specific Fabs blocked MAbs to N. In addition, N-specific Fabs bound to denatured MV N protein in Western blotting. The specificity of the fifth Fab, MV4, could not be determined. By plaque reduction assays, three of the five Fabs, MV4, MV12, and MT14, exhibited neutralizing activity (80% cutoff) against MV (LEC-KI strain) at concentrations ranging between ~2 and 7 µg ml⁻¹. Neutralization capacity against MV strains Edmonston and Schwarz was also detected, albeit at somewhat higher Fab concentrations. In conclusion, three neutralizing Fabs were isolated, two of them reactive against the H glycoprotein of MV and another reactive against an undefined epitope. This is the first study in which MV-neutralizing human recombinant Fab antibodies have been isolated from phage display libraries.

Measles virus (MV) infection is rare in industrialized countries today, thanks to the safe and effective live attenuated vaccine. However, measles is still one of the most serious infectious diseases in children in developing countries, causing more than one million deaths annually (9). Measles is characterized by high fever, cough, coryza, conjunctivitis, and Koplik’s spots, followed by a maculopapular rash about 2 weeks after initial exposure (18). Immunization at an early age is necessary in countries with high levels of MV transmission and where MV infection is a serious and life-threatening disease. However, because of maternal antibodies and immaturity of the neonatal immune system, early immunization can result in low seroconversion rates, resulting in inadequate levels of immune protection (16). The World Health Organization has proposed a plan for the eradication of measles, but to achieve this goal, new alternative vaccination strategies and/or vaccines that are safe for young infants and not inhibited by maternal antibodies are needed (38).

MV has a negative-sense RNA genome encoding six structural proteins. The hemagglutinin (H) and fusion (F) envelope glycoproteins and the nucleocapsid (N) protein surrounding the genome have been shown to be the most important proteins in terms of raising immunity against the virus (16). Both the cellular immune response, which is thought to be directed predominantly against the N protein (12, 13), and the humoral immune response are important during an MV infection. As demonstrated by passive immunization against measles, antibodies alone are capable of protection against and contribute to the control of and recovery from MV infection (22). The importance of antibodies in the immunity against MV is also exemplified by protection of infants by maternal antibodies in the first months of life (16). Antibodies are induced to most viral proteins, but the major targets for the protective antibody responses are directed against the MV H and F proteins (5, 37). Although MV is generally considered to be an antigenically conserved virus, differences in the presence of specific epitopes defined by the binding of monoclonal antibodies (MAbs) have been described, showing that the H protein has the widest degree of variation between MV strains, while the F and N proteins are antigenically more conserved (34). This conclusion is supported by studies characterizing sequences of different MV strains (27, 28). H protein-specific MAbs have been shown to provide passive protection against encephalitis in rodents (14, 41), and vaccinia virus recombinants encoding H and F proteins have been shown to induce neutralizing antibodies in mice and protect them from lethal MV challenge (11, 39). Furthermore, in a cynomolgus monkey model similar results were obtained with recombinants expressing H and F proteins (36). Therefore, any new MV vaccine, be it a recombinant vector/protein, recombinant protein, or DNA vaccine,
should induce neutralizing antibodies to the H and F proteins in addition to stimulating the cellular immune response.

The preparation of combinatorial libraries from variable heavy- and light-chain antibody genes provides an efficient route for the isolation of human antibody Fab fragments (Fabs). Using antigen binding as a means of selection, Fab molecules of interest can be rescued from such libraries. The construction of antibody libraries on the surface of M13 phages has been described (2, 21), as well as their application for the generation of a large range of human MAbs against a variety of viruses (1, 4, 8, 10, 20, 32, 40). However, only three studies have generated recombinant human Fab molecules against MV (3, 6, 24), and none of them were able to neutralize the virus. Studies on the biological functions of generated Fabs have the potential to allow detailed analysis of human antibody responses, such as epitope specificities and neutralization mechanisms. In addition, identification of protective B-cell epitopes of specific viral proteins is vital for the development of rationally designed vaccines.

This study describes the generation of three neutralizing and two nonneutralizing MV-specific human antibody Fab molecules by panning selection of combinatorial Fab libraries against viral proteins directly adsorbed or adsorbed with MV-specific MAbs onto microtiter surfaces. Additionally, studies of their biological properties and neutralization mechanism are described and discussed.

MATERIALS AND METHODS

Antibody phage display libraries. Three cDNA libraries generated from RNAs derived from three different donors (A, B, and C) were utilized in the present study. Two libraries were prepared from bone marrow RNAs recovered from two donors (A and B) possessing serum antibody titers of 1:400 against lysates of MV-infected Vero cells. In addition, serum immunoglobulin G (IgG) from these patients was demonstrated to specifically recognize viral proteins from MV-infected-cell lysates. The RNA source of the third library originated from splenocytes from a splenectomized patient (donor C) possessing a serum antibody titer of 1:19,000 against MV measured in a standard diagnostic enzyme-linked immunosorbent assay (ELISA) performed at the Swedish Institute for Infectious Disease Control (SIIDC), Stockholm, Sweden. The same library has previously been used for isolation of Fab molecules specific for Puumula virus (10, 29). The IgG1 heavy-chain Fd regions and whole κ and λ light chains from the three libraries (A, B, and C) were expressed in the phage display vector pComb3H essentially as described earlier (8, 26, 29).

Selection of antigen-binding clones by panning. Two different strategies were used for panning of the libraries. Libraries A and B were panned against MV antigen directly adsorbed or adsorbed with MV-specific MAbs onto microtiter surfaces. Additionally, studies of their biological properties and neutralization mechanism are described and discussed.

(i) ELISA for determination of MV specificity. Microtiter wells were coated with sucrose gradient-purified MV (SIIDC) at 2.5 μg ml⁻¹ or with MV lysate antigen prepared as described above. After blocking, undiluted crude Fab supernatants or diluted purified Fabs were added. Bound Fabs were detected by horseradish peroxidase-labeled goat anti-human IgG F(ab')² (Pierce, Rockford, Ill.) coupled to Affi-Gel 10 (Bio-Rad, Richmond, Calif.) as previously described (10, 30). The purity of Fab preparations was determined by ratio of antibody-coated wells to the total number of wells coated with the same antigen. Fab concentrations were determined by ELISA, including a standard (Fab fragment of human IgG; Nordic Immunology, Tiltburg, The Netherlands), as described before (10).

(ii) Cross-reactivity with unrelated proteins. The antigenic specificities of affinity-purified Fab fragments were evaluated against unrelated proteins to assess the reactivities to tetanus toxoid (SBL Vaccin, Stockholm, Sweden), ovalbumin (Worthington Biochemical Corp., Lakewood, N.J.), and BSA. The antigens (10 μg ml⁻¹) were applied directly to microtiter plates, and the ELISA was performed as described above.

(iii) Competitive binding assays. Twenty well-characterized MV H, F, and N protein-specific mouse MAbs were used in competitive binding assays to determine which of their epitopes were blocked by the Fabs generated in this study. Preparation and characterization of the MAbs have been described previously (23, 33, 34, 35). Seven H-specific (1-2, 2-9, 1-4, 1-44, 7-AG11, 16-CD11, and 16-DE6), eight F-specific (16-AG5, 16-AE7, 16-DC9, 16-EE8, 19-FF4, 19-FF10, 19-GD1, and 19-BH4), and five N-specific (16-ACS, 16-CE7, R1B5, R2C2, and R2C3) MAbs were utilized. All MAbs were first titrated against sucrose gradient-purified MV (SIIDC) in ELISA, with the titer being determined by inhibition of Fab antigenic specificity, and competition studies were performed by ELISA. For this assay, the following reagents were used. Antigens or antibodies were diluted in 50 mM sodium carbonate buffer (pH 9.6), applied to microtiter plates, and left overnight at 4°C. Coated plates were blocked with 3% BSA in PBS. All reagents were diluted in dilution buffer, and the plates were washed six times in PBS-T between each step. All incubations were carried out at 37°C for 1 h. The assays were developed with either o-phenylenediamine dihydrochloride (Sigma, St. Louis, Mo.) or p-nitrophenyl phosphate (Sigma) in diethanolamine buffer. Absorbances were measured at 490 or 405 nm, respectively.

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ELISA. Screening of crude Fab preparations, determination of Fab concentrations, confirmation of Fab antigenic specificities, and competition studies were performed by ELISA. For this assay, the following reagents were used. Antigens or antibodies were diluted in 50 mM sodium carbonate buffer (pH 9.6), applied to microtiter plates, and left overnight at 4°C. Coated plates were blocked with 3% BSA in PBS. All reagents were diluted in dilution buffer, and the plates were washed six times in PBS-T between each step. All incubations were carried out at 37°C for 1 h. The assays were developed with either o-phenylenediamine dihydrochloride (Sigma, St. Louis, Mo.) or p-nitrophenyl phosphate (Sigma) in diethanolamine buffer. Absorbances were measured at 490 or 405 nm, respectively.

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Western blotting. The antigenic reactivities of Fab fragments against denatured antigen were evaluated by western blotting. Detergent-treated, sucrose gradient-purified MV antigen (SDC) was mixed with Laemmli sample buffer, electrophoresed in a Tris-HCl–4 to 20% polyacrylamide gel (Bio-Rad), and transferred electrophoretically to a nitrocellulose membrane. Nitrocellulose strips blocked with 5% nonfat milk in PBS were incubated overnight at 4°C with Fab (20 μg ml⁻¹, Mabs, or sera diluted 1:200). After washing, an ALP–goat anti-human IgG F(ab')₂ (Pierce) or ALP–goat anti-mouse IgG antibody (Sigma) was added at a dilution of 1:1,000 in 5% nonfat milk in PBS. Strips were developed, after additional washings, with 5-bromo-4-chloro-3-indolylphosphatase–tetrahydrofilm (Sigma). All MV-specific Mabs used in this study were tested and included as controls in addition to MV-antibody positive serum.

Virus neutralization assay. The ability of Fab to inhibit MV infectivity was assayed by the plaque reduction neutralization test (PRNT). Fab were serially diluted in Dulbecco’s minimal essential medium (DMEM) supplemented with 1% fetal calf serum (FCS), 2 mM l-glutamine, 10 μU of penicillin ml⁻¹, and 30 μg of streptomycin ml⁻¹ and mixed with an equal volume of MV strain LEC-KI, Edmonston, or Schwartz [34] at 1,000 PFU ml⁻¹. The mixture was incubated under rotation for 1 h at 37°C and subsequently inoculated (200 μl) into 12-well tissue culture plates containing confluent Vero cell monolayers. After adsorption for 1 h at 37°C, wells were overlaid with a 42°C 1:1 (vol/vol) mixture of 1% low-melting-point agarose and DMEM supplemented with 6% FCS, 20 mM HEPES, 2 mM l-glutamine, and antibiotics as described above. Tissue culture plates were incubated at 37°C in a humidified 5% CO₂ atmosphere for 7 days. For plaque detection, the agarose was gently removed and cells were fixed and stained with 0.15% crystal violet in 20% ethanol, followed by air drying. Plaques were enumerated, and the neutralization activity was expressed as the amount of Fab necessary to reduce the number of plaques by 80% compared to wells incubated with MV and medium only. As a negative control a Puumala virus-specific Fab was included, and neutralization assays were repeated at least twice with reproducible results.

Radioimmunoprecipitation of [³⁵S]-labeled MV proteins with recombinant Fab. For radioimmunoprecipitation assays (RIPA), a confluent monolayer of Vero cells (225 cm²) was infected with MV LEC-KI strain. The infected cells were incubated at 37°C with DMEM supplemented with 1% FCS, 2 mM l-glutamine, and antibiotics as described above until a 50 to 60% cytopathic effect was reached. At that time the cell culture medium was replaced with cysteine- and methionine-free DMEM supplemented with 0.5% FCS, 2 mM l-glutamine, 60 μg of cysteine ml⁻¹, and antibiotics as described above. Cells were incubated for 1 h at 37°C, after which they were radiolabeled with 1 μCi of [³⁵S]methionine ([³⁵S]Met) (Rediue; Amesher, Little Chalfont, United Kingdom). After 24 h, PMSF (2 mM) and aprotinin (1% Sigma) were added to the cell flask, and the cells were harvested and pelleted. Cells were resuspended in 4 ml of RIPA buffer (10 mM Tris-HCl [pH 7.8], 150 mM NaCl, 0.6 M KCl, 5 mM EDTA, 2% Triton X-100, 1 mM PMSF, and 1% aprotinin), lysed by freeze-thawing five times, and centrifuged at 60,000 × g for 45 min in a Beckman SV60 rotor. A mixture of 150 μl of radiolabeled MV antigen, 20 μg of Fab in 100 μl of Tris-HCI buffer (10 mM Tris-HCl [pH 7.4], 150 mM NaCl), and 250 μl of RIPA buffer was incubated at 4°C overnight. Precipitations of Fab were performed by addition of 100 μl of protein G-coupled agarose beads (50% slurry) (Amersham Pharmacia Biotech, Upsalla, Sweden) with 10 μg of goat anti-human IgG Fab (λ), (Pierce) ml⁻¹ and incubation with rotation at 4°C for 4 h. For Mabs and control serum, only protein G-agarose beads were added. The beads were pelleted and washed six times with RIPA buffer and once with Tris-HCI buffer. Samples were resuspended in 30 μl of Laemmli sample buffer and boiled for 5 min, and after a brief centrifugation the samples were electrophoresed on a Tris-HCl–4 to 20% polyacrylamide gel (Bio-Rad). The gel was fixed in a solution of 20% methanol and 10% acetic acid for 30 min, washed twice with distilled deionized H₂O, and then incubated in Amplify solution (Amersham) for 30 min. After drying, the gel was visualized by autoradiography on a phosphorimager.

Nucleic acid sequencing. Cycle sequencing was carried out on phagemid DNA using the PRISM Ready BigDye Terminator Cycle sequencing kit with AmpliTaq DNA polymerase FS (Perkin-Elmer/Applied Biosystems Division, Foster City, Calif.). Amplification was performed using the GeneAmp PCR system 2400 (Perkin-Elmer, Norwalk, Conn.). The reaction mix (20 μl) contained 8 μl of PRISM premix, 3.2 pmol of sequencing primer, and 500 ng of DNA. The PCR products were analyzed using an ABI377 sequencer (Perkin-Elmer/Applied Biosystems Division). The following primers were used: Pelseq, ACC TAT TGC CTA CGG CAG CCG; Segazz, GAA GTA GTC CTT GAC CAG; Sceq, GAA GTC ACT TAT GAC ACA CAC; and SceqkB, ATA GAA GTC GTT CAG CAG GCA.

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<th>TABLE 1. Amino acid sequences of heavy-chain complementarity-determining region 3 (CDR3) and flanking framework regions FR3 and FR4 of generated MV-specific Fab fragments</th>
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RESULTS

Isolation of MV-specific Fab fragments by panning. Three IgG1 λ and κ antibody libraries, A, B, and C, expressed on the surface of filamentous phage and derived from bone marrow or spleen lymphocyte RNAs from three MV-seropositive donors, were used for selection of MV-specific Fabs. Panning was performed on five different occasions, twice with library A, once with library B, and twice with library C. The second panning of library A was performed by first masking the panning antigen with the purified Fab generated in the first panning of the same library. The input phage library consisted of 8 × 10⁹ PFU ml⁻¹ for panning of library A, 5 × 10⁹ PFU ml⁻¹ for panning of library B, and 7.3 × 10⁹ and 4.5 × 10⁹ PFU ml⁻¹, respectively, for the two panning occasions for library C. Libraries A and B were panned against viral proteins applied directly to microtiter wells, while library C was panned against viral proteins immobilized in microtiter wells with MV H and F protein-specific Mabs. Four or five rounds of panning resulted in 10⁷ to 10⁸ PFU of eluted phage ml⁻¹ in all panning experiments. Phagemid DNA was prepared from the fourth or fifth round of panning, and the gene III fragment was removed by NheI and SpeI enzyme digestion followed by religation. The resulting phagemid was transformed into XL1-Blue E. coli cells to produce clones secreting soluble Fab fragments. To screen for antigen-specific Fab clones, 20 colonies each from the first and second pannings of library A, 30 colonies from the panning of library B, and 80 and 120 colonies from the first and second pannings of library C, respectively, were randomly picked, and freeze-thawed culture supernatants were screened for reactivity against MV in ELISA. Five of 20, 1 of 20, 2 of 30, 2 of 80, and 79 of 120 crude Fab bacterial lysates from the five different panning occasions reacted positively with MV antigen. Twenty-eight of the 79 ELISA-positive crude Fab suspensions from the second panning of library C were additionally screened by PRNT, where they showed 52 to 82% plaque reduction at a 1:2 dilution (data not shown). Five positive clones from the first and one positive clone from the second panning of library A, two positive clones from panning of library B, and two positive clones from the first panning of library C and eight of the clones with highest neutralizing capacity in PRNT from the second panning of library C were sequenced.

Nucleic acid sequencing of Fab heavy- and light-chain variable regions. To evaluate whether the isolated Fab fragments represent individual clones, complementarity-determining regions 1 to 3 of the heavy and light chains were sequenced. This analysis revealed five predominant sequences with different heavy-chain sequences (Table 1) and light-chain sequences...
MT14 and MT64 from the filtered MV antigen. Purified MV12 (E) against sucrose gradient-purified MV. All clones were amplified and affinity purified for further characterization.

**Fab specificity for MV.** Fab clones MV4, MV12, MT14, MT64, and GL29 were tested for MV specificity in different assays. Affinity-purified Fabs from each of the five clones were tested for MV binding by titration of equivalent amounts of each Fab against sucrose gradient-purified MV. All clones bound to MV in a concentration-dependent manner but at various levels, suggesting different affinities for the antigen. Fabs MV12 and MT14 showed the highest and Fabs MV4 and MT64 showed the lowest reactivity (Fig. 1). Antigen specificity of the Fab was verified by reactivity at the background level (optical density of <0.1) in ELISA against tetanus toxoid, ovalbumin, and BSA (data not shown).

**Western blotting.** A Western blot assay was applied to determine the nature of the epitopes recognized by the Fabs. Two of the clones, MT64 and GL29, visualized protein bands corresponding to H11001/H11002 and H11015/H11016 as that seen with MV N protein. All clones were included as controls (data not shown). This indicated that these two Fabs were N protein specific and most likely recognized linear epitopes within this protein.

**RIPA with Fabs.** Fabs were run in a RIPA to assess the MV specificity. Four of the isolated Fab clones were found to precipitate MV-specific proteins, identifying two different protein specificities (summarized in Table 2): those reactive with N protein and those reactive with H protein. The RIPA reactivities of Fabs MV12 and MT14 suggested that they were specific to H in that they precipitated a band of ~80 kDa from MV-infected Vero cell lysates labeled with [35S]Met (Fig. 2). A band of the same size was also precipitated by the MV H-specific MAb (I-41). Fabs MT64 and GL29 immunoprecipitated an ~60-kDa band corresponding to N (Fig. 2). The same band was also seen when an MV N-specific MAb (16-CF7) was used. Fab MV4 did not immunoprecipitate any clearly identifiable MV proteins with this method.

**Epitope mapping of Fabs.** Cross-competition studies between Fabs and biotin-labeled Fabs were performed to investigate the presence of potentially shared epitopes among the different Fabs generated. All of the Fabs were blocked by the homologous Fab by 80% or more of maximal binding (Table 3). The two Fabs, MV12 and MT14, blocked each other’s binding to MV as efficiently as they blocked homologous binding, indicating that they had overlapping or shared epitopes. MV12 also blocked MV4 but to a much lower extent. In addition, Fab MV4 partially blocked GL29 binding.

**Virus neutralization by Fabs.** Affinity-purified recombinant Fabs MV4, MV12, MT14, and GL29 were tested for their capacity to neutralize MV strains LEC-KI (laboratory strain), Edmonston (laboratory strain), and Schwarz (vaccine strain) by PRNT. Virus neutralization was set as the end point concentration of the Fab required to reduce the number of plaques by 80%. Three of five characterized Fabs had neutralizing activity against MV. Fab clones MV12 and MT14 neutralized an in that they precipitated a band of ~80 kDa from MV-infected Vero cell lysates labeled with [35S]Met (Fig. 2). A band of the same size was also precipitated by the MV H-specific MAb (I-41). Fabs MT64 and GL29 immunoprecipitated an ~60-kDa band corresponding to N (Fig. 2). The same band was also seen when an MV N-specific MAb (16-CF7) was used. Fab MV4 did not immunoprecipitate any clearly identifiable MV proteins with this method.

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**Virus neutralization by Fabs.** Affinity-purified recombinant Fabs MV4, MV12, MT14, and GL29 were tested for their capacity to neutralize MV strains LEC-KI (laboratory strain), Edmonston (laboratory strain), and Schwarz (vaccine strain) by PRNT. Virus neutralization was set as the end point concentration of the Fab required to reduce the number of plaques by 80%. Three of five characterized Fabs had neutralizing activity against MV. Fab clones MV12 and MT14 neutralized an in that they precipitated a band of ~80 kDa from MV-infected Vero cell lysates labeled with [35S]Met (Fig. 2). A band of the same size was also precipitated by the MV H-specific MAb (I-41). Fabs MT64 and GL29 immunoprecipitated an ~60-kDa band corresponding to N (Fig. 2). The same band was also seen when an MV N-specific MAb (16-CF7) was used. Fab MV4 did not immunoprecipitate any clearly identifiable MV proteins with this method.
neutralized the MV LEC-KI strain at \( \approx 7 \mu g \) ml\(^{-1} \), while Fab MV4 neutralized the same strain at a concentration of below \( \approx 2 \mu g \) ml\(^{-1} \) (Fig. 4). When the neutralization cutoff was reduced to 50%, less than \( \approx 0.5 \mu g \) of Fabs MV4 and MT14 ml\(^{-1} \) was needed for neutralization, while \( \approx 1.5 \mu g \) of Fab MV12 ml\(^{-1} \) was needed (Fig. 4). Neutralization of the Edmonston strain was seen with Fab concentrations similar to those for the LEC-KI strain. Somewhat higher concentrations of Fabs MV4, MT14 were needed to neutralize the Schwarz LEC-KI strain. Neutralization of the Edmonston strain was needed (Fig. 4). Neutralization of the Edmonston strain was seen with Fab concentrations similar to those for the LEC-KI strain. Somewhat higher concentrations of Fabs MV4, MT14 were needed to neutralize the Schwarz strain (Table 4).

**DISCUSSION**

The use of combinatorial libraries displayed on the surface of filamentous phage offers an efficient means of obtaining a diverse set of human MAbs from an immune donor (7). In this study we have used combinatorial phage antibody libraries generated from RNAs derived from bone marrow or splenic lymphocytes, which were selected against MV for the isolation of neutralizing H-specific and nonneutralizing N-specific recombinant human Fab molecules.

Numerous murine MAbs specific for all of the structural proteins of MV have been isolated to date by using conventional hybridoma technology (15, 23, 31, 34). These MAbs are widely used as research tools and reagents for diagnosis. However, human MAbs are scarce, and only three earlier studies have generated human Fab molecules specific for MV proteins from recombinant antibody libraries. In the first of these studies, Bender et al. isolated three MV-specific Fabs, two of which were cross-reactive with a number of other antigens, from a library originating from bone marrow RNA from an individual with low antibody titers against MV (3). In the two remaining studies, an antibody library was prepared from the brain tissue of a patient with subacute sclerosing panencephalitis (SSPE) and selected either against MV-infected cells yielding one N-specific and three phosphoprotein-specific Fab molecules (6) or against SSPE brain tissue sections, generating N-specific Fabs (24).

Neutralizing antibodies are directed predominately against the surface glycoproteins of a virus, while intracellular proteins usually are more important for stimulation of the cellular immunity. However, isolating recombinant Fab molecules, from phage display libraries, directed against surface glycoproteins by selection against cellular extracts of virus-infected cells can be technically challenging. These selection procedures usually result in the isolation of antibodies specific for immunodominant proteins present in large amounts, such as the nucleocap-

![FIG. 2. Radioimmunoprecipitation of MV strain LEC-KI, illustrating the specificities of anti-MV recombinant Fabs. Lanes: 1, Fab MV4; 2, MV12; 3, MT14; 4, MT64; 5, GL29; 6, Puumala virus-specific Fab; 7, Tris-HCl buffer; 8, anti-H MAb I-41; 9, anti-N MAb 16-CF7; 10, anti-F MAb 16-AG5; 11, MV antibody-positive serum; 12, MV antibody-negative serum. The migration of molecular weight standards (in thousands) is indicated on the left.](http://jvi.asm.org/)

**TABLE 3. Cross-competition between Fabs for MV binding**

<table>
<thead>
<tr>
<th>Fab</th>
<th>MV4 % Inhibition of binding(^b) with blocking Fab</th>
<th>MV12</th>
<th>MT14</th>
<th>MT64</th>
<th>GL29</th>
</tr>
</thead>
<tbody>
<tr>
<td>MV4</td>
<td>81</td>
<td>34</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>MV12</td>
<td>—(^a)</td>
<td>100</td>
<td>100</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>MT14</td>
<td>—</td>
<td>96</td>
<td>98</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>MT64</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>85</td>
<td>—</td>
</tr>
<tr>
<td>GL29</td>
<td>34</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>100</td>
</tr>
</tbody>
</table>

\(^a\) Biotin labeled.

\(^b\) Boldface values indicate homologous inhibition.

\(^c\) —, <20% inhibition of binding.
and 532 to 533, indicating that this MAb has a conformational epitope. Together these data suggest that the epitopes of the Fabs generated in this study are located within these areas of the H protein, but further studies are needed to confirm this finding. It has not been possible to show by MAb competition studies to which epitopes the two N-specific Fabs are directed, as the MAbs used have not been previously mapped to specific epitopes within the N protein.

Of the five isolated Fab clones, three conferred neutralizing activity against MV in PRNT. Two of three (MV12 and MT14) were shown by RIPA to be H specific, while the third Fab (MV4) was interpreted to be H or F specific, as these are the proteins responsible for inducing neutralizing antibodies. The fact that Fab MV4 could not precipitate any protein by RIPA using $[^{35}\text{S}]$Met-labeled antigens may indicate that this Fab has low affinity for its target protein or that MV4 is specific for the F$_2$ part of the F protein, which is not detectable with this method as it has a very low methionine content. The low MV reactivity of Fab MV4 as seen by ELISA titration could depend on small amounts of F$_2$ and/or F in the MV antigen preparation used. In addition, an F-specific MAb (19-FF10) was used for the selection of Fab MV4, adding support to this conclusion.

The lowest Fab concentrations were needed for neutralization of the MV LEC-KI strain, which may have been expected since the Fabs were selected against this strain. Somewhat higher concentrations were needed for neutralizing the Edmonston and Schwarz strains. Differences in the amount of Fab needed for neutralization between strains could possibly reflect sequence differences between the H proteins of these three strains. While LEC-KI and Edmonston are laboratory strains, from an SSPE patient and a measles patient, respectively, the Schwarz strain is a vaccine strain. Although MV is a relatively conserved virus with low sequence diversity between strains, some differences occur, which could be of importance for neutralization.

Neutralizing Fab fragments have been isolated for a number of viruses. Here we describe the first isolation of a number of human neutralizing Fab fragments against MV. A possible
mechanism by which these Fab fragments neutralize is by coating of the virus surface leading to inhibition of attachment or interference with virus-cell fusion, which has been proposed to be a major mechanism of neutralization (25). It may be interesting to study this in more detail. The corresponding whole antibodies may inhibit MV in vivo by invoking additional, Fc-dependent, mechanisms such as complement activation and antibody-dependent cellular cytotoxicity.

Cloned antibodies from immune donors are helpful tools in the study of humoral responses. The analysis of antigen and epitope specificities of recombinant human antibodies during different infections can generate information about which proteins, and specifically which parts of proteins, are important for the generation of humoral immune responses. Particularly interesting are differences in antigen recognition in special forms and/or complications of a disease, such as atypical measles and SSPE. This approach was applied by Burgoon et al. when identifying the specificity of cloned antibodies from an SSPE brain (6). In the same line of thought, recombinant antibodies could be used to study differences in immunity induced by natural infection or vaccination. This information could be important when trying to circumvent the maternal antibodies present in the infant at the time for vaccination. In addition, Fab molecules could be reconstituted to whole immunoglobulins and be used for prophylaxis and/or immunotherapy in individuals in whom an MV infection could lead to serious complications. Phage display antibody libraries could also be utilized in the search for antigens related to diseases with unknown etiology.

In conclusion, this is the first report that describes the isolation of MV-specific neutralizing human Fab fragments. These antibodies, which were directed against the H (MV12 and MT14) and possibly the F (MV4) proteins of MV, could block the binding of MAbs to MV proteins (MV12 and MT14) and were able to neutralize heterologous MV strains in PRNT (MV4, MV12, and MT14). Resolving the epitope specificities of these antibodies within their target proteins could aid

FIG. 4. Neutralization of MV by purified Fab fragments MV4 (■), MV12 (●), MT14 (♦), MT64 (▲), and GL29 (○) in PRNT using MV strain LEC-KI.

TABLE 4. Fab neutralization of MV

<table>
<thead>
<tr>
<th>Fab</th>
<th>LEC-KI</th>
<th>Edmonston</th>
<th>Schwarz</th>
</tr>
</thead>
<tbody>
<tr>
<td>MV4</td>
<td>≤0.625</td>
<td>≤0.625</td>
<td>≥2.5</td>
</tr>
<tr>
<td>MV12</td>
<td>≥2.5</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
<tr>
<td>MT14</td>
<td>≤0.625</td>
<td>≤0.625</td>
<td>≥2.5</td>
</tr>
<tr>
<td>MT64</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>GL29</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*—, no neutralization.
ACKNOWLEDGMENTS

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REFERENCES


