A Simple and Reproducible Method for Collecting Nasal Secretions in Frail Elderly Adults, for Measurement of Virus-Specific IgA

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The standard method for collection of respiratory secretions, by use of a nasal wash (NW) to measure virus-specific IgA, is problematic in frail elderly adults. Therefore, a simplified collection approach using a nasal swab (NS) is described. NW and NS samples were collected from healthy young and frail elderly adults, and IgA titers to respiratory syncytial virus (RSV) fusion and attachment glycoproteins were determined by enzyme immunoassay. Correlation between IgA titers in NW and NS was excellent for each of the antigens (correlation coefficients, .71–.93). In addition, NS results were reproducible when frail elderly subjects were sampled several weeks apart and were nearly equivalent to results from NW samples. The ability to sample nasal secretions by use of an NS when an NW is not technically feasible will facilitate the study of mucosal immunity to RSV as well as the study of mucosal response to candidate RSV vaccines in frail elderly populations.

Evaluation of immunity to many viral pathogens often involves measurement of virus-specific IgA in mucosal secretions. IgA at the mucosal surface is considered a primary defense against initiation of infection by viruses that enter via the mucosal surfaces, such as influenza virus, respiratory syncytial virus (RSV), and other respiratory viruses [1–3]. Several investigators have demonstrated an inverse relationship between infection rates in adults after experimental challenge and the level of preexisting RSV-specific nasal antibody [4, 5]. Although primarily considered a pediatric problem, RSV has recently been recognized as an important cause of serious respiratory tract infection in the elderly, including those with underlying cardiopulmonary disease and residents of nursing homes [6–8].

Measurement of RSV-specific IgA in nasal secretions from infants, children, and young adults has been reported by use of a nasal wash (NW) technique [4, 9, 10]. In adults, the nares are rinsed with 10–20 mL of saline solution while the subject expels the fluid into a collection device. An alternative method for collection of nasal secretions uses tampons made from gauze, which are inserted into the nasal cavity and left in place for up to 2 h [11]. Although the results are satisfactory, these methods are cumbersome and generally require full cooperation from the subject. Repeated washes may also be necessary because of erratic recovery of samples.

Frail older subjects pose unique problems in collection of nasal secretions since they may have difficulty cooperating with the NW procedure because of cognitive deficits, and the procedure may present some hazard to those with tenuous cardiopulmonary status. To facilitate the investigation of mucosal immunity to RSV infection in the frail elderly, a more practical method for collection of nasal specimens is desirable. This paper describes a simple, efficient, and reproducible method for the collection of nasal secretions for determination of virus-specific IgA in frail adults.

Methods

Subjects. During February 1998, 16 healthy adult volunteers, ages 21–50 years, were recruited from the staff of the Rochester General Hospital. In May, 15 frail elderly volunteers, ages 66–96 years (mean 81), were enrolled from an adult day care center that has participated in ongoing epidemiology studies of respiratory illness [12]. Frail elderly subjects were chosen if they could cooperate with the NW procedure. Informed consent was obtained from all volunteers.

Collection of nasal secretions. NW samples were obtained from the left naris of all subjects by slowly instilling 5 mL of sterile phosphate-buffered saline (PBS; pH = 7.4) and collecting the expelled fluid in a plastic petri dish. The recovered volume varied from 1.5 to 4.0 mL (30%–80% by volume). The opposite naris was then sampled with a sterile cotton-tipped wooden swab that was premoistened with sterile PBS. The swab was placed ~2 cm into the anterior nares and rolled across the mucosa for 5 s with moderately constant pressure. None of the samples was visibly tinged with blood to suggest excessive trauma. The swab was then placed in 2 mL of PBS and vigorously shaken and wrung out. The wash and swab samples were immediately centrifuged to remove cellular debris, and the supernatant was frozen at −70°C until assayed for total protein and RSV-specific IgA. A second pair of samples was obtained from the frail elderly subjects 3 weeks after the first. None
of the subjects reported any symptoms of a respiratory illness between sampling.

**RSV-specific IgA enzyme immunoassay (EIA).** An EIA to detect IgA specific for the envelope glycoproteins of RSV was developed by use of a method similar to an assay for the detection of IgG in serum [7]. The fusion protein (F) from the Long strain of RSV (group A virus) and the attachment proteins (Gα and Gβ) from Long and CH18537 strains (group B virus) were purified by affinity chromatography according to published methods and were coated onto microELISA plates (Immulon 1; Dynex, Chantilly, VA) [13, 14]. Samples were mixed with an equal volume of 2× sample buffer (PBS; 0.6% tween–20, 0.02 M EDTA), serially diluted in 1× sample buffer and incubated in duplicate overnight at 30°C in antigen-coated wells. Bound IgA was detected by use of alkaline phosphatase-conjugated goat anti-human IgA antiserum (α-chain–specific; BioSource International, Camarillo, CA) for 3 h at 30°C, followed by substrate (phosphatase 104; Sigma, Deisenhofen, Germany). The end-point titer was defined as the sample dilution with an optical density (OD) >0.10 on the antigen plate and at least twice the background reading on an antigen-free control plate. Each assay included a standard NW sample to control for intra-assay variability. Total protein in samples was determined by use of a microBCA (bicinchoninic acid) kit (Pierce, Rockford, IL). The log₂-transformed IgA titer for each sample was adjusted to a total protein of 100 μg/mL. For statistical analysis, samples with no detectable IgA were assigned a value of 0.5 μg/mL before adjustment.

Antigen specificity of the EIA was confirmed by a blocking assay. The standard control NW or nasal swab (NS) specimen was incubated at a 1:4 dilution with serial 2-fold dilutions of soluble F, Gα, or Gβ in the antigen-coated wells, followed by development as described above.

**Statistical analysis.** Mean IgA titers from the NWs were compared with those from the NSs, by use of the t test, and the correlation coefficient between paired sets of samples was determined. Titers from samples collected at the 2 time points were similarly analyzed to assess reproducibility of the assay over time.

**Results**

**Antigen specificity of the IgA assay.** After the assay working conditions were determined, we demonstrated antigen specificity of the IgA EIA by using a blocking assay. At a concentration of ~8 μg/mL of soluble antigen, binding of IgA to solid-phase F protein was significantly blocked by soluble F (87% blocking) but not by Gβ (7%), binding to solid-phase Gα was blocked by soluble Gα (78%) but not by F (11%), and blocking of solid-phase Gβ was seen with soluble Gβ (68%) but not F (0%).

**Comparability of RSV-specific IgA titers in nasal secretions obtained by NW and NS.** The IgA titer to F, Gα, and Gβ in NW and NS specimens for 16 younger subjects is shown in figure 1 (panels A–C). The IgA titer in the sample was corrected to the approximate mean total protein for the group (100 μg/mL). The mean total protein was 138 μg/mL for the NW and 150 μg/mL for the NS. The mean log₂ ± standard deviation of IgA titers (for the NS and NW, respectively) were 1.70 ± 1.56 and 1.81 ± 1.40 for F, 1.31 ± 0.75 and 1.52 ± 0.64 for Gα, and 1.58 ± 0.87 and 1.66 ± 0.70 for Gβ. None were statistically different. The correlation coefficients for F, Gα, and Gβ IgA titers between NS and NW samples were .93, .85, and .81, respectively. Overall, for all 3 antigens, the titers were within a 2-fold dilution in 41 of 48 paired tests, and there was no consistent trend for higher or lower titers when either collection method was used. Although unadjusted IgA titers also had reasonable correlation coefficients, for each antigen the correlation coefficient improved after correction for total protein (data not shown).

Since there was reasonable correlation between the NS and NW in young subjects, we collected NS and NW samples from 15 frail elderly persons. The correlation coefficients between the paired NW and NS samples were slightly lower than those seen in the younger group, ranging from 0.75 to 0.79 (figure 2, panels A–C). Overall, 32 of 45 paired IgA determinations were within a 2-fold dilution. Although the absolute titers appear higher in the elderly group than in the young group, the results are not directly comparable since the same standard sample was not used for the 2 different groups.

To determine whether RSV-specific IgA titers were reproducible over time, a second set of NW and NS samples were obtained from the elderly subjects 3 weeks after the first samples were collected. The correlation coefficients between IgA titers in the paired NS samples were 0.85–0.86 and in the paired NW samples were 0.71–0.75 (figure 3). In general, mean IgA titers were similar at both time points, a time frame that did not include the RSV season, although the mean IgA titers to Gβ were statistically lower in the second NS samples (5.53 vs. 4.79; P < .001, t test). Of the 45 paired NS determinations, 32 of 45 were within a 2-fold dilution, compared with 29 of 45 for the paired NW specimens.

One NW and 7 NS samples had no detectable IgA titers and contained low total protein (<50 μg/mL). Adjustment of these 8 titers for total protein concentration could result in incorrect results. Therefore, the data were reanalyzed after exclusion of the pairs that included these samples (indicated by the dashed lines in figure 3). After elimination, the correlation coefficient between the first and second NS samples increased from .85 to .96 for F and was unchanged for Gβ.

**Discussion**

Resistance to reinfection by RSV and other respiratory viruses has been directly correlated to the level of virus-specific IgA in nasal secretions. Traditionally, nasal secretions are collected by NW, a technique that is readily performed in infants [9]. Although a modified procedure for obtaining NWs can be done in adults, even in the frail elderly, it is technically difficult and requires significant cooperation by the subject to obtain satisfactory specimens. Alternative methods such as insertion of a nasal tampon for 2 h may be effective but are not practical.
Figure 1. Correlation of IgA titers to respiratory syncytial virus antigens in nasal wash (NW) and nasal swab (NS) samples from younger adults. IgA titers in NS and NW samples from a single time point, adjusted to a total protein of 100 µg/mL are plotted for each subject. Results for F antigen (A), Gα (B), and Gβ (C) are shown.
Figure 2. Correlation of IgA titers to respiratory syncytial virus antigens in nasal wash (NW) and nasal swab (NS) samples from frail elderly adults. IgA titers in NS and NW samples from a single time point, adjusted to a total protein of 100 µg/mL are plotted for each subject. Results for F antigen (A), Gα (B), and Gβ (C) are shown.
In contrast, the NS method is simple, quick, and well tolerated in frail elderly subjects. Side effects other than minor nasal irritation are negligible. The procedure can easily and reliably be performed by study personnel with minimal training and, most importantly, requires little coordination or cooperation from the subject.

We found that virus-specific IgA titers were similar regardless of the collection technique. In addition, titers were equally consistent by either method when measured 3 weeks apart in the frail elderly. Adjustment of the IgA titer for total protein in both NS and NW samples was found to optimize correlation between samples obtained by either method. However, adjust-
ment for total protein in those samples without detectable IgA may result in erroneous data, especially if the adjustment is large because of very low protein content. Therefore, samples with low protein (i.e., <50 μg/mL) in which virus-specific IgA is not measurable should probably be considered inadequate and disregarded. By use of this criteria, 13% of the paired NS samples were not able to be evaluated, in contrast to 2% of the NW samples. After elimination of these samples, tight agreement between first and second specimens improved slightly, from 32 (70%) of 45 to 30 (75%), of 39 for the paired NS samples. None of the frail elderly had a 4-fold rise in virus-specific titer between samplings, which is the generally accepted increase attributable to recent infection. The correction of IgA titers to a fixed concentration of either total protein or total IgA has been used by some investigators to minimize the effect of sample dilution of NWs, since adequacy of the wash may be erratic [10, 15]. We too found a wide variation in the total protein concentration in both the NS and NW, but they were similar. Ideally, a measurement of IgA would not require correction, and the issue of the most precise correction method remains to be clearly defined.

Correlation of virus-specific IgA in nasal secretions and protection from infection has been demonstrated in certain situations, but the number of reports are few and are, in general, limited to experimental challenge studies. In addition, the mucosal IgA response to infection with respiratory viruses has rarely been described in elderly populations. Collection of mucosal secretions by NW is the most desirable technique and is most commonly used. However, in noncooperative subjects or in those with respiratory compromise, the NS technique provides a satisfactory alternative method that yields comparable results. The ability to easily sample nasal secretions from frail elderly subjects will facilitate evaluation of mucosal immunity to RSV and possibly other respiratory viruses in this unique, at-risk population. This method of sample collection will also allow repeated measurements to assess variation of nasal IgA titers in subjects over time. Finally, a practical collection method of nasal samples will also enhance the analysis of mucosal immune responses to natural infection and of protective efficacy of candidate vaccines for these viruses in the elderly.

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References