Anti–G Protein Antibody Responses to Respiratory Syncytial Virus Infection or Vaccination Are Associated with Inhibition of G Protein CX3C-CX3CR1 Binding and Leukocyte Chemotaxis

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Respiratory syncytial virus (RSV) is an important cause of severe lower respiratory tract illness in infants and the elderly. Presently, no safe and efficacious RSV vaccine exists; however, advances in our understanding of immunity and the pathogenesis of disease associated with RSV infection may lead to new vaccine strategies. RSV G protein contains a CX3C chemokine motif that interacts with the CX3CR1 chemokine receptor and modifies the activities of fractalkine. In the present study, we show that anti–RSV G antibody responses after recent RSV infection or vaccination are associated with inhibition of RSV G protein CX3C-CX3CR1 interaction and RSV G protein–mediated leukocyte chemotaxis.

Subjects, materials, and methods. Paired serum specimens were collected from 6–41-month-old children enrolled in studies of live attenuated RSV vaccines and from adults experimentally infected with wild-type RSV [11]. Children in these studies were classified as RSV seropositive (with serum 60% plaque reduction neutralization titer ≥1:40) or as RSV seronegative (with serum plaque reduction neutralization titers ≤1:40). Serum specimens were stored at −20°C, with Johns Hopkins University institutional review board approval. Group A (n = 4) included 7–20-month-old RSV-seronegative children with very low anti–RSV G antibody titers who received placebo. Group B (n = 4) included 15–41-month-old RSV-seropositive children with relatively high anti–RSV G antibody titers both before and after vaccination who were not infected with the vaccine strain of virus, as determined by the absence of shedding of vaccine virus and the absence of a ≥4-fold rise in RSV antibody titers. Group C (n = 3) included 15–36-month-old RSV-seropositive children with low anti–RSV G antibody titers before vaccination who were infected with 1 of 2 experimental live attenuated RSV A2 vaccines, cpts 248/955 or cpts 530/1009, as determined by recovery of vaccine virus from nasal washes after inoculation. Group D (n = 9) included 6–17-month-old RSV-seronegative children who became naturally infected with wild-type RSV, as determined by isolation of RSV during post-
vaccination surveillance [11]. Group E (n = 5) included seropositive adults experimentally infected with wild-type RSV [11]. To determine whether IgG contributed to inhibition of RSV G protein CX3-CX3CR1 interaction, IgG was depleted from these adult serum specimens (group F; n = 5) using Gammabind G Sepharose (Amersham Biosciences). The depletion process was repeated until the reciprocal titer of anti–RSV G protein IgG antibody was ≤5.1 log2. All anti–RSV G protein antibody titers were determined by ELISA [11].

Potential endogenous chemotactic components in serum specimens were microdialyzed in a 10-well Spectra/Per Micro-dialyzer, using a 100-kDa molecular weight cutoff (MWCO) membrane (Spectrum) against serum-free Dulbecco’s modified Eagle medium (DMEM; Invitrogen) for 24 h at 4°C. Microdialyzed serum specimens had no change in anti–RSV G antibody titer and did not mediate measurable human leukocyte chemotaxis.

RSV G protein was purified from RSV A2–infected Vero E6 cells, as described elsewhere [9]. The purified RSV G protein revealed 2 major bands (90 kDa and 45 kDa) that were detected by Western blotting using anti–RSV G monoclonal antibody (131-2G); no bands were detected by Western blotting using anti–RSV F protein (anti–RSV F) monoclonal antibody (131-2A) [9].

For isolation of leukocytes, heparinized serum specimens from 10 adults were pooled. Leukocytes were isolated by use of density-gradient separation medium (ICN Biomedicals) in accordance with the manufacturer’s instructions, were resuspended in 90% fetal bovine serum (HyClone Laboratories) plus 10% dimethyl sulfoxide (Sigma), and were stored at −70°C until use. Leukocytes recovered from frozen specimens were >95% viable, as determined by trypan blue dye exclusion analysis.

For leukocyte chemotaxis assays, a chemotaxis chamber (NeuroProbe) was used to determine human leukocyte chemotaxis toward 10 nmol/L recombinant human fractalkine (R&D Systems), 10 nmol/L purified RSV G protein, and DMEM, as described elsewhere [9]. For antibody-blocking assays, microdialyzed serum specimens were diluted 1:200 in DMEM and then added to the upper and lower wells of the chemotaxis chamber. The percentage of inhibition of chemotaxis was determined by the following formula: 100 × [(1 − (total number of cells that migrated toward chemoattractant—i.e., RSV G protein or fractalkine—in the presence of serum antibodies))/(total number of cells that migrated toward chemoattractant in the absence of serum antibodies)].

For CX3CR1 binding assays, recombinant fractalkine (R&D Systems) was conjugated to AlexaFluor-488 (Molecular Probes) in accordance with the manufacturer’s instructions. Unreacted AlexaFluor-488 was removed by dialysis (7-kDa MWCO Slide-A-Lyzer; Pierce) against 3 L of PBS for 24 h at 4°C. AlexaFluor-488–fractalkine (488-FKN) was used in binding assays with CX3CR1–transfected 293 cells (CX3CR1.293 [9]). Preliminary binding assays using purified RSV G protein or 488-FKN diluted in fluorescence-activated cell-sorter buffer (Dulbecco’s PBS and 1% bovine serum albumin) containing 10 μg/mL heparin showed that RSV G protein saturated CX3CR1.293 binding at 1000 nmol/L and 488-FKN binding at 47 nmol/L; thus, these concentrations were used in all binding and inhibition assays, as described elsewhere [9]. The percentage of inhibition of RSV G protein or fractalkine binding to CX3CR1 was determined by the following formula: 100 × [(1 − (percentage of specific binding to CX3CR1.293 cells in the absence of serum antibodies))/((percentage of specific binding to CX3CR1.293 cells in the absence of serum antibodies))].

Data on leukocyte chemotaxis and binding inhibition are presented as the median ± SD of 3 assays performed in triplicate. Differences between absolute pre- and postvaccination percentage of inhibition within each group were determined by use of a paired, 2-tailed t test and were considered to be significant if P < .05.

Results. To determine the relationship between anti–RSV G antibody titers and inhibition of RSV G protein binding to CX3CR1, blocking assays were performed using serum specimens from infants and young children (groups A–D) and from adults (groups E and F) as described in Subjects, materials, and methods. The serum specimens from the children who received placebo (group A) had low anti–RSV G antibody titers (figure 1C) and inhibited 25%–30% of RSV G protein binding before administration of placebo, and no significant change in inhibition of RSV G protein binding was observed in postadministration specimens (figure 1A). The observed inhibition of RSV G protein binding by the serum specimens from these RSV-seronegative children may, in part, have been due to maternally derived antibody, or the inhibition may have been nonspecific. The serum specimens from the RSV-seropositive children with high titers of anti–RSV G antibody (group B) inhibited RSV G protein binding to a greater extent than did the serum specimens from the children in group A, suggesting that higher anti–RSV G antibody titers are associated with greater inhibition of RSV G protein binding. Consistent with this hypothesis, the serum specimens from the RSV-seronegative children infected with an experimental RSV vaccine (group C) or naturally infected with RSV (group D) exhibited significant increases in inhibition of RSV G protein binding after vaccination or infection (group C, 9.4% to 27.9% inhibition [P < .001]; group D, 24.4% to 44.1% inhibition [P < .001]). Increased inhibition of RSV G protein binding was also observed after experimental infection of adults with RSV (group E, 27.8% to 34.5% inhibition), but this change was not statistically significant. Negligible inhibition of RSV G protein binding by the IgG-depleted adult serum specimens (group F) suggests that inhibition of RSV G binding to CX3CR1 is primarily mediated by IgG.
Fractalkine binds specifically to CX3CR1 [12]. To determine whether anti–RSV G antibodies inhibit the binding of fractalkine to CX3CR1, similar assays were performed using the serum specimens from groups A–F (figure 1B). As expected, no appreciable inhibitory effects were detected. Because fractalkine has a CX3C site, these results indicate that inhibition of RSV G protein binding by antibodies is specific and is directed at epitopes proximal to the CX3C motif. These results are consistent with our previous findings from assays using anti–RSV G monoclonal antibodies that are reactive to sites proximal to the CX3C site [9].

RSV G protein CX3C-CX3CR1 interaction has been shown to mediate human leukocyte chemotaxis [9]. To determine whether the serum specimens from groups A–F inhibited RSV G protein–mediated leukocyte chemotaxis, blocking assays were performed (figure 2A). The serum specimens from the children receiving placebo (group A) did not inhibit RSV G protein–mediated leukocyte chemotaxis and in some instances enhanced it (as indicated by a negative percentage of inhibition values), suggesting the presence of chemotactic components that were not removed by dialysis. The inability of low-titer serum spec-
imens (group A) to inhibit leukocyte chemotaxis (figure 2A) but partially inhibit RSV G protein binding (figure 1A) suggests that higher anti–RSV G antibody titers may be required to inhibit the RSV G protein signaling threshold. In contrast, high titers of anti–RSV G antibodies in the pre- and postvaccination serum specimens from the RSV-seropositive children in group B inhibited leukocyte chemotaxis, and, similar to what was found in the binding assays (figure 1A), no significant increase in inhibition occurred after vaccination. High titers of anti–RSV G antibodies induced by RSV vaccination (group C) or by RSV infection (group D) were associated with increased inhibition of leukocyte chemotaxis ($P = .008$ and $P < .001$, respectively), although this effect was more pronounced in naturally infected children (figure 2A). The serum specimens from adults experimentally infected with wild-type RSV (group E) showed a modest increase in inhibition of leukocyte chemotaxis (figure 2A). As with inhibition of RSV G protein binding, adsorption of these adult serum specimens abolished inhibition of leukocyte chemotaxis, suggesting that this effect is also mediated by IgG. These results suggest that inhibition of RSV G protein binding (figure 1A) and leukocyte chemotaxis (figure 2A) is associated with increased titers of anti–RSV G antibodies induced by recent RSV infection or vaccination.

To determine whether anti–RSV G antibodies affected fractalkine-mediated leukocyte chemotaxis, similar assays were performed using the serum specimens from groups A–F (figure 2B). These specimens did not substantially inhibit leukocyte chemotaxis and in some cases enhanced it (as indicated by a negative percentage of inhibition values), suggesting that antibody inhibition is specific and is associated with reactivity to epitopes proximal to the CX3C motif [9].

Discussion. Humoral immunity is important in protection against RSV infection; however, it is unclear whether it can also protect against virus-specific immunomodulatory effects that may contribute to disease pathogenesis. Antibodies to RSV are induced in infants and young children after RSV infection or vaccination with live attenuated RSV vaccine candidates [10, 11], and it appears that anti–RSV G antibodies, not anti–RSV F antibodies, may be preferentially induced in the youngest vaccinated infants [13]. Studies in animal models have suggested that RSV G protein affects aspects of immunity and disease pathogenesis [6, 7] and that RSV G protein CX3C-CX3CR1 interaction contributes to enhanced disease associated with formalin-inactivated RSV vaccination [14]. Thus, a cautious approach toward RSV vaccine development should be taken; however, the results of the present study suggest that anti–RSV G antibodies induced by recent RSV infection or vaccination may be important in protection against unwanted immunomodulatory effects that may be associated with RSV G protein CX3C-CX3CR1 interaction. In addition, our results show that anti–RSV G antibodies do not appreciably affect fractalkine-mediated responses; this finding is significant, because fractalkine is important in antiviral immunity, functioning as both a chemoattractant and an adhesion molecule for CX3CR1+ leukocytes, which include cytotoxic T cells and NK cells [15].

The present study demonstrated that inhibition of RSV G protein CX3C-CX3CR1 interaction was mediated by serum specimens from all but the placebo group. The serum specimens from the RSV-seronegative children infected with wild-type RSV (group D) exhibited the greatest inhibition of RSV G protein–mediated binding and leukocyte chemotaxis, suggesting that anti–RSV antibodies associated with recent infection may be directed to epitopes that are proximal to the CX3C site in the RSV G protein and that more effectively block CX3C-CX3CR1 interaction [9]. One implication of these results is that humoral immunity to RSV infection or vaccination may protect against immunomodulatory effects associated with RSV G protein.

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References


