Rhinology, 34, 2-8, 1996

Pattern of nasal secretions during experimental influenza virus infection*

William J. Doyle[1], David P. Skoner[2], Marta White[3], Frederick Hayden[4], Allen P. Kaplan[5], Michael A. Kaliner[3], Yoji Shibayama[5], Philip Fireman[2]

[1] Department of Otolaryngology, Children's Hospital of Pittsburgh, University of Pittsburgh School of Medicine, Pittsburgh, USA
[2] Department of Pediatrics, Division of Allergy/Immunology/Rheumatology, Children's Hospital of Pittsburgh, University of Pittsburgh School of Medicine, Pittsburgh, USA
[3] Allergy Diseases Section, Laboratory of Clinical Investigation, National Institutes of Health, Bethesda, USA
[4] Department of International Medicine and Pathology, University of Virginia, Charlottesville, USA
[5] Department of Medicine, State University of New York at Stoney Brook, New York, USA

SUMMARY

To define the pattern of secretion production during influenza virus infection, 28 adult subjects were inoculated with influenza-A virus (H1N1) and cloistered for a period of 8 days. On each day, symptoms associated with virus infection were scored, nasal secretions were collected and nasal lavages were performed. Recovered lavage fluids were submitted for virus culture and assayed for proteins, histamine, and bradykinin. Twenty-one subjects were infected with influenza-A virus and had significant increases in daily secretion weights and symptom scores extending from day 2 to 7, post-inoculation. Plasma-derived proteins in the nasal lavage fluids showed an early increase to peak at day 4 and then decreased. Glandular proteins showed a later increase to peak at day 5. Bradykinin but not histamine was significantly elevated and tracked the changes in the glandular proteins. In contrast, a shallow increase in symptoms confined to day 2 post-inoculation, but no increase in daily secretion weights was documented in the seven uninfected subjects. There, an increase in plasma proteins was observed on days 1 and 2, but no change in glandular proteins was obvious. These results support a biphasic secretory response during influenza-virus infection with transudation dominating the early period and glandular secretions contributing later.

Key words: influenza-A virus, nasal secretions, vascular permeability, glandular secretions

INTRODUCTION

Increased nasal secretion production is a common expression of diseases that provoke nasal mucosal inflammation such as allergic rhinitis and upper respiratory viral infections. Indeed, rhinorrhoea is one of the three nasal symptoms along with congestion and sneezing which are most commonly reported by patients with these diseases and targeted for amelioration by symptomatic therapies (Borum et al., 1981; Konno et al., 1987; Gaffey et al., 1987, 1988). An analysis of the biochemical components of nasal secretions has been used to provide insights into the pathogenic process and the mechanisms of host defense (Remington et al., 1964; Norman et al., 1985; Proud et al., 1992).

In that regard, the source of nasal secretions for different nasal inflammatory diseases has become a focus of study. Earlier studies showed that intranasal challenge with histamine provoked secretions rich in plasma proteins such as IgG and albumin, while challenge with methacholine caused increased concentrations of glandular proteins including sIgA, lactoferrin, and lysozyme (Raphael et al., 1988a, 1989; Meredith et al., 1989). Nasal allergen challenge of sensitized subjects resulted in the rapid accumulation of plasma proteins in the secretions and a secondary, reflex-mediated increase in glandular proteins (Raphael et al., 1988b, 1991). In those studies, the fold increase in the plasma proteins was much greater than that of the glandular proteins suggesting that the volume of provoked secretions was attributable primarily to transudation from the vascular compartment. More recently, nasal lavage fluids from a large cohort of subjects experimentally infected with rhinovirus

* Received for publication August 8, 1994; accepted October 10, 1994

were assayed for plasma and glandular proteins in an effort to define the source of secretions in that disease (Igarashi et al., 1993). The results documented an early increase in the concentrations of plasma proteins which peaked on day 3 after inoculation and a later increase in glandular proteins which peaked on day 5 after inoculation. These results were interpreted as evidencing a biphasic secretory response of the nasal mucosa to viral infection consisting of an early transudative phase associated with increased vascular permeability and a later secretory phase attributed to increased glandular activity.

In contrast to the effects of other respiratory viruses, rhinovirus infection results in minimal cytotoxicity and mucosal disruption (Turner et al., 1982). Most investigators attribute disease expression to secondary effects of the host inflammatory response. Elaboration of bradykinin, mast cell products and cytokines are likely candidates for this role in that they can provoke nasal response, modulate the inflammatory process and activate parasympathetic responses (Naclerio et al., 1988; Proud et al., 1990). Consequently, the temporal pattern of secretion production documented during rhinovirus infection may reflect the sequential elaboration of these and other inflammatory chemicals during the host response. However, this pattern of response may not be generalizable to other respiratory virus infections that result in more significant mucosal pathology. In a recent study, significant nasal signs and symptoms, throat symptoms as well as more generalized symptoms were documented for infected subjects inoculated with influenza-A virus (Doyle et al., 1994). Since infection with influenza virus is associated with marked cytopathology, nasal lavage fluids collected from this cohort of subjects were assayed for glandular and serum proteins and inflammatory mediators. This report describes the temporal pattern of secretion production for influenza virus infection and compares it to that for rhinovirus infection.

MATERIAL AND METHODS

Experimental plan

Adult subjects previously enrolled in a study designed to determine the nasal and otologic effects of experimental influenza-A infection served as the source material for this extension of that protocol (Doyle et al., 1994). Thirty-three (20 males, 13 females) healthy, adult subjects (20–39 years old) were enrolled after providing a written informed consent. The study was approved by the Human Rights Committee at the Children's Hospital of Pittsburgh.

Enrolled subjects were cloistered in individual rooms of a local hotel for an 8-day period (study days 0 through 7). Twenty-four hours after admission to the cloister site (end study day 0), the subjects were intranasally inoculated with 10^7 TCID50 of a safety-tested, clinical isolate of influenza A/Kawasaki (H1N1) supplied by the National Institute of Allergy and Infectious Disease (wild-type lot E-262). On each study day, the subjects were instructed to expel all nasal secretions into preweighed tissues and seal the expended tissues in plastic bags of known weight. The weight of expelled secretion for each 24-h period was calculated by subtraction. Eight specific symptoms (including sneezing, nasal discharge, nasal congestion, malaise, headache, chilliness, sore throat, and cough) were rated daily by the subject on a four-point, 0–3 scale corresponding to none, mild, moderate, or severe. Also, on each day a nasal lavage was performed during the morning hours using methods previously described. Blood was collected from the subjects on study days 0, 3, 4, 6, 7, and 21.

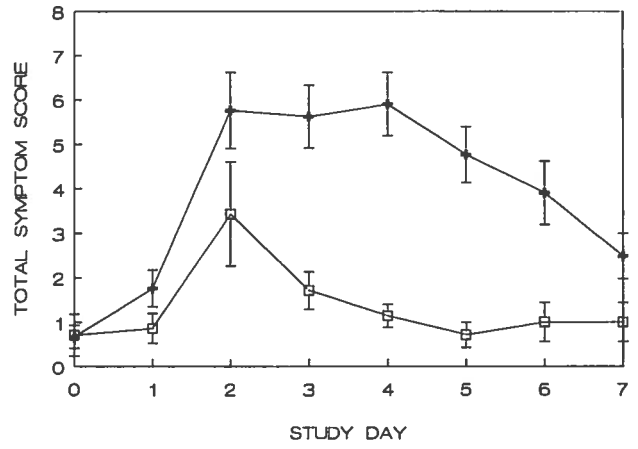
Assessments of infection

At the termination of the 8-day period of study, samples of nasal lavage fluids for every subject on each study day were submitted to the Respiratory Disease Study Unit (University of Virginia) for culture of influenza-A virus in triplicate monolayers of Madin-Darby-Canine-Kidney (MDCK) cells according to previously described methods (Tobita et al., 1975). Paired serum samples for all subjects were submitted to those laboratories for assay of haemagglutination-inhibition (HI) antibodies to influenza A/Kawasaki virus using standard methods (Dowdle et al., 1979). A person was considered to be infected with influenza-A virus if that virus was isolated from the lavage fluids on at least one post-challenge day of cloister or a 4-fold increase in serum antibody titer between pre-inoculation and convalescent samples was documented.

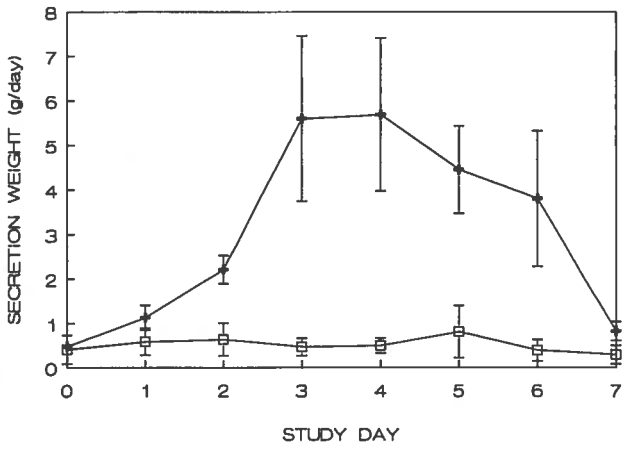
Lavage fluid assays

Total protein concentration was measured by the BCA protein assay (Pierce Chemical Corp.) using bovine serum albumin as a standard (Smith et al., 1985). Albumin was measured by a competitive enzyme-linked immunosorbent assay (ELISA), modified from a previously described method (Raphael et al., 1988). Lactoferrin, IgG and sIgA were measured by a modified non-competitive ELISA as previously described (Igarashi et al., 1993). Lysozyme activity was measured by a turbidimetric assay based upon the enzymatic hydrolysis of bacterial cell walls (Raphael et al., 1989). Histamine was measured by a competitive radioimmunoassay (McBride et al., 1988).

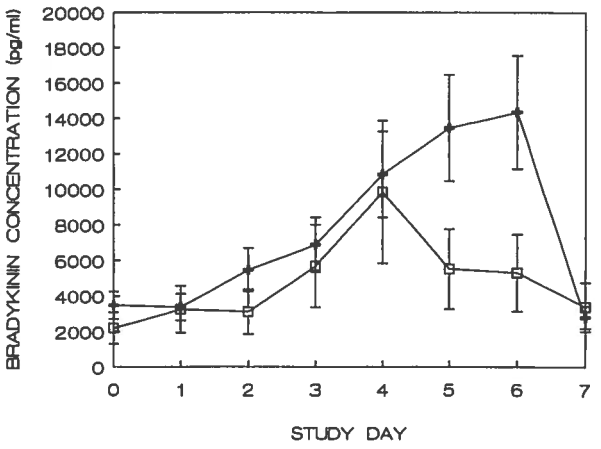
Bradykinin samples were collected in an inhibitor mixture which included 20 mg/ml EDTA, 4 mg/ml polybrene, 10 mg/ml 1,10-phenanthroline, 5 mg/ml SBTI, and 1.67 mg/ml aprotinin. Samples sent as a CO_2 pellet were thawed at room temperature and 600 µl ice-cold ethanol was added to 300 µl nasal wash with continuous stirring. The samples were incubated for 60 min at 4°C. The supernatants were decanted into 3-ml polypropylene tubes and dried by centrifugal evaporator. The residue was resuspended into 300 µl of buffer (0.1 M sodium phosphate, pH 7.4, containing 3 mM 1,10-phenanthroline, 10 mM EDTA, and 0.1% gelatin) and 150 µl 1,1,2-trichloro-1,2,2-trifluoroethane. After centrifugation at 3,000 rpm for 20 min at 4°C, 100 µl of the upper aqueous phase was transferred to a new tube and bradykinin concentration determined by radioimmunoassay (Nishikawa et al., 1992). A correction solution to control for any substance in nasal washes that might affect bradykinin analysis was prepared. Ten microlitres of nasal wash from 60 randomly selected samples were mixed and prepared for assay as described. After resuspending in 300 µl of water (in place of buffer) the suspension was incubated with 20 µl of 2 mg/ml chymotrypsin overnight to digest bradykinin. The sample was



1a



1b



1c

Figure 1. Average values and standard errors of total symptom score (a), secretion weights (b) and urea-adjusted bradykinin concentration (c) for each of the days of cloister in the infected (plus) and uninfected (square) groups.

boiled at 100°C for 10 min to inactivate the enzyme, dried, resuspended, and assayed. The value was subtracted from the samples. Urea was measured employing a spectrophotometric assay based on the enzymatic hydrolysis of urea (Rock et al., 1987; Sigma Diagnostics Kit No. 66-UV, Sigma Chemical Co.). For serum samples, 5-µl samples were incubated with 1 ml reagent. For nasal lavages, 20-µl samples were incubated with 300 µl reagent. After 30 min, the absorbance was read at 340 nm, and urea concentrations in the samples were calculated.

Statistical methods

Because virologic assays were performed after completion of the cloister phase of the study, investigators and participants were blinded to the individual's pre-challenge HI antibody titer as well as the results regarding viral shedding or seroconversion. At the completion of the virologic assays, laboratory specific data were exchanged between the Children's Hospital of Pittsburgh and the University of Virginia. The primary analysis planned was a comparison of the response of the various measures to viral inoculation in infected and uninfected subjects. As described in an earlier report (Doyle et al., 1994), the former group showed a marked differential response to infection associated with the pre-existing HI antibody status. In the present study, the results for two unambiguous subgroups are presented; a high-titer (>16) uninfected group (n=7) and a low-titer (<8) infected group (n=21).

Data for the primary variables related to infection, signs, symptoms and pathophysiologies were previously reported. In this paper, the data for secretion components are presented. The infected and uninfected groups were analyzed separately. For protein concentrations in lavage fluids, the levels at each of the post-inoculation study days were compared to the respective pre-inoculation (day 0) values using the Student's paired t-test evaluated for significance at $\alpha < 0.05$ (one-tailed). In a secondary analysis, the data for protein concentrations were transformed to correct for possible differences in dilution between lavages using methods previously described (Kaulbach et al., 1993). Briefly, the protein concentrations were multiplied by the ratio of urea concentration in the serum to urea concentration in the lavage fluids. The statistical methods described above were then applied to these transformed data. In the presentation, the convention mean±standard error is used throughout.

RESULTS

Measures of illness

Figure 1a shows the daily average values of the total symptom score for the infected and uninfected subgroups. The infected group showed a significant rise in total symptom score by day 1, a period of increased scores lasting from day 2 through day 4 before a gradual decrease to approach baseline values by day 7. An examination of the temporal pattern for individual symptoms showed that the throat and general symptoms peaked earlier (day 2) than the nasal symptoms of sneezing, congestion and rhinorrhoea (day 4). For the uninfected subjects, the total symptom score showed a significant increase on day 2 and a more rapid decrease to baseline values by day 4. The major contributors to this increase in magnitude order were the symptoms of sore throat, headache, congestion and cough.

Figure 1b shows the average daily secretion weights for the two groups. For the uninfected group, no changes in that measure were observed over the course of the study. However, beginning on the day following viral inoculation and extending for

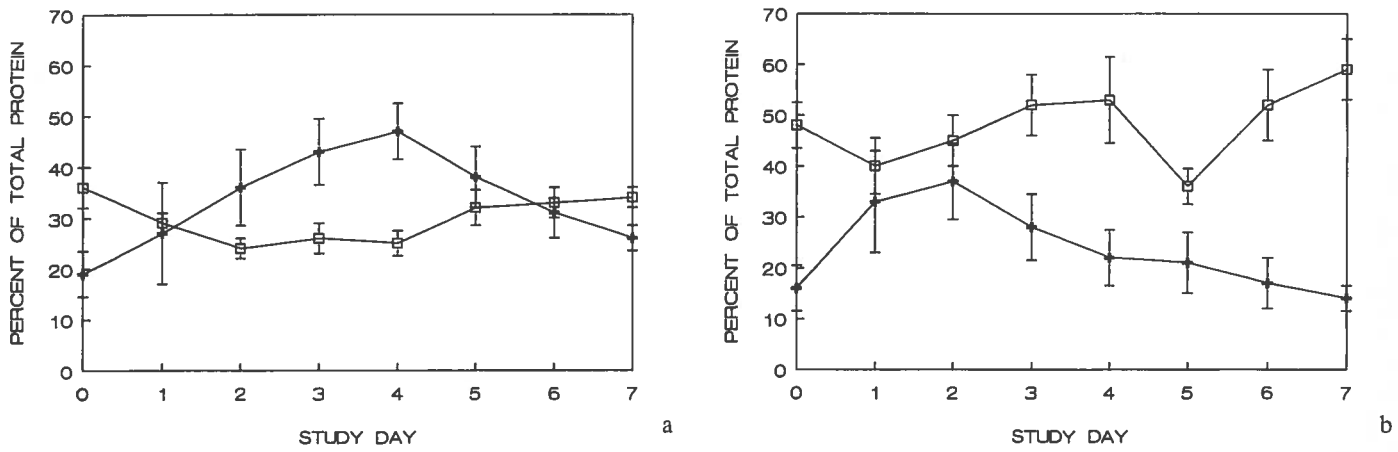


Figure 2. Averages and standard errors of the fraction of total protein represented by the glandular proteins (squares) and plasma proteins (plusses) on each of the days of cloister for the infected (a) and uninfected (b) groups.

6 days, the average daily secretion weights in the infected group were significantly greater than that recorded on study day 0. Secretion weights showed a temporal pattern similar to that of nasal symptoms, peaking on days 3 to 4 and then decreasing to approximate the baseline value by study day 7.

Mediator concentrations

Bradykinin has been used as a marker of inflammation during rhinovirus colds and nasal allergic reactions. Figure 1c shows the average urea-adjusted secretion concentrations of bradykinin in the two groups on all study days. Bradykinin was measurable in the baseline, pre-inoculation lavage fluids of both groups. After inoculation, bradykinin concentration showed a significant progressive increase in the infected group to peak on day 6 post-inoculation, and then a rapid decrease to baseline values by day 7. In contrast, for the uninfected group a transient 3-fold rise in bradykinin concentration peaking on days 3 and 4 was observed. Histamine was measurable in the lavage fluids on all study days. However, no consistent increases were observed in either group following virus inoculation. The range of average lavage concentrations for the different study days was 3.3±1.6 to 13.5±11.0 ng/ml for the uninfected group, and 4.6±1.5 to 11.1±4.4 ng/ml for the infected group. The transformation to correct for differences in dilution did not change these results.

Lavage proteins

The average concentrations of total protein and the glandular and plasma proteins in nasal lavage fluids are presented in Table 1. Total lavage protein in the infected group was significantly increased after viral inoculation to peak on study day 4 and then decrease to baseline values. In the uninfected group, the total protein increased on study days 1 and 2 and then decreased to baseline values. For both groups, the temporal patterns of change in the concentrations of the two plasma proteins assayed, albumin and IgG, tracked the respective patterns observed for total protein. The concentrations in the lavage fluids of the three glandular proteins, lactoferrin, lysozyme and sIgA from the infected subgroup were increased after viral inoculation to peak on study day 5 and then decreased to baseline values. However, the concentrations of these three proteins in the uninfected group did not show a well-defined temporal pattern and were highly variable.

Table 1. Average and standard error of the concentrations (µg/ml) of proteins in the nasal lavage fluids of infected (n=21) and uninfected (n=7) subjects.

study day	albumin	IgG	lactoferrin	lysozyme	sIgA	total protein
infected group:						
0	53±18	17±6	13±4	32±10	4±14	245±49
1	189±130	43±27	11±2	26±6	63±20	445±203
2	206±101	47±20	12±3	22±3	55±14	449±154
3	412±187*	81±34*	18±4	29±6	70±17*	741±253*
4	442±162*	79±29*	20±4*	29±4	92±18*	770±222*
5	226±100*	46±17*	21±4*	43±14	99±30*	511±152*
6	121±49	24±7	18±4	32±10	77±19	350±76*
7	52±8	22±7	14±2	22±2	58±11	280±49
uninfected group:						
0	80±49	29±15	27±10	52±17	166±78	461±157
1	437±298	70±36	30±34	42±10	190±67	881±390
2	412±229	73±25*	39±12	108±61	232±68	887±284
3	170±91	38±13*	26±4	71±35	176±59	538±166
4	101±44	37±16	21±6	54±24	206±90	452±110
5	79±38	21±7	18±4	34±5	80±30	346±78
6	80±44	25±11	24±7	63±25	53±44	435±116
7	43±13	20±6	26±7	61±24	186±49	423±79

* significant $p < 0.05$; paired t-test, one-tailed

In the infected subjects, the increases in the plasma proteins relative to baseline were approximately 4-fold for IgG and 8-fold for albumin, while fold increases of less than 2 were documented for the glandular proteins. These differences are graphically depicted in Figure 2. There, the relative contributions of the glandular and plasma proteins to total protein concentration are shown for the infected (Figure 2a) and uninfected subgroups (Figure 2b). For the infected group, the glandular protein fraction decreased from a baseline value of 0.36 to a value of approximately 0.25 on days 2 through 4 and then increased to 0.34 by study day 7. In contrast, plasma protein fraction

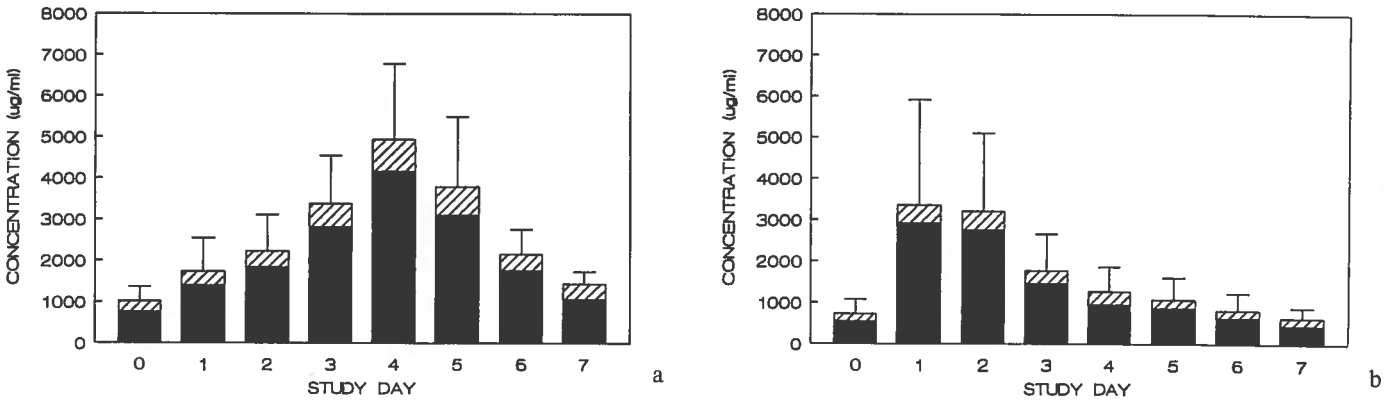


Figure 3. Bar graph showing the averages of the urea adjusted summed concentrations of the two plasma proteins assayed and the standard error of the total for each of the days of cloister in the infected (a) and uninfected (b) groups (solid bars: albumin; striped bars: IgG).

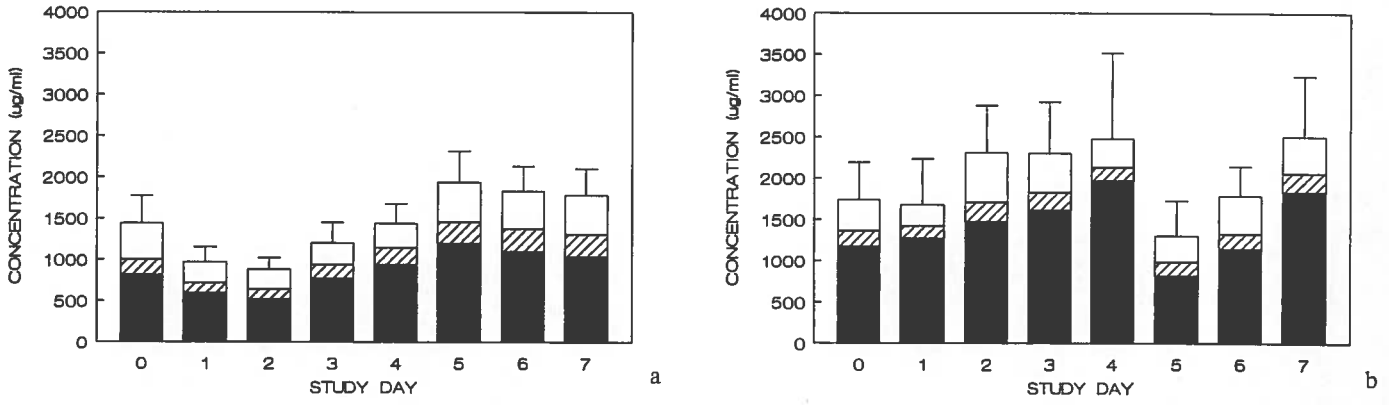


Figure 4. Bar graph showing the averages of the urea-adjusted summed concentrations of the three glandular proteins assayed and the standard error of the total for each of the days of cloister in the infected (a) and uninfected (b) groups (solid bars: IgA; striped bars: lactoferrin; open bars: lysozyme).

increased from a baseline value of 0.19 to peak on day 4 at a value of 0.47 and then decreased over the remainder of the study period. For uninfected subjects, no directional changes in the glandular protein fraction were obvious with average daily values varying about a value of 0.48. In that group, plasma protein fraction increased from 0.16 at baseline to peak at 0.37 on day 2 and then decreased to a value of 0.17 by day 6.

Using the serum/lavage urea ratio, the protein concentrations in nasal lavage fluids were corrected for possible differences in dilution. The adjusted concentrations of the plasma and glandular proteins for each study day are shown in Figures 3 and 4, respectively. The temporal patterns for these protein concentrations are similar to those discussed above for the unadjusted values. Specifically, plasma protein concentrations in the lavage fluids of infected subjects (Figure 3a) increased to peak on day 4 and then decreased, while those in the uninfected subjects (Figure 3b) were increased on days 1 and 2 and then decreased to baseline. The concentrations of the glandular proteins in the lavage fluids of the infected group (Figure 4a) were greatest on days 5, 6 and 7, but the increases with respect to baseline were not significant. Glandular protein concentrations of uninfected subjects (Figure 4b) were variable and showed no clear temporal pattern of change.

DISCUSSION

In this study, adult subjects experimentally infected with influenza-A virus developed early throat and general symptoms followed in time by signs and symptoms of nasal inflammation. The latter included sneezing, nasal congestion, and significant nasal secretion production as documented objectively by increased daily secretion weights and subjectively by increased symptoms of rhinorrhoea. The temporal patterning of protein concentrations in the secretions was consistent with a biphasic secretory response to influenza-A virus infection characterized by an early period of transudation and a later period of active glandular secretion. Moreover, these results suggest that for the symptomatic period of illness, the total volume of secretions was primarily of plasma origin, with a minimal glandular contribution. This pattern of secretion production is similar to that reported in an early study of a small number of subjects experimentally infected with Coxsackie A-21 virus or rhinovirus type-15 (Butler et al., 1979), and for a large cohort of subjects experimentally infected with rhinovirus 39 (Igarashi et al., 1993). The primary difference in the secretory pattern between these viruses is the timing of the onset of glandular secretory activity with rhinovirus provoking an earlier response, influenza virus an intermediate, and Coxsackie-A virus a late response. These data suggest that this pattern of secretion production is a generalized response to viruses that infect the nasal mucosa.

These results have implications for the treatment of rhinorrhoea during a respiratory virus infection. Specifically, most therapies have focussed on the suppression of glandular secretions using agents with pronounced anticholinergic activities. However, the results of this and earlier studies show that the major source of secretion during the symptomatic period of viral illness is transudation secondary to increased vascular permeability. An increase in glandular secretions was observed only during the convalescent period and after the peak increases in the objective measure of secretion weight and subjective measure of rhinorrhoea symptom score. Thus, anticholinergic compounds would be expected to exert their effect late in the disease process and at a time of limited illness. Indeed, a re-examination of the data for secretion weights from two previous treatment trials with topical anticholinergic compounds shows a suppressive effect of the active medication primarily confined to the later period of a rhinovirus cold (Gaffey et al., 1987, 1988). Recognizing the importance of transudation in secretion production, Persson (1990) suggested alternative approaches to the treatment of rhinorrhoea that target endothelial cells of the post-capillary venules and thereby prevent gap formation and the attendant increase in vascular permeability.

However, the advisability of suppressing the exudative, transudative and glandular contributions to nasal secretions during viral infections has been questioned. Butler et al. (1979) suggested that rhinorrhoea may be an adaptive response facilitating delivery of host-defense components to the mucosal surface and thereby limiting viral spread. Igarashi and colleagues (1993) suggested that the vascular leakage contributes to the resolution of infection by washing out newly formed viruses from the mucosal surface. After reviewing the recent literature, Persson (1990) concluded that one source of secretions, plasma exudation, is an important first line of mucosal defense against viral and bacterial infections. The results of the present study for uninfected subjects are interpretable as evidencing an acute virally-mediated inflammation with consequent exudation of plasma proteins and subsequent suppression of the viral infection. Specifically, in seven subjects with elevated pre-inoculation HI antibody titers, early symptoms associated with headache and sore throat were reported two days after virus inoculation despite the lack of objective evidence of viral infection. The expression of these symptoms was limited in time and temporally associated with increased concentrations of the plasma proteins, IgG and albumin in nasal lavage fluids. In contrast to the exaggerated responses of the low-titer infected subjects, the prompt resolution of the symptoms and lack of significant increases in secretion weight or rhinorrhoea symptoms suggest that this possible defense mechanism is self-limiting when effective.

The significance of the late rise in glandular proteins in the secretions accompanying a viral infection is not known. For the infected subjects in the present study, the glandular proteins including sIgA peaked on day 5 post-inoculation. In a previous study of subjects infected with rhinovirus 39, sIgA also peaked on day 5 while lysozyme and lactoferrin concentrations peaked one day earlier (Igarashi et al., 1993). Butler et al. (1979) reported peaks of IgA on day 8 for rhinovirus 15 and a biphasic increase with peaks on day 6 and 14 for Coxsackie A-21 infected subjects. In all three studies, the increase in glandular proteins occurred after the peak period of symptoms and signs of illness. Thus, Butler et al. (1979) suggested that the increase in these proteins is associated with the synthesis of specific anti-viral antibodies, while Igarashi et al. (1993) suggested that the antimicrobial activities of these proteins may protect from secondary infections and aid in the healing of the mucosa. This issue was not specifically addressed by the design of the present study. Future studies incorporating assays of virus-specific antibodies and bacterial colonization are planned to test these hypotheses.

Of considerable interest is the biochemical agent responsible for initiating the generalized transudative process accompanying a viral infection of the nasal mucosa. In that regard, a number of bioactive chemicals have been shown to be capable of increasing vascular permeability following topical application to the nasal mucosa (Baumgarten et al., 1986; Konno et al., 1987; Doyle et al., 1990; Proud et al., 1992). These include bradykinin, histamine, prostaglandin D_2, leukotrienes, and platelet-activating factor. Moreover, many of these substances when administered to the nasal mucosa provoke signs, symptoms and pathophysiologies characteristic of respiratory viral infections (Doyle et al., 1990). However, with the exception of bradykinin, none of these substance have been detected in increased concentrations in nasal lavage fluids of patients with respiratory virus infections (Naclerio et al., 1988). In the present study, a significant increase in the secretion concentration of bradykinin but not histamine was reported for subjects with confirmed influenza virus infection. Because the increase in secretion bradykinin concentration was a relatively late event, a causative role of bradykinin in initiating the transudative process or in provoking symptoms is not supported by these data. The possibility that increased bradykinin concentration is a marker of nasal inflammation rather than an active agent in disease expression was raised previously by Proud et al. (1990), who noted the parallel increases in bradykinin and albumin for subjects challenged with allergens or infected with rhinovirus. These observations suggest that bradykinin concentration is increased in the nasal secretions by local activation of kinin-generating enzymes acting on plasma-derived substrates supplied in association with other plasma proteins by transudation.

If the substrate for enzymatic conversion to bradykinin is supplied by transudation, the concentration of that mediator would be expected to reflect the degree of increased vascular permeability. However, that same process would decrease by dilution with the transudate the concentration of locally synthesized or released inflammatory substances. The lavage method for recovery of secretions further dilutes locally synthesized chemicals. In this study, estimates for the lavage fluid dilution factor from paired measurement of plasma and lavage urea concentrations averaged between 10- and 20-fold on different days. These dilution factors may explain the apparent absence during viral infections of highly potent initiators of

increased vascular permeability such as histamine, prostaglandin D_2 and leukotrienes. In light of these limitations to identifying the presence of locally produced chemicals by assay of lavage fluids, the use of specific, therapeutic probes may hold the most promise for elucidating the biochemical cascade responsible for initiating the inflammatory process during respiratory virus infections.

ACKNOWLEDGEMENTS

The authors would like to thank the nursing staff of the General Clinical Research Center at the Children's Hospital of Pittsburgh for assistance with phlebotomies, and Carolyn Crump, James T. Seroky and Ernest Tanner for assistance with the technical aspects of the study.

Supported in part by a grant from the National Institutes of Health (AI 19262), and General Clinical Research Centers Grant MO/RR 00084.

REFERENCES

1. Baumgarten CR, Nichols RC, Naclerio RM, Proud D (1986) Concentrations of glandular kallikrein in human secretions increase during experimentally induced allergic rhinitis. J Immunology 137: 1323–1328.
2. Borum P, Olsen L Winther B, Mygind N (1981) Ipratroprium nasal spray. A new treatment for rhinorrhea in the common cold. Am Rev Respir Dis 123: 418–420.
3. Butler WT, Waldmann TA, Rossen RD, Douglas RG, Couch RB (1979) Changes in IgA and IgG concentration in nasal secretions prior to the appearance of antibody during viral respiratory infection in man. J Immunol 105: 584–591.
4. Dowdle WR, Kendal AP, Noble GR (1979) Influenza viruses. In: EH Lennette, NJ Schmidt (Eds.) Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections, 5th Edition. American Public Health Association, Washington, pp. 585–609.
5. Doyle WJ, Boehm S, Skoner DP (1990) Physiologic responses to intranasal dose-response challenges with histamine, methacholine, bradykinin and prostaglandin in adult volunteers with and without nasal allergy. J Allergy Clin Immunol 86: 924–935.
6. Doyle WJ, Skoner DP, Hayden F, Buchman CA, Seroky JT, Fireman P (1994) Nasal and otologic effects of experimental influenza A virus infection. Ann Otol Rhinol Laryngol 103: 59–69.
7. Gaffey MJ, Gwaltney JM, Dressler WE, Sorrentino JV, Hayden FG (1987) Intranasally administered atropine methonitrate treatment of experimental rhinovirus colds. Am Rev Respir Dis 135: 241–244.
8. Gaffey MJ, Hayden FG, Boyd JC, Gwaltney JM (1988) Ipratropium bromide treatment of experimental rhinovirus infections. Antimicrob Agents Chemother 32: 1644–1647.
9. Igarashi Y, Skoner DP, Doyle WJ, White MV, Fireman P, Kaliner MA (1993) Nasal secretions during experimental rhinovirus upper respiratory infection: Comparison of non-allergic and allergic rhinitis subjects. J Allergy Clin Immunol 92: 722–731.
10. Kaulbach HC, White MV, Igarashi Y, Hahn BK and MA Kaliner (1993) Estimation of nasal epithelial lining fluid using urea as a marker. J Allergy Clin Immunol 92: 457–465.
11. Konno A, Terada N, Okamoto Y, Togawa K (1987) The role of chemical mediators and mucosal hyperreactivity in nasal hypersecretion in nasal allergy. J Allergy Clin Immunol 79: 620–626.
12. McBride P, Bradley D, Kaliner M (1988) Evaluation of a radioimmunoassay for histamine measurement in biologic fluids. J Allergy Clin Immunol 82: 638–645.
13. Meredith SD, Raphael GD, Baraniuk JN, Banks SM, Kaliner MA (1989) The pathophysiology of rhinitis. III. The control of IgG secretion. J Allergy Clin Immunol 84: 920–930.
14. Naclerio RM, Proud D, Lichtenstein LM, Kagey-Sobotka A, Hendley JO, Sorrentino J, Gwaltney JM (1988) Kinins are generated during experimental rhinovirus colds. J Infect Dis 157: 133–142.
15. Nishikawa K, Reddigari SR, Silverberg M, Kuna PB, Yago H, Nagaki Y, Toyomaki Y, Suehiro S, Kaplan AP (1992) Effect of neurotropin on the activation of the plasma kallikrein-kinin system. Biochem Pharmacol 43: 1361–1369.
16. Norman PS, Naclerio RM, Creticos PS, Togias A and Lichtenstein LM (1985) Mediator release after allergic and physical challenges. Int Arch Allergy Appl Immunol 77: 57–63.
17. Persson CGA (1990) Plasma exudation in tracheobronchial and nasal airways: A mucosal defense mechanism becomes pathogenic in asthma and rhinitis. Eur Respir J 3: 652–672.
18. Proud D, Naclerio RM, Gwaltney JM, Hendley JO (1990) Kinins are generated in nasal secretions during natural rhinovirus colds. J Infect Dis 161: 120–123.
19. Proud D, Bailey GS, Naclerio RM, Reynolds CJ, Cruz AA, Eggleston PA, Lichtenstein LM, Togias AG (1992) Tptase and histamine as markers to evaluate mast cell activation during the response to nasal challenge with allergen, cold, dry air and hyperosmolar solutions. J Allergy Clin Immunol 89: 1098–1110.
20. Raphael GD, Druce HM, Baraniuk JN, Kaliner MA (1988) Pathophysiology of rhinitis. I. Assessment of the sources of protein in methacholine induced nasal secretions. Am Rev Respir Dis 138: 413–420.
21. Raphael GD, Meredith SD, Baraniuk JN, Druce HM, Banks SM, Kaliner MA (1989a) The pathophysiology of rhinitis. II. Assessment of the sources of protein in histamine induced nasal secretions. Am Rev Respir Dis 139: 791–800.
22. Raphael GD, Jeney EV, Baraniuk JN, Kim J, Meredith SD, Kaliner MA (1989b) Pathophysiology of rhinitis. Lactoferrin and lysozyme in nasal secretions. J Clin Invest 84: 1528–1535.
23. Raphael GD, Igarashi Y, White MV, MA Kaliner (1991) The pathophysiology of rhinitis. V. Sources of protein in allergen induced nasal secretions. J Allergy Clin Immunol 88: 33–42.
24. Remington JS, Vostri KL, Lietze A, Zimmerman AL (1964) Serum proteins and antibody activity in human nasal secretions. J Clin Invest 43: 1613–1624.
25. Rock RC, Walker WG, Jennings CD (1987) Nitrogen metabolites and renal function. In: NW Dietz (Ed). Fundamentals of Clinical Chemistry. WB Saunders, Philadelphia, pp. 669–704.
26. Tobita K, Sugiura A, Enomoto C, Furuyama M (1975) Plaque assay and primary isolation of influenza A viruses in an established line of canine kidney cells (MDCK) in the presence of trypsin. Med Microbiol Immunol (Berlin) 162: 9–14.
27. Turner RB, Hendley JO, Gwaltney JM (1982) Shedding of infected ciliated epithelial cells in rhinovirus colds. J Infect Dis 145: 849–853.

William J. Doyle, PhD
Dept. of Pediatric Otolaryngology
One Children's Place
3705 Fifth Avenue at DeSoto Street
Pittsburgh, PA 15213-2583
USA