Respiratory syncytial virus (RSV) is recognized as the most important cause of serious lower respiratory tract illness in infants and young children worldwide (17). Most children are infected with RSV by age two, and repeated infections can occur throughout life, with serious complications most often occurring in elderly patients and patients with compromised cardiac, immune, or pulmonary systems (7, 8, 10, 13, 38). RSV infection in infants and young children is often associated with bronchiolitis and an increase in respiratory rates; apnea can also occur (3). In one study of 274 infants under 6 months of age, 56 infants (20.4%) had apnea with RSV infection (3). The mechanisms by which RSV infection causes apnea are not understood. One potential mechanism for RSV-associated alteration in respiratory rates is induction of pulmonary substance P (SP) and G glycoprotein-CX3CR1 interaction, an effect that is inhibited by treatment with anti-G glycoprotein, anti-SP, or anti-CX3CR1 monoclonal antibodies. These data suggest new approaches for treating some aspects of RSV disease.

RSV infection in the neonate can alter respiratory rates, i.e., lead to episodes of apnea. We show that RSV G glycoprotein reduces respiratory rates associated with the induction of substance P (SP) and G glycoprotein-CX3CR1 interaction, an effect that is inhibited by treatment with anti-G glycoprotein, anti-SP, or anti-CX3CR1 monoclonal antibodies. These data suggest new approaches for treating some aspects of RSV disease.

*Corresponding author. Mailing address: Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333, and Department of Pediatrics, National Jewish Medical and Research Center, Denver, Colorado 80206.

Received 31 October 2002/Accepted 6 March 2003

The G Glycoprotein of Respiratory Syncytial Virus Depresses Respiratory Rates through the CX3C Motif and Substance P

Ralph A. Tripp,1* Azzeddine Daklama,2 Les P. Jones,1 Albert Barskey,1 Erwin W. Gelfand,2 and Larry J. Anderson1

Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333,1 and Department of Pediatrics, National Jewish Medical and Research Center, Denver, Colorado 802062

Copyright © 2003, American Society for Microbiology. All Rights Reserved.
then averaged for each group, and the mean values were expressed for each treatment. Statistical significance with a P value of <0.05 was determined by analysis of variance with the Bonferroni correction for multiple comparisons of the means. Baseline respiratory rates (350 to 370 breaths/min) did not differ significantly among any of the groups examined. Purification of RSV G glycoproteins was carried out as previously described (44). Western blot analysis of the G glycoprotein preparations with anti-G glycoprotein monoclonal antibody (131-2G) yielded two distinct bands at approximately 90 and 45 kDa, and no detectable bands were revealed with anti-F glycoprotein monoclonal antibody (131-2A). Treatment with 10 nM G glycoprotein or phosphate-buffered saline (PBS) had no significant effect on respiratory rates; however, treatment with 100 or 1,000 nM G glycoprotein dramatically reduced respiratory rates beginning at 0.5 h postinjection, suggesting a dose-dependent effect on respiratory rates. The peak reduction in respiratory rate occurred at 1 h postinjection, but some reduction in respiratory rate was observed through the 4-h-postinjection period. The respiratory rates in mice treated with 100 nM G glycoprotein returned to baseline at 2 h posttreatment, i.e., respiratory rates of PBS-treated mice, but were reduced below the baseline rate at 3 and 4 h postinjection. Experiments examining the duration of this effect through 12 h postinjection showed that respiratory rates returned to baseline by 6 h postinjection. Mice treated i.v. with 100 nM purified, endotoxin-free uninfected Vero cell lysate (VCL) isolated in a similar manner as F and G glycoproteins showed no decrease in respiratory rate (Fig. 1B). Purification of RSV F glycoprotein was carried out as previously described (20). Western blot analysis of the purified F glycoprotein detected by anti-F glycoprotein monoclonal antibody (131-2A) yielded a distinct band at approximately 70 kDa and no distinct bands detected by anti-G glycoprotein monoclonal antibody (131-2G). These results indicate that RSV G glycoprotein rapidly (within 1 h) decreases respiratory rates in mice.

To better understand the mechanisms associated with G glycoprotein depression of respiratory rates, mice were intraperitoneally (i.p.) administered 10 μg of anti-SP (clone NC1/34; PharMingen, San Diego, Calif.) or anti-G glycoprotein (clone131-2G) or anti-CX3CR1 (clone 2A9-1; MBL, Nagoya, Japan) monoclonal antibodies diluted in PBS prior to treatment with 100 nM G glycoprotein (Fig. 2). Eight mice were examined for each treatment. Neither PBS nor 10 μg of anti-F glycoprotein monoclonal antibody altered the G glycoprotein-associated reduction in respiratory rates (Fig. 2A). In contrast, treatment with anti-G glycoprotein (Fig. 2A), anti-SP (Fig. 2B), or anti-CX3CR1 monoclonal antibodies (Fig. 2C) abolished G glycoprotein-associated reduction in respiratory rates. A study of SP levels in cell-free bronchoalveolar lavage (BAL) specimens from the monoclonal antibody-treated mice supports the role of SP in G glycoprotein-mediated reduction in
respiratory rates. SP levels in cell-free BAL fluid were analyzed by a competitive enzyme-linked immunoassay kit per the manufacturer’s instructions (Cayman Chemical, Ann Arbor, Mich.) as previously described (46). The assay is based on the competition between free SP and an SP tracer for a limited number of SP-specific binding sites. SP levels were examined prior to treatment and between 1 and 2 h posttreatment. The baseline level of SP in BAL specimens from naïve or PBS-treated naïve mice ranged from 200 to 350 pg/ml. SP levels posttreatment with 10 nM G glycoprotein increased slightly and ranged from 300 to 750 pg/ml; however, SP levels dramatically increased to 2,200 to 2,800 pg/ml in mice treated with 100 nM G glycoprotein. In contrast, SP levels were considerably reduced in 100 nM G glycoprotein-treated mice pretreated with anti-SP (250 to 500 pg/ml), anti-G glycoprotein (400 to 800 pg/ml), or anti-CX3CR1 (250 to 800 pg/ml). These results indicate that G glycoprotein treatment induces SP through CX3CR1 and that SP expression contributes to lower respiratory rates.

To determine if the G glycoprotein-associated reduction in respiratory rates was linked to the CX3C motif in G glycoprotein, we examined the respiratory rates in mice given (i.v.) 100 nM FKN (R&D Systems, Minneapolis, Minn.) or 100 nM RSV G glycoproteins with mutations at the CX3C motif, i.e., a deletion in the CX3C motif (GDCYS) or an Ala insertion in the CX3C motif (G-CX4C) (Fig. 3). Purified G glycoprotein mutants, i.e., GDCYS and G-CX4C, were prepared from Vero cells stably transfected with plasmid DNA encoding the G glycoprotein mutants under G418 selection as previously described (44). As hypothesized, FKN-treated mice had reduced respiratory rates beginning at 2.0 h posttreatment that lasted through 4 h posttreatment.
posttreatment. BALB/c mice were determined at time points between 0.25 and 4 h. The mean respiratory rates have been shown elsewhere to express CX3CR1 and SP receptors (14, 24, 26), and G glycoprotein has been shown previously to be involved in the CX3C motif in the G glycoprotein results in less SP expression of FKN may occur through induction of SP.

The link established by the data in this study among G glycoprotein binding to CX3CR1, induction of SP, and subsequent depressed respiratory rates and the previously established link between SP and increased pulmonary inflammation (46) support the concept that the G glycoprotein CX3C motif is likely important to some aspects of RSV disease and suggest new approaches for preventing and treating RSV disease. The apparent dose-dependent effect of G glycoprotein on respiratory rates suggests that attenuated RSV vaccine candidates would be less likely to alter respiratory rates through this mechanism. Structural modifications to the G glycoprotein CX3C motif to prevent binding to CX3CR1 may improve the safety of live and/or subunit RSV vaccines. In addition, administration of antibodies, drugs, or agents that inhibit the interaction between G glycoprotein and CX3CR1 or the actions of SP may be beneficial in treating some aspects of RSV disease.

R. A. Tripp and A. Dakhama contributed equally to the work from their respective laboratories.
This research was supported in part by grants from the National Institutes of Health (HL-60015 and HL-36577) and by Environmental Protection Agency grant R825702 to E.W.G.

We thank Annette Ballhorn (National Jewish Medical and Research Center, Denver, Colo.) for her technical assistance.

REFERENCES


