Relationship of Serum Antibody to Risk of Respiratory Syncytial Virus Infection in Elderly Adults

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The relationship of serum antibody to respiratory syncytial virus (RSV) and risk of RSV infection was prospectively evaluated in frail elderly persons. Baseline blood samples from 22 subjects who developed symptomatic RSV infection during the 26-month study and from 22 control subjects who did not become infected with RSV were compared. The mean serum IgG titer to RSV fusion protein was significantly lower in the RSV-infected group than in the controls (15.4 ± 1.6 vs. 16.4 ± 1.8, P = .05), as were the neutralizing titers to group A RSV (12.4 ± 2.2 vs. 14.2 ± 2.2, P = .008) and group B virus (9.1 ± 2.1, vs. 10.3 ± 1.5, P = .01). These results suggest that older adults with low titers of serum neutralizing antibody may be at greater risk of developing symptomatic RSV infection than those who have high antibody titers.

Respiratory syncytial virus (RSV), which was once thought to be a significant pathogen only in young children, is now appreciated as a serious problem in elderly adults [1, 2]. Similar to influenza, RSV has been associated with excess morbidity and mortality in persons aged >65 years, particularly those with underlying heart and lung disease [3]. The reasons for severe illness in older persons are not completely understood but may be related to a declining immune system and the presence of chronic medical conditions. Protective immunity against RSV infection is complex, and the importance of serum antibody is controversial [4]. However, animal and human data suggest that serum neutralizing antibody to RSV may modify disease severity and play a role in prevention of reinfection [5]. Few data are available on the immune status in response to RSV in the older adult. The purpose of this study was to examine the relationship of serum antibody to RSV and the risk of RSV infection in frail elderly persons.

Materials and Methods

Subjects. Volunteers were recruited from three adult day care centers in Rochester. The centers provide social and medical services to frail elderly persons who are eligible for nursing home admission by New York Medicaid standards. Participants attend the centers for an average of 3 days per week. Enrollment was ongoing throughout the study period.

Study design. Serum for immunologic assays was obtained from volunteers on enrollment. Subjects were evaluated for any acute respiratory illness that occurred during the following 26-month study (February 1992 to April 1994). When a respiratory illness was identified by the day care staff, a nasopharyngeal sample was obtained for viral culture, and a convalescent blood sample was taken 4 weeks later. During the 2 years of the study, 28 RSV illnesses were identified by either positive culture or serology. Six subjects who had a single illness associated with a significant rise in antibody to RSV and to another viral pathogen were excluded from analysis since the timing of their RSV illness was uncertain. Twenty-two subjects were selected as a control group and were matched by age, sex, and the availability of a baseline serum drawn at approximately the same time as the subjects with RSV. To maximize the likelihood that controls were exposed to RSV, they were also selected on the basis of attendance at the same center during the same 2-week period. Controls also included subjects who had an illness evaluated during that period and whose convalescent sera did not show a 4-fold rise in RSV titers. This reduced the possibility that controls could have had undetected or asymptomatic RSV infection. Pre-illness serum IgG to specific RSV proteins (by EIA) and serum RSV neutralizing activity were compared between groups.

Cultures. Nasopharyngeal secretions for viral culture were inoculated onto HEp-2 cell cultures within 4 h of collection, incubated at 35°C, and examined for cytopathic effect daily for 10 days. All terminal cultures were examined for RSV by an IFA using a mixture of RSV-specific monoclonal antibodies. RSV isolates were typed as group A or B using group-specific monoclonal antibodies to the attachment (G) protein.

EIA. Serum IgG to the purified fusion (F) and attachment proteins of group A (Gₐ) and group B (G₈) RSV was determined by EIA, using standard methods [6]. Two-fold dilutions (1:800–1:102,400) of serum were incubated at 37°C overnight on 96-well plates coated with either F or G antigens or bicarbonate buffer as a control. Bound IgG was detected by alkaline phosphatase conjugated goat anti–human IgG, followed by substrate. The IgG titer was defined as the highest dilution with an OD ≥0.10 and a...
value at least twice that of the bicarbonate plate. For the serologic
diagnosis of RSV infection, baseline and convalescent sera were
screened at a single dilution of 1:3000. If the convalescent-to-
baseline optical density ratio was ≥2 for any antigen, then all
dilutions were performed. Serologic evidence of RSV infection
was defined as a ≥4-fold rise in IgG to any RSV antigen.

Neutralization assay. Serum neutralizing activity was deter-
mimed by a modified microneutralization method as described by
Andersen et al. [7]. Long strain virus was used for group A neutral-
ization (MNA) and 18537 strain for group B (MNB) assays. Fifty
microliters of 2-fold dilutions (1:50–1:6400) of each serum in
MEM was incubated in microtiter plates with ~100 pfu of virus
and 1:10 dilution of rabbit complement in 50 µL. After 30 min,
100 µL of HEp-2 cells (10^3/mL) was added, and plates were incu-
bated at 37°C. After 3 days, the plates were acetone-fixed and
neutralization titer was defined as the highest dilution that
resulted in a 50% reduction in color compared with control
wells containing virus without serum.

Data analysis. Proportions were compared using χ² with
Yates’s correction factor. Comparisons of mean log₂ titers were
done using t test, and correlations were determined using linear
regression.

Results

A total of 244 subjects was enrolled in the study and experi-
enced 265 respiratory infections during the 26 months of study.
Nine men and 13 women had a respiratory illness with RSV
documented as the sole pathogen. Of the 22 illnesses, 9 were
culture-positive and 13 were culture-negative and demonstrated a
≥4-fold rise in RSV-specific IgG as measured by EIA. Of the
9 culture-positive subjects, 8 had convalescent sera avail-
able and all showed a ≥4-fold rise in RSV antibody by EIA.
The 22 control subjects had paired sera from illnesses during
the same time period that were negative for RSV infection by
the screening EIA assay. The mean number of days in surveil-
 lance was not significantly different between the RSV and the
control groups (543 vs. 527).

Nine (41%) of 22 illnesses could be typed by RSV group;
4 RSV isolates were group A and 5 were group B. The 2
positive cultures from year 1 were group B isolates, and both
group A and B viruses were identified during the second season.
Of the 5 group B–infected patients, 3 had ≥4-fold responses
to GA and 4 had GB responses. Similarly, of the 4 group A–infected
subjects, 4 had a ≥4-fold response to GA and 2 had
significant rises in titers to GB. Thus, it is not possible to
differentiate group A and B infection by serologic response
alone in RSV reinfections.

Fifty-five percent of both the RSV-infected and control
groups were women; the mean age for both cohorts was 78 ±
9 years. Baseline blood samples were collected for all subjects
135 ± 122 (mean ± SD) days prior to the illness (range, 0–
383), and there was no significant difference between the RSV-
infected and control groups (123 ± 127 vs. 148 ± 119).

<table>
<thead>
<tr>
<th>RSV-infected (n)</th>
<th>Controls (n)</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>EIA-F</td>
<td>15.4 ± 1.6</td>
<td>16.4 ± 1.8</td>
</tr>
<tr>
<td>EIA-G_{α}</td>
<td>12.1 ± 1.3</td>
<td>12.4 ± 1.2</td>
</tr>
<tr>
<td>EIA-G_{β}</td>
<td>14.3 ± 1.5</td>
<td>14.9 ± 1.5</td>
</tr>
<tr>
<td>MNA</td>
<td>12.4 ± 2.2</td>
<td>14.2 ± 2.2</td>
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<tr>
<td>MNB</td>
<td>9.1 ± 2.1</td>
<td>10.3 ± 1.5</td>
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NOTE. EIA-F were used to measure serum IgG to purified fusion (F) and
attachment proteins of groups A (G_{α}) and B (G_{β}). MNA and MNB, group A
and B neutralization assays, respectively.

EIA and RSV serum neutralizing titers for each group are
shown in table 1. The mean baseline EIA-F titer of the RSV-
infected group was 2-fold lower than that of the control group
(15.4 ± 1.6 vs. 16.4 ± 1.8, P = .05), whereas the EIA-G_{α} and
G_{β} titers were similar for the 2 cohorts. The mean MNA and
MNB titers were also significantly lower in the RSV-infected
group than in the controls. The MNA and MNB titers of all
44 subjects correlated well (r = .67, P < .001), as did the
MNA and EIA-F titers (r = .54, P < .001). There was a weaker
but still significant correlation between MNB and EIA-F titers
(r = .33, P = .03). In addition, MNA correlated with EIA-G_{α}
titers (r = .33, P = .03), and MNB titers correlated with EIA-
G_{β} titers (r = .42, P = .004). As expected, there was no
correlation of G_{α} and G_{β} titers with the neutralizing titers of
the opposite group. Although there was substantial overlap of
serum neutralizing titers between the 2 groups, there was
segregation at the lowest and highest titers for both MNA and
MNB (figure 1). At the low end of the MNA spectrum (titer
≤10.64), 6 (86%) of 7 became infected with RSV, while at the
high end (≥15.64), only 2 (18%) of 11 became infected
(P = .02, χ²).

The neutralizing and EIA titers of the RSV culture–positive
persons and seropositive-only persons were analyzed separately
to determine if there was a difference between these groups.
Both the mean MNA and MNB titers of the 9 culture-positive
subjects were lower than the titers of the 13 persons with
serologically diagnosed infections (MNA: 11.4 ± 2.2 vs. 13.1
± 2.1; MNB: 8.6 ± 2.4 vs. 9.8 ± 1.5); however, these
differences were not significant.

Discussion

The results of this study demonstrate that the mean baseline
neutralizing titers to RSV were lower in subjects who subse-
sequently experienced symptomatic RSV infection than in
matched controls who had non-RSV illnesses during the same
time period. Despite the small number of RSV illnesses evalu-
ated, there was a statistically significant difference in the mean
EIA-F and neutralization titers of the infected group compared
with titers in the uninfected group. These findings suggest that elderly adults with low serum neutralizing titers to RSV may be more likely to develop symptomatic RSV infection than elderly persons with higher titers.

Although controversial, current data suggest that circulating neutralizing antibody to RSV is beneficial. Passive transfer of neutralizing monoclonal antibodies to the F and G proteins protects rodents against RSV challenge, as does immunization with these glycoproteins [8]. Glezen et al., in a study of infants, demonstrated that neutralizing titers in cord sera correlated inversely with risk of hospitalization due to RSV infection [9]. In a subsequent study, the same investigators showed that the risk of reinfection with RSV was inversely related to the level of neutralizing antibody [10]. In a study of experimental rein-

Figure 1. Distribution of individual neutralization titers for group A (MNA) and group B (MNB) virus. Titers are expressed as log2.

A. MNA

B. MNB
Infection of young adult volunteers, Hall et al. also showed that antibody titers directly correlated with protection [11]. In addition, recent reports of the benefits of IgG prophylaxis for RSV infections in high-risk children suggest that serum antibody may ameliorate severe infection [12].

Despite repeated studies, correlates of immunity remain elusive in children. Even less is known about the immune status to RSV in the elderly adult except that all adults have detectable serum RSV antibody, and older persons are capable of mounting an IgG response to infection [13, 14]. In a previous study of nursing home patients, we found no relationship between serum neutralization titers and risk of infection, although a trend toward low neutralization titers and more severe disease was noted [13]. However, that study was hampered by the fact that sera were obtained after illness was identified, and antibody levels may have already begun to rise. In the current study, the blood samples were obtained well in advance of the illnesses and provide a more accurate representation of baseline immunity.

The ideal method for assessing the protective effect of serum antibody in RSV reinfection would be a challenge study to insure equal exposure to the virus in all subjects. This approach is obviously unacceptable in frail elderly persons. Therefore, we attempted to minimize the effect of variable exposures by choosing a relatively small, closed population. One possible limitation of the study was that over half the infections were diagnosed serologically and that a potential bias towards low titers in that group existed. However, when culture-positive persons were compared with seropositive-only subjects, the latter group was found to have higher baseline neutralization and EIA titers. It is possible that lower titers of RSV antibodies in the culture-positive group may have permitted higher titers of virus in their nasopharyngeal secretions and thus were more likely to be detected by culture.

RSV antibody levels may wane in some older persons due to immunosenescence and the lack of recent exposure and infection. The results of the current study suggest that low serum neutralizing antibody may predispose elderly adults to RSV infections and support the view that boosting antibody levels by vaccination may be beneficial. Larger, prospective studies will be required to confirm these preliminary findings.

Acknowledgments

We thank Maria Formica for technical assistance, Mary Criddle for assistance with specimen collection, and Joanne Prives for manuscript transcription. We also thank the participants and staff of the Independent Living for Seniors for their help in conducting the study.

References