Nasal Secretion Protein Responses in Patients with Wild-Type Adenovirus Disease

DAVID P. MCCORMICK, RICHARD P. WENZEL, JOHN A. DAVIES, AND WALTER E. BEAM, JR.
Virology Division, Naval Medical Field Research Laboratory, Camp Lejeune, North Carolina 28542
Received for publication 9 December 1971

Proteins were studied in nasal secretions obtained from Marine Corps trainees infected with wild adenovirus type 7 both during the acute phase of illness and after recovery. Illness was associated with a marked increase in the concentration of serum proteins in the secretions, and during inflammation there was no apparent barrier to the passage of large molecules (molecular weight 775,000) from the serum into the respiratory passages. At the time of virus isolation, trainees requiring hospitalization had less immunoglobulin A (IgA) in their secretions even though they had greater quantities of immunoglobulin G (P < 0.05) and albumin than trainees followed in the field, whose secretions were also tested at the time of virus isolation. Base-line IgA and protein concentrations were lower (P < 0.05) in hospitalized trainees than in trainees followed prospectively in the field. The results suggest a nonspecific protective function for secretion protein, although we have not excluded the possibility that field study trainees were protected by specific neutralizing antibody present in the nasal secretion.

A protective role for nasal secretion antibody has been shown in experimental respiratory infection with rhinovirus (14), parainfluenza (21), and influenza infections (8). Neutralizing antibodies reside primarily in the 11S immunoglobulin A (IgA) fraction of the secretion proteins (8, 12), although virus-specific antibody can also be detected in the immunoglobulin G (IgG) fractions (1, 2). In addition, a number of other serum proteins enter the nasal passages as a result of the inflammation induced by viral infection (15).

The effect of pre-existing or "base-line" secretion IgA on response to respiratory tract infection has been most extensively studied in experimental influenza and respiratory syncytial virus infections (9, 12, 16). In men infected intranasally with live A2 influenza virus, the severity of clinical illness was inversely related to the quantity of base-line secretion IgA recovered, and men with the greatest quantity of base-line nasal secretion IgA responded with the highest levels of neutralizing secretory IgA after infection. It appeared that even in the absence of specific neutralizing antibodies, large quantities of IgA present in the mucus before infection were associated with protection against respiratory disease caused by influenza virus. By contrast, patients with chronic lung disease have recently been shown to have a decreased concentration of IgA in their sputum, and they respond to acute infections with smaller increases in sputum IgA when compared with controls having minimal chronic lung disease (11).

To ascertain whether the relationship between quantitative secretion IgA and illness held true "in the field" as well as in experimental infections and in patients with chronic lung disease, we studied 17 trainees over a 3-month period and 10 trainees hospitalized with adenovirus disease. The results of our study of the development of serum antibodies and quantitative changes in nasal secretion proteins in relation to clinical illness provide the material for this report.

MATERIALS AND METHODS

Camp Lejeune respiratory virus surveillance. A throat gargoyle and rectal swab for virus isolation were obtained from all trainees hospitalized for acute respiratory disease at Camp Lejeune, and from up to 40 trainees per week who reported to the Camp Geiger dispensary (Camp Lejeune complex). Acute and convalescent blood specimens were collected for serological analysis. Convalescent blood samples were drawn not sooner than 14 days after the acute blood specimens. All trainees were between the ages of 17 and 22.

Virus isolation, complement fixation, and neutralization tests. Virus was isolated from throat gargoyle specimens by using HEK or WI-38 cells, and viruses were identified by neutralization. Throat gargoyle specimens obtained from the trainees studied in the field were passed three times in HEK and WI-38 if no cytopathogenic effect was observed after the first passage. The
methods for virus isolation and identification and for detection of serum complement-fixing antibody to adenovirus type 7 have already been described (18, 20). Serum neutralizing antibodies were detected by using 32 TCID<sub>50</sub> of adenovirus type 7 in microtiter plates with HEp-2 cells. Nasal secretion neutralizing antibody was not studied.

Hospital study. An acute serum, a nasal secretion collection, and a throat gargoyle for virus isolation were obtained on admission from 10 trainees hospitalized for acute respiratory disease at the Naval Hospital, Camp Lejeune. Convalescent sera and secretions were collected 17 to 19 days after the initial specimen.

Prospective field study. A total of 17 Marine trainees were examined once each week during the months of November, December, and January 1970–1971, except for a 3-week period from 13 December to 3 January when the men were on Christmas leave (see Fig. 1). Otherwise, each week a nasal secretion specimen, a blood specimen, and a throat gargoyle for virus isolation were obtained.

Nasal secretions. A 5-ml amount of 0.15 M sodium chloride solution was repeatedly instilled in each nostril and forcibly expelled into a sterile container until a total of 100 ml was collected from both nostrils. Secretions were shaken with glass beads and centrifuged at 2,000 rev/min for 20 min. The fluids were dialyzed for 24 hr against distilled water at 4°C, concentrated 10-fold in 40% polyethylene glycol, lyophilized, and reconstituted in 3.0 ml of sterile phosphate-buffered saline, pH 7.4, with 5,000 U of penicillin and 5,000 μg of streptomycin added to each specimen.

Nasal secretion proteins. IgA, IgG, albumin, and alpha-2-macroglobulin were determined by radial immunodiffusion. Protein standards used were Hyland 75 IgA lot no. 7012M01301, Hyland low-level IgG lot no. 7072M004A1, Hyland albumin lot no. 7102M0031A, and Hyland alpha-2-macroglobulin lot no. 7066M003B1. Plates containing a low level of anti-IgG accurately measured small quantities of secretion IgG, but alpha-2-macroglobulin levels were estimated from an extrapolation of the curve derived from the control values. Nasal secretion total protein was determined on a Technicon Autoanalyzer by a modification of the biuret reaction (7).

Nasal secretion IgA was measured by using the Hyland 75 IgA standard. We recognized that the secretions contained a mixture of 11 and 7S IgA and that 11S IgA diffuses more slowly through the agar than 7S IgA. Thus, total IgA may have been up to threefold higher than that measured.

Nasal secretion hemoglobin was measured qualitatively with Hemastix reagent strips (Miles Laboratories, Elkhart, Ind.).

Statistical methods. Arithmetic mean, standard deviation, Student's t test, and the Mann-Whitney U statistic were calculated by the usual methods. Nine acutely ill hospitalized patients did not tolerate the full 100-ml nasal wash. The protein values measured on the secretions from these men were corrected by an appropriate factor so that they could be compared on the same scale with the 100 ml of saline and secretions obtained from the other trainees.

RESULTS

Epidemiological background of the prospective study. The prospective field study was carried out during the early stages of an adenovirus type 7 epidemic (Fig. 1) (20). Adenovirus type 7 was isolated from all 10 of the hospitalized patients studied and from 6 of the 17 trainees observed prospectively in the field (Tables 1 and 2). Only one trainee in the field study from whom virus was isolated developed a fourfold rise in complement-fixing antibodies to adenovirus, whereas none of the ten hospitalized trainees developed a fourfold rise in adenovirus type 7 antibody. Of the six trainees studied in the field from whom virus was isolated, all had mild symptoms of respiratory disease and all developed a significant inflammatory protein response as determined by an increase in albumin in the nasal secretions. No serum neutralizing antibodies were detected in any of the six men studied in the field from whom virus was isolated, either in acute-phase serum or in serum tested from 4 to 8 weeks after virus isolation.

Quantitative secretion proteins in hospitalized patients. (Table 3). When compared with convalescence, the acute phase of adenovirus disease was associated with a marked increase in serum IgA (molecular weight 160,000), IgG (molecular
weight 153,000), albumin (molecular weight 68,460), and alpha-2-macroglobulin (molecular weight 775,000). During illness, the albumin to IgG ratio was 6.7:1, whereas during convalescence the ratio dropped to 1.7:1, suggesting that in the absence of disease a portion of the IgG present in the secretions was synthesized locally.

The proportion of IgA in the secretions was greater in both the acute and convalescent specimens than would have been expected if it were present in the secretion merely as a transudate. The albumin to IgA ratio in the acute specimens was 7.47:1 and in the convalescent was 2.34:1. In addition, the increment in IgA in the secretions during the acute phase could not be accounted for on the basis of transudation alone. The ratio of albumin to IgA in serum was 16:1, whereas the ratio of the increment in albumin to the increment in IgA during the acute phase of illness was 11:1.

**Relationship of secretion proteins to severity of illness.** In the trainees followed in the field prospectively, “base-line IgA” was defined as the IgA concentration of a specimen chosen specifically for its low albumin concentration to minimize the possibility of contamination by serum proteins, and in the hospitalized patients base-line IgA was defined as the IgA concentration of the convalescent specimen. Base-line IgA concentrations corre-
TABLE 3. Concentrations of albumin, IgG, IgA, and alpha-2-macroglobulin in acute and convalescent nasal secretions obtained from trainees hospitalized with adenovirus disease

<table>
<thead>
<tr>
<th>Protein</th>
<th>Acute (mg/100 ml)</th>
<th>Convalescent (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>522.4 ± 291a</td>
<td>67.3 ± 50.3a</td>
</tr>
<tr>
<td>IgG</td>
<td>77.7 ± 36.0</td>
<td>39.3 ± 21.0a</td>
</tr>
<tr>
<td>IgA</td>
<td>69.9 ± 39.5</td>
<td>28.7 ± 17.3a</td>
</tr>
<tr>
<td>Alpha-2-macroglobulin</td>
<td>36.0 ± 16.3</td>
<td>11.3 ± 2.30a</td>
</tr>
</tbody>
</table>

a Arithmetic mean ± one standard deviation.
b t-test acute versus convalescent: P < 0.05.

related inversely with the severity of illness as measured by a need for hospitalization and the development of serum complement-fixing antibodies. Base-line secretion IgA (P < 0.05) and base-line protein (P < 0.05) were lower in the 10 trainees requiring hospitalization than in the 17 trainees from the field, none of whom required hospitalization, and 6 of whom excreted adenovirus type 7. As a check on the possibility of contamination by a serum transudate, there was no significant difference in nasal secretion albumin in the base-line secretions of the hospitalized versus the field study trainees (P > 0.05).

Mean secretion IgA at the time of virus isolation was lower in hospitalized trainees (69.9 ± 39.5 mg/100 ml) when compared with nonhospitalized trainees (118.3 ± 53.07 mg/100 ml), even though secretion albumin in hospitalized trainees was higher (522.4 ± 291 mg/100 ml) than in nonhospitalized men (336 ± 310 mg/100 ml), but the differences were not statistically significant (P > 0.05). However, the ratio of albumin to IgA at the time of virus isolation was less in the secretions of trainees who shed virus but did not require hospitalization than in the secretions of hospitalized trainees (Table 1). In the hospitalized trainees the ratio approaches the ratio present in serum. By contrast, secretion IgG during acute illness requiring hospitalization was significantly higher (77.7 ± 36 mg/100 ml, P < 0.05) than the IgG concentration in secretions of nonhospitalized trainees (27.16 ± 21.5 mg/100 ml). The difference could be accounted for on the basis of transudation from the serum.

Week-by-week changes in secretion protein concentrations during adenovirus infection in two men are illustrated in Fig. 2. Neither trainee required hospitalization, both excreted virus, and serum complement-fixing antibodies developed only in trainee "Re." Secretion albumin and IgA rose to a peak over a 3-week period and was temporally related to virus isolation. At the time of virus isolation, no virus-specific serum antibodies could be detected. The duration of symptoms correlated well with upper respiratory tract inflammation detected by changes in the secretion protein pattern. The increment in IgA concentration in each case was greater than would have been expected from transudation alone.

In contrast is the pattern of trainee "Gr" (Fig. 3). This man had a relatively high base-line secretion IgA concentration. The duration of symptoms in this trainee was 10 weeks, and virus was isolated on two separate occasions. However, he did not develop a significant increase in secretion albumin until a late stage of illness, and he never developed complement-fixing or neutralizing serum antibodies.

Nasal secretion hemoglobin. Nasal secretions were monitored for hemoglobin throughout the duration of the study, but the presence or absence...
of hemoglobin did not correlate with secretion protein concentrations.

**DISCUSSION**

Secretions collected from normal volunteers on a day-to-day basis have previously been analyzed for protein content, and such information provides a background for a better understanding of the results of this study (17). In general, a given volunteer secreted a relatively constant quantity of IgA on a day-to-day basis, but volunteers differed as much as fivefold in the amount that each secreted. Thus, "hyposecretors" and "hypersecretors" could be identified, and each group tended to maintain approximately the same level of total protein secretion over a 1-month period.

It is important to mention at the outset that we did not study secretion neutralizing antibodies. However, the total quantity of IgA present in secretions may be related to the clinical and immunological response of patients to respiratory virus infection. This has been shown to be true both in experimental influenza virus infection (8) and in experimental respiratory syncytial virus infection (12). Our findings would suggest that, at least with respect to clinical illness, similar mechanisms affect the host response to wild-type adenovirus infection. Trainees requiring hospitalization had lower mean base-line nasal wash IgA and protein levels than those in the prospective field study who had mild symptoms but did not require hospitalization ($P < 0.05$). Despite the fact that hospitalized trainees secreted more albumin and IgG at the time of virus isolation, they secreted less IgA at that time than trainees in the prospective study. We have shown that some, but not all, of the increment in IgA during the acute phase of the disease comes from the serum. We concluded, therefore, that local mechanisms for the maintenance of IgA levels in the secretions were different in the hospitalized trainees, during both the acute and convalescent phases of illness.

It is interesting that nasal secretion IgA increases at or about the time that virus is isolated, and that some of this increment may be due to increased local production or release. In coxsackievirus A-21 and rhinovirus disease, IgA increases even before transudation occurs (4), and in our study secretion IgA in trainee "Yo" doubled even though no serum antibody could be detected up to 4 weeks after virus isolation. Such phenomena would support the theory that preformed IgA resides in a "compartment" separate from the secretion and is released at the first stage of infection. Such IgA stores might be released from glandular type epithelial cells in the respiratory mucosa (3, 10, 19) or from IgA-synthesizing plasma cells in the mucosa, or it may be mobilized from additional sites in the respiratory passages such as the nasal sinuses, which may offer a relatively large additional surface area.

It would be mistaken to assume that molecules of the IgA type play the only role or even the major role in local protection against viral respiratory disease. Studies have shown that only 31 to 73% of the total protein in nasal secretions can be identified as belonging to a specific class of proteins such as IgA, IgG, albumin, or siderophilin (17). Other proteins such as alpha-1-glycoprotein, haptoglobin, orosomucoid, immunoglobulin M, and $\beta$-lipoprotein have also been identified, and a portion of the total protein present remains unidentified. It is possible that one or more non-immunoglobulin viral inhibitors such as interferon (5) assist in the local defense against viral respiratory tract infection. The role of cellular immunity in this context also awaits evaluation.

An unexpected finding was the failure of the men in the prospective field study to develop serum antibodies even though virus was isolated many weeks before the convalescent serum was obtained. A representative case is illustrated in Fig. 3. This anomalous situation may be similar to that noted in studies of secretion antibody induced by local application of inactivated poliovirus vaccine (13). Two of eight children given intranasal trivalent inactivated polio vaccine failed to develop serum antibodies even though specific nasal secretion antibody of the IgA type did develop. It is likely that, given a small enough dose, adenovirus could proliferate in the upper respiratory tract without stimulating the synthesis of serum antibody. Large quantities of heterospecific nasal secretory IgA or other secretion proteins causing nonspecific inhibition might in addition have confined adenovirus proliferation to the upper respiratory tract or inactivated the virus so that systemic antibodies were not formed.

In addition to pre-existing host immunity, the method of virus transmission from man to man might also have been important in determining the severity of disease and the immunological response to infection. It is known that epidemics of viral respiratory disease among military recruits can spread by aerosol in the confined and crowded barracks situation (6), but the factors critical for the spread of disease by aerosol may vary from month to month depending on the indoor relative humidity (which is low in the winter months), the number of men in each barracks, or the stage of training. It is important to remember that the trainees followed prospectively were infected at a different point in the epidemic than the hospitalized trainees (Fig. 1). Although we do not have direct evidence, it is possible that adenovirus aero-
sols present during the peak of the epidemic may have been responsible for the more severe illness which the hospitalized trainees developed.

Our data illustrate again the marked transudation of proteins from serum into the respiratory passages which occurs during acute respiratory disease. Serum proteins readily pass into the nasal passages, large molecules as well as small. It is easy to see how pre-existing serum antibody and complement, even in the absence of local specific antibody, might modify the local inflammatory process during respiratory infection, and such a process provides, at least in part, a rationale for giving parenteral vaccines for respiratory pathogens.

The concept that transudation may play a role in protection against disease has been vividly amplified in a recent study by Woodhour et al. (22). When monovalent A2/Aichi influenza virus vaccine was administered to volunteers intramuscularly with peanut oil adjuvant 65, 100% of recipients developed high titers of serum antibody. Immunization by the intramuscular route with vaccine plus adjuvant resulted in higher serum antibody titers than immunization with the aqueous vaccine either by the intramuscular or intranasal route. Unexpectedly, the vaccine given parenterally with adjuvant also gave a higher titer of nasal antibody in a larger number of recipients than when the vaccine was given by the nasal route. The authors did not determine the antibody class responsible for the neutralizing effect of the nasal secretion, nor did they determine the amount of transudate present in their routine nasal secretion collections. However, their work would suggest that transudation occurs even in the absence of overt clinical respiratory disease, in which case pre-existing serum antibody might play a role in preventing the establishment of a viral upper respiratory infection. This phenomenon would be in addition to any neutralizing effect which serum antibodies might have as upper respiratory symptoms develop and when full-blown transudation is taking place.

Work is presently underway in our laboratory in an attempt to answer some of the questions which this study raised. Was specific neutralizing secretory IgA responsible for protection against severe disease in trainees infected with adenosivirus but not requiring hospitalization? Did the trainees studied in the field produce more secretion IgA and protein because they were healthy and had not recently required hospitalization? Do secretory proteins exert a nonspecific protective effect in other respiratory illnesses such as *Mycoplasma pneumoniae* infection?

**ACKNOWLEDGMENTS**

We are indebted to Carl L. Berling and Raymond S. Combs for excellent technical assistance; to E. P. Smith and J. Russell for performing adenosivirus identification and serum neutralizations; to H. B. Burns for complement fixation tests; to Q. P. Galapon for assisting with statistical analysis; and to J. P. Roberts and R. F. Rhoads, Jr., who made the drawings.

**LITERATURE CITED**


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