Multiplex Assay for Detection of Strain-Specific Antibodies against the Two Variable Regions of the G Protein of Respiratory Syncytial Virus

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The role of strain differences in respiratory syncytial virus (RSV) disease has not been clearly defined. To investigate the possibility that strain differences contribute to susceptibility to repeated infections, we developed assays to detect antibodies to the two variable regions of the RSV G protein by cloning and expressing the internal variable region at amino acids (aa) 60 to 172 (g1) and the carboxy-terminal variable region at aa 193 to the carboxy terminus (g2) from different genotypes of RSV. The purified proteins were covalently linked to beads with different proportions of red and orange fluorescent dyes and reacted against serum specimens. Antibody reacting against the differently colored beads, and thus against different G polypeptides, was detected by use of flow cytometry and the Luminex system. This assay system detected group- and, to some extent, genotype-specific responses to RSV infection and can be used to investigate the role of strain differences in RSV disease.

Respiratory syncytial virus (RSV) is the single most important lower respiratory tract pathogen of infants and young children worldwide, and its priority for vaccine development is high (22, 23). Infections in older children and adults can also be associated with severe disease, especially in those with compromised pulmonary, cardiac, or immune systems and in the elderly (17, 19, 20, 28). To date, efforts to develop a safe and effective vaccine have been unsuccessful (12). Several features of RSV disease present difficulties in developing a vaccine, including the virus’ ability to cause repeat infections and disease throughout life (16, 20), its ability to cause yearly community outbreaks, and its propensity to cause disease in infants despite the presence of maternal neutralizing antibodies (35). Genetic and antigenic differences between RSV strains may contribute to some of these limitations in the RSV protective immune response. Although differences among RSV isolates were noted in the 1960s (9), they were not studied until monoclonal antibodies, and later, genetic studies, made it possible to clearly and consistently distinguish between different strains (2, 32). Most of the studies of RSV strains have focused on 1 of the 11 RSV proteins, the G protein. The G protein is the most variable of the RSV proteins (25) and, along with the F protein, induces long-term protective immunity in animals (10, 38). Both antigenic and genetic studies of RSV have demonstrated two major groups of strains, A and B, and multiple distinct strains within each group (1, 5, 6, 25). Sequence studies can be used to place strains within each group into clusters of closely related strains (e.g., genotypes) (6, 14, 36, 37).

Although multiple investigators have studied RSV strains, the importance of strain differences to protective immunity, RSV disease, and vaccine development has not been clearly defined. It is likely that antigenic differences between the group A and group B strains do affect susceptibility to infection. In animals, the titer of neutralizing antibody is higher and the protection from virus challenge is better when the immunizing and challenge viruses are from the same group (24). In primary infections in humans, the neutralizing antibody response against a strain from the infecting group is different from that against a strain from the other group (21, 30), and in one study, a child’s second infection often occurred with a strain from a group different from that of the strain causing the primary infection (31). It is less clear whether differences between strains within the same group affect susceptibility to infection. In animals, the titers of neutralizing antibody and degrees of protection from virus challenge are not significantly different when strains from different genotypes within the same group are used (40). On the other hand, the yearly shift in the group or genotype of viruses causing outbreaks in communities suggests that differences between strains in the same group may affect susceptibility to infection (4, 18, 37, 42). It is possible that a virus from a new genotype can transmit more efficiently or be more pathogenic because the RSV immunity in the population is most effective against a recently circulating genotype.

Given the potential importance of strain differences to vaccine development, we felt it important to develop serologic assays that detect genotype-specific immune responses to investigate the role that strain differences play in RSV disease. We focused these efforts on the most variable of the RSV proteins, protein G. The G protein contains two relatively conserved regions: the cytoplasmic and transmembrane regions (amino acids [aa] 1 to 66) and a central conserved region (aa 153 to 221). The two remaining regions, from aa 70 to 150 and aa 222 to the carboxy terminus, are highly variable (5, 25,
TABLE 1. Fluorescence of RSV G polypeptides reacted against an anti-histidine tag monoclonal antibody

| Strain | Source, yr of isolation | MFI ± SD for polypeptide: a | g1 | g2 |
|--------|-------------------------|-----------------------------|-----|-----|
| GA1    | Australia, 1961         | 8,120 ± 314                 | 1,189 ± 191 |
| A2     | New York, 1993–1994     | 8,632 ± 120                 | 3,580 ± 471 |
| CH34   | Missouri, 1984–1985     | 8,853 ± 148                 | 5,771 ± 367 |
| 10849  |                        |                             |     |     |
| GA2    | New York, 1993–1994     | 5,922 ± 312                 | 2,700 ± 69  |
| 2008.1 | New York, 1985–1986     | NA b                        | 3,002 ± 148 |
| GB     | Wisconsin, 1976         | 6,872 ± 622                 | 2,057 ± 265 |
| 334,4  | West Virginia, 1987     | 8,737 ± 657                 | 7,734 ± 613 |
| 2B     | New York, 1994–1995     | 6,715 ± 809                 | 3,948 ± 387 |
| CH41   | New York, 1992–1993     | 7,780 ± 483                 | 2,345 ± 97 |
| CH53   | New York, 1992–1993     |                             |     |     |
| DHFR   | control                 |                             |     |     |

a Data are for triplicate samples.

b NA, not available.

In this report, we describe the characteristics of assays that use expressed polypeptides representing both G protein variable regions from different genotypes from groups A and B. These assays detect an antibody response that is group and partially genotype specific.

MATERIALS AND METHODS

The RSV strains used to clone the G polypeptides (Table 1) were selected to represent distinct genotypes within the two major groups of RSV, groups A and B. In addition, two group A strains, CH06 (genotype GA1) and CH20 (genotype GA6), and two group B strains, B1 (genotype GB1) and CP52 (derived from B1 and lacking the G and SH genotypes) (26), were used to induce RSV antibodies in mice. All viruses were grown in Vero cells as previously described (41) and were stored at −70°C.

Serum specimens. Three sets of serum specimens were used for this study: (i) serum from mice immunized with different RSV strains or control antigens, (ii) pre- and postvaccination serum specimens from 6- to 60-month-old children who participated in clinical trials of live RSV vaccines, and (iii) serum specimens from patients exposed to RSV during a nosocomial outbreak in a chronic-care facility. With Institutional Review Board approval, the sources of the human specimens were made anonymous and the study was exempted from Institutional Review Board approval.

The mouse antisera were generated by intranasal inoculation of 4- to 6-week-old BALB/c mice with 50 μl of tissue culture material containing 10^7 PFU of live RSV or a comparable concentration of uninfected tissue culture material on day 0 and intraperitoneal inoculations on days 7 and 14. Sera from the immunized mice were harvested 4 weeks after the last immunization. The titer of RSV antibodies was determined by use of an indirect enzyme-linked immunosorbent assay with RSV-infected tissue culture as the positive standard and uninfected tissue culture as the negative control antigen as previously described (11).

Serum specimens from 20 children participating in trials of two cold-passaged, temperature-sensitive (CPTS) attenuated strains derived from the A2 strain of RSV, CPTS 248/955 and CPTS 530/1009 (27), were tested for G polypeptide antibodies. Three of the 20 children received a placebo, and 17 received the vaccine. Serum specimens were obtained from all children just before vaccination and 4 weeks postvaccination, and the seronegative vaccine recipients had a third serum specimen collected 8 weeks after vaccination. Before vaccination, 8 of the 17 vaccine recipients were negative and 9 were positive for anti-RSV antibodies by a complement-enhanced 60% plaque reduction assay (8). Fourteen of the children responded to the vaccination with a fourfold or greater rise in neutralizing antibodies, and 12 of these also shed vaccine virus. No vaccinee without an antibody response shed virus.

Acute- and convalescent-phase serum specimens were available from 14 children and adults (mean age, 20 years) exposed to one of two strains of RSV during two distinct nosocomial outbreaks in one chronic-care facility (13). Eight patients were infected indirectly, and no evidence of RSV infection, and the amounts of specimens collected from two patients were insufficient to determine whether they were infected. The infecting RSV strains are indicated in Table 1.

G protein polypeptides. RNA was isolated from RSV-infected cell culture supernatants by use of RNAzol (Tel-Test Inc., Friendswood, Tex.) according to the manufacturer’s instructions. The two hypervariable regions of the G gene of RSV, g1, corresponding to aa 60 to 172, and g2, corresponding to aa 193 to the C terminus, were amplified by reverse transcription-PCR. The PCR primers were designed to incorporate a SpeI restriction site at the 5’ end of the coding sequence and a HindIII restriction site at the 3’ end of the coding sequence. The 338-nucleotide product corresponding to the g1 fragment of the RSV G gene was ligated into the expression vector pQE42, and the 292-nucleotide g2 fragment was ligated into the expression vector pQE42 (Qiagen, Valencia, Calif.). These vectors express the respective g1 and g2 coding sequences fused in frame with the coding sequences for the 25-kDa mouse dihydrofolate reductase (DHFR) protein and an N-terminal six-histidine tag. The polyhistidine tag was used to purify the expressed polypeptides by metal chelate chromatography. The cloned DNA was sequenced with a model ABI 377 sequencer (PerkinElmer) to ensure authenticity. The plasmids containing the recombinant DHFR-G polypeptides were expressed in Escherichia coli, and the proteins were purified on Ni-nitrosoacetic acid spin columns (Qiagen) used according to the manufacturer’s instructions.

Antibody assays. We used the Lumines (Austin, Tex.) Multiplex analysis system to test the serum specimens for anti-G g1 and g2 antibodies. Briefly, each polypeptide antigen (250 μg/ml) was covalently linked by a carbodiimide conjugation method as described by the manufacturer (Pierce, Rockford, Ill.) to beads of uniform size (diameter, 5.5 μm), which were colored with different amounts of red and orange fluorescent dyes to allow for discrimination based upon the relative emission intensities at the wavelengths of the two fluorescent dyes. The fluorescence emission of each bead and, therefore, of the specific peptide antigen, was determined with a fluorescence-activated cell scanner (FACSscan). The antibody that reacted against antigen attached to the specific bead was detected by the addition of an antigenspecific antibody labeled with biotin and fluorescein-labeled streptavidin (Molecular Probes, Inc., Eugene, Ore.). For the antibody assays, 2,000 coupled beads of one color (i.e., a specific G polypeptide) were combined with 2,000 beads each of one or more other colors and reacted with diluted serum specimens. The total reaction mixture volume was 100 μl, and the diluent was phosphate-buffered saline (PBS)-TBN (0.02% Tween 20, 0.1% bovine serum albumin [BSA], 0.02% azide). The serum-bead mixture was incubated at 37°C for 2 h, and the beads were washed once with PBS-TBN, resuspended in PBS-TBN, reacted against a biotinylated antibodies for 1 h at 37°C (Accurate Antibodies, Westbury, N.Y.), washed once with PBS-TBN, suspended in PBS-TBN, and reacted against 5 μg of fluorescein-conjugated Alexa-streptavidin (Molecular Probes) for 15 min at room temperature. The bead-seum reaction mixture was diluted 1:1 with PBS-TBN and subjected to two-color FACSscan analysis. We used the mean fluorescence intensity (MFI), the average green fluorescence signal for a bead with a specific red color, as our indicator of antibody reactivity against a specific G polypeptide.

RESULTS

Characteristics of the peptide-specific antibody assays. The DHFR-G polypeptides were effectively linked to the beads as indicated by their reactivities to a monoclonal antibody, RGS-His (Qiagen), that reacts with the histidine tag on the DHFR-G polypeptide fusion proteins. The efficiency of the labeling, however, varied among the G polypeptide-specific beads (Table 1). The average MFI signals for individual G polypeptide for three tests done on three successive days ranged from 1,189 for the beads labeled with the g2 peptide from RSV A2 to 8,853 for the beads labeled with the g1 polypeptide from RSV 10849. The standard deviations (SD) for the three successive tests suggest that these assays give highly reproducible readings; the median SD for the 18 different polypeptide-labeled beads was 6.3% of the MFI (range, 1.4 to 16.1%).
We next tested the ability of these beads to specifically detect RSV antibodies. We used the DHFR-only beads to detect the reactivity of a given serum sample against the DHFR protein or the beads. We used mouse serum raised against uninfected Vero cell lysate and against the CP52 strain of RSV (this strain lacks the G and SH genes) to determine background reactivity with the DHFR-G polypeptide beads. To allow comparison of signals between beads and to account for variation in bead labeling efficiency, we chose to standardize the test serum signal (MFI sample) to the signal for the GA1 genotype strain (CH06) reacted against all three g1 and all three g2 peptides from the GA1 strains, one of one g1 peptide and zero of two g2 peptides from the GA2 strains, and one of four g1 and zero of four g2 peptides from group B strains (Table 2).

The sera raised against different RSV strains had high SF indices against polypeptides from the homologous genotype and varied reactivities to polypeptides from heterologous genotypes. For example, the serum specimen raised against the GA1 genotype strain (CH06) reacted against all three g1 and all three g2 peptides from the GA1 strains, one of one g1 peptide and zero of two g2 peptides from the GA2 strains, and one of four g1 and zero of four g2 peptides from group B strains (Table 2). Mouse sera raised against a strain from the GA6 genotype as well as those raised against a group B strain reacted with similar patterns of specificity (Table 3). The anti-GA6 genotype (CH20) serum failed to react with GA1 peptides and group B peptides but reacted against the g2 peptides from the GA2 genotype (GA2 strains are more closely related to GA6 strains than to GA1 strains). The anti-group B serum failed to react with the GA1 or GA2 peptides but had good SF indices against both g1 and g2 group B peptides.

**Peptide-specific antibody response to a live-attenuated RSV vaccine in children.** The G peptide antibody response in recipients of an RSV vaccine (attenuated strain of RSV A2 [GA1 genotype]) followed a pattern similar to that seen with the RSV-infected mice; that is, the G peptide response was group and, to some extent, genotype specific. After reviewing the distribution of SF index values from human serum specimens, we chose to consider SF indices of <2.5 to be negative, SF indices between 2.5 and <3.0 to be indeterminant, and SF indices of ≥3.0 to be positive. Since a twofold increase in SF index values was always >3 SD above the variation in repetitive tests, we considered a twofold increase between pre- and postvaccination serum specimens to be a significant increase and indicative of a response to the vaccine.

The prevaccine serum specimens that were negative by an RSV neutralization test were also negative for most G peptide antibodies (Table 4); four of these specimens had antibody to one or more group B peptides. In contrast, 7 of the 10 prevaccination serum specimens positive for RSV-neutralizing antibodies had a positive reaction against at least one and often several groups of G polypeptides, usually with different patterns of polypeptide reactivities. Most (12 of 14) of the children who had a neutralizing antibody response to the vaccine also had a response to the G polypeptides. The most frequent

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**TABLE 2. Antibody responses (in the sera of RSV CH06 [genotype GA1]-infected mice) against RSV G polypeptides**

| Strain | MFI ± SD (SFI)* | g1 region | g2 region |
|--------|-----------------|-----------|-----------|
| GA1    |                 |           |           |
| A2     | 8,427 ± 1,471 (15.4) | 488 ± 42 (7.0) |           |
| CH34   | 8,948 ± 353 (14.6)   | 3,397 ± 541 (19.3) |           |
| 10849  | 7,454 ± 593 (16.0)   | 2,934 ± 494 (9.3)  |           |
| GA2    |                 |           |           |
| CH25   | 7,846 ± 748 (24.6)   | 74.1 ± 8.9 (0.6)   | 90.4 ± 6.7 (0.6) |
| 2008.1 |                 |           |           |
| GB     |                 |           |           |
| 2334.4 | 259 ± 15 (0.7)      | 132 ± 34 (1.1)    |           |
| 2B     | 157 ± 12 (0.3)      | 286 ± 32 (0.6)    |           |
| CH41   | 556 ± 15 (1.3)      | 193 ± 10.5 (1.1)  |           |
| CH53   | 2,676 ± 44 (6.1)    | 53.4 ± 8.2 (0.7)  |           |
| DHFR control | 224 ± 14.2 |           |           |

* Data are for triplicate samples. SFI, standardized fluorescence index.

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**TABLE 3. Antibody responses against G polypeptides in sera from mice infected with different RSV strains and genotypes**

| RSV G protein polypeptide | No. of samples tested | % of RSV polypeptides yielding positive SFI (mean SFI)* in the sera of mice infected with: |
|--------------------------|-----------------------|------------------------------------------------------------------------------------------|
|                         |                       | CH06 (GA1) | CH20 (GA6) | BI (group B) | CP52 (G deleted) | Uninfected Vero cells |
| g1 GA1                   | 3                     | 100 (15.3) | 0 (1.0)    | 0 (0.8)      | 0 (0.9)        | 0 (0.4)               |
| g2 GA1                   | 3                     | 100 (12)   | 0 (1.0)    | 0 (0.9)      | 0 (1.3)        | 0 (0.8)               |
| g1 GA2                   | 1                     | 100 (18.4) | 0 (2.1)    | 0 (0.6)      | 0 (0.6)        | 0 (0.5)               |
| g2 GA2                   | 2                     | 0 (0.6)    | 100 (23)   | 0 (1.0)      | 0 (1.0)        | 0 (0.5)               |
| g1 GB                    | 4                     | 25 (2.1)   | 0 (0.7)    | 75 (8.7)     | 0 (0.7)        | 0 (0.5)               |
| g2 GB                    | 4                     | 0 (0.9)    | 0 (1.0)    | 100 (7.3)    | 0 (1.0)        | 0 (1.0)               |

* Positive SFI (SF index) was ≥3.0.
response was to the g1 A2 polypeptide (10 of 12 children), followed by the g1 GA1 polypeptides and the g2 A2 polypeptide. One vaccinee had a response to only the g2 A2 polypeptide, and one had a response to only one of the g2 GA1 polypeptides. None of the vaccinees without a neutralizing antibody response had an antibody response to the G polypeptides.

In postvaccination serum specimens, both the rate of positivity and the amount of antibody, as indicated by the SF index, were indicative of a group- and partially genotype-specific response to the vaccine. The SF indices were highest for the A2 polypeptides, second highest for the other GA1 polypeptides, third highest for the GA2 polypeptides, and lowest for the group B polypeptides (Table 4). The percentage of vaccinees who responded to vaccination with a significant increase in polypeptide antibodies also suggested a group- and partially genotype-specific response. Among the 11 children with an RSV A2 polypeptide response, 9 also had a response to one of the other GA1 genotype polypeptides, 4 had a response to GA2 genotype polypeptides, and 2 had a response to group B polypeptides. Only one of the five vaccinees with no antibody response to the RSV A2 polypeptides had a response to another polypeptide. As noted above, one vaccinee had a response to only one of the g2 GA2 polypeptides.

**G polypeptide antibody response in persons naturally infected with RSV.** The G polypeptide response to natural RSV infection in older children and adults also tended to be group specific, but it was not genotype specific. All patients had RSV antibody in their acute-phase serum specimens, and all had antibody against at least one of the G peptides (data not shown). Six of the eight with documented infection had a response to GA1 and GA2 peptides, and one of the four without documented infection had a response to GA1 and GA2 peptides (Table 5). One of the seven patients with GA1 and GA2 peptide responses also had a group B response. Three of the three persons infected after exposure to the GA1 virus had GA1 peptide antibodies in their acute-phase serum specimens, and two of the five persons infected after exposure to the GA5 virus had GA2 antibodies in their acute-phase serum specimens (GA2 strains are more closely related to GA5 strains than to GA1 strains).

### DISCUSSION

The findings in this study demonstrate a group- and partially genotype-specific antibody response to variable regions of the G protein. Our data suggest that people with RSV infections are likely to have an antibody response against one or both of the two variable regions of the G protein of the same genotype or group and most consistently to the internal g1 variable region. Although our data are limited to group A infections, we suspect that the response to group B infections will be similar. In primary infection, the response rate decreases as the genetic distance between the infecting virus and source virus for the test polypeptide increases. With infections in older children

### TABLE 4. Antibody responses against G polypeptides before and after vaccination in the sera of infants who were RSV seropositive (n = 9) or RSV seronegative (n = 8) before vaccination

| RSV G protein polypeptide | No. of polypeptides tested | % of specimens yielding positive SFI against one or more polypeptides (mean SFI)* |
|---------------------------|---------------------------|---------------------------------------------------------------------------------|
|                           |                           | Before vaccination | After vaccination | Before vaccination | After vaccination |
| g1 A2                     | 1                         | 0 (0.3)            | 75 (5.3)           | 11 (1.0)           | 67 (3.4)           |
| g2 A2                     | 1                         | 0 (1.3)            | 63 (5.2)           | 56 (3.1)           | 44 (3.8)           |
| g1 GA1                    | 3                         | 0 (0.5)            | 75 (5.1)           | 0 (1.0)            | 33 (2.7)           |
| g2 GA1                    | 3                         | 0 (0.9)            | 13 (3.0)           | 11 (1.7)           | 22 (2.0)           |
| g1 GA2                    | 1                         | 0 (0.4)            | 13 (1.7)           | 0 (0.9)            | 0 (1.4)            |
| g2 GA2                    | 2                         | 0 (0.7)            | 25 (2.8)           | 22 (1.7)           | 22 (1.6)           |
| g1 group B                | 4                         | 13 (0.7)           | 0 (0.8)            | 11 (1.0)           | 11 (1.0)           |
| g2 group B                | 4                         | 38 (1.6)           | 63 (1.7)           | 44 (1.8)           | 44 (1.7)           |

*Prevaccination seropositivity and seronegativity were determined by a complement-enhanced 60% plaque reduction neutralization assay. Infants were vaccinated with a live-attenuated strain of RSV A2. SFI, standardized fluorescence index.

### TABLE 5. G polypeptide antibody responses in persons exposed to RSV during a nosocomial outbreak (13)

| Patient Infected* | GA1 | GA2 | Group B |
|-------------------|-----|-----|---------|
|                   | g1(3) | g2(3) | g1(3) | g2(3) | g1(3) | g2(3) |
| GA1               |       |       |       |       |       |       |
| 1                 | -     | +     | -     | +     | -     | -     |
| 2                 | -     | -     | -     | -     | -     | -     |
| 3                 | -     | -     | -     | -     | -     | -     |
| 4                 | +     | -     | -     | +     | -     | -     |
| 5                 | +     | +     | -     | +     | -     | -     |
| 6                 | +     | -     | -     | -     | -     | -     |
| 7                 | ND    | -     | -     | -     | -     | -     |
| GA5               |       |       |       |       |       |       |
| 8                 | ND    | -     | -     | -     | -     | -     |
| 9                 | +     | +     | -     | +     | -     | -     |
| 10                | +     | +     | -     | +     | -     | -     |
| 11                | +     | +     | -     | +     | -     | -     |
| 12                | -     | -     | -     | -     | -     | -     |
| 13                | +     | -     | -     | +     | -     | -     |
| 14                | +     | -     | -     | -     | -     | -     |

* A + indicates a >2-fold rise in SFI index between the pre- and postinfection serum specimens against one or more of the indicated polypeptides (e.g., a positive response to one or more of the three g1 polypeptides from GA1 RSV strains).

**A + indicates that the patient had virus isolated or ≥4-fold rise in RSV antibodies between the pre- and postinfection serum specimens. ND, not determined (the volume of the specimen was insufficient to determine if the patient was infected or not).
and adults, our limited data suggest that the response to infection is still group specific but less likely to be genotype or strain specific.

The findings in this study are consistent with those of previous studies of antibody responses to G peptides or G polypeptides. Cane et al. (7) studied the primary antibody response in young children to RSV infection of known genotype against expressed polypeptides comprising the terminal 84 to 85 aa of six RSV genotypes. They found that the response detected against polypeptides from the carboxy-terminal variable region of G was varied (i.e., a response was detected in only ~50% of children) and highest to the polypeptide from the infecting genotype. Cane (3) also studied a series of 12- and 9-mer synthetic peptides from the carboxy-terminal regions of different genotypes of RSV. She found that antibody responses against these peptides after primary infection tend to be genotype, and often, strain specific. Norby et al. (33) tested paired serum specimens from adults and children against a series of 15-mer peptides and found responses only to peptides representing the internal conserved regions. They, however, did not account for genotype differences between the test peptides and infecting strains.

One limitation in using peptides and expressed polypeptides to study the antibody response to the G protein is that these peptides are not glycosylated, and glycosylation has previously been shown to alter antigenicity, as indicated by reactivity against mouse monoclonal antibodies to G protein (15, 34). It seems likely that glycosylation is also important to the human response to some G epitopes during RSV infection. Consequently, it is likely that the polypeptides used in this study detected some but not all genotype-specific antibodies induced by RSV infection in humans. Although further study is needed to clearly determine how good an indicator of genotype-specific immunity these assays are, they do provide a means to further explore this important part of the RSV immune response. Additional expressed polypeptides are being developed so that the known genotypes can be represented in future studies by this assay system.

The Luminex assay proved to be an efficient way to detect genotype-specific antibodies. We were able to test six polypeptides in the same assay with a <15% loss in signal compared with signals in assays using individual polypeptides. Since only 1 μl of serum specimen is needed to test one set of six beads, only a small volume of serum specimens is needed to complete testing. Consequently, this assay system allows us to test multiple specimens against multiple genotypes while conserving specimen, reagents, and personnel resources.

In summary, we have developed an assay that efficiently measures antibodies against the two variable regions of the G protein. This assay can detect a group- and partially genotype-specific response to RSV infection that should provide a new way to study the role of strain differences in RSV disease.

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