Oral prednisone therapy in experimental rhinovirus infections

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This study was designed to test the effects of oral steroid therapy on the kinin levels and symptoms of experimental rhinoviral colds. Forty-seven men were randomized to receive prednisone (20 mg) or placebo. Therapy was administered three times a day for 5 days, after one dose was given 11 hours before inoculation with rhinovirus. Viral titers, symptom scores, and kinin and albumin concentrations in nasal washes were monitored. The mean kinin levels were lower in the steroid group (287 vs 449 pg/ml, $p = 0.005$) with significant differences in kinin levels on days 3 and 4 ($p < 0.01$). No significant difference in total symptom scores was seen between the two groups. Except for increased sneezing ($p < 0.01$) and mucus weights ($p < 0.05$) on day 1 in patients treated with prednisone, there were no significant differences in individual symptom scores. Headache tended to be less prominent in steroid recipients. Mean viral titers were higher in the steroid group (1.13 vs 0.79, $p = 0.03$) with significant differences in the daily viral titers on days 3 ($p < 0.05$) and 4 ($p < 0.01$). Steroids reduced kinin levels in rhinoviral infections, but that reduction was not associated with a significant reduction in symptoms. This study also provides evidence for the enhancement of viral growth in steroid recipients. (J ALLERGY CLIN IMMUNOL 1996;97:1009-14.)

Key words: *Prednisone, corticosteroids, rhinovirus, kinins, viral titers*

The pathogenesis of rhinovirus colds is poorly understood. Although rhinovirus is cytolytic in human embryonic lung cell culture, attempts to demonstrate cellular destruction in nasal biopsy specimens[1] or in nasal organ culture[2] have proven unsuccessful. There is a demonstrable rise in mucosal polymorphonuclear neutrophils during naturally occurring colds,[3] and patients with experimentally induced colds show increased levels of kinins in their nasal secretions.[4] Thus the symptoms commonly associated with colds including rhinorrhea, sore throat, and nasal obstruction may be the result of a host inflammatory response, rather than the viral infection itself.

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Received for publication May 5, 1995; accepted for publication June 12, 1995.

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0091-6749/96 $5.00 + 0 **1/1/67508**

Steroids are known antiinflammatory agents with pleiotropic effects. They can inhibit production or activity of IL-1, IL-2, tumor necrosis factor, kinins, arachidonic acid derivatives, and other cytokines. In addition, steroids can decrease plasma levels of eosinophils, basophils, and monocytes and reduce vascular permeability.[5] Steroids have been shown to be effective for treatment of allergic rhinitis in which the major symptomatic mediator is histamine.[6] In rhinovirus infections, increased nasal histamine levels have not been measured,[4, 7] although antihistamines do reduce sneezing and rhinorhea in rhinovirus colds.[8] The contribution of other inflammatory mediators in rhinovirus infection has yet to be fully elucidated. Because of the effect of steroids on a wide variety of inflammatory mediators, a trial determining the effectiveness of steroids in rhinoviral infections seems warranted.

It has been suggested that kinins may be involved in the pathogenesis of rhinovirus infections. High levels of bradykinin and lysyl bradykinin have been found in the nasal secretions of individuals with experimental rhinovirus infections[4] and in those with natural rhinovirus colds.[7] Naclerio et al.[4] described a correlation between kinin levels

and cold symptom scores, and Proud et al.[9] demonstrated that kinins applied to nasal mucosa induce symptoms of nasal obstruction, rhinorrhea, and sore throat.

Steroids have been shown to inhibit kinin formation from kininogens[10] and to reduce the level of kinins in individuals experiencing allergic reactions.[11] A recent study has shown that prophylactic treatment with a combination of systemic and intranasal steroids delayed kinin generation and had a modest effect on the symptoms associated with experimental rhinovirus colds.[12] However, the study did not determine whether the results were due to systemic or topical therapy or the combination of the two. This double-blind, randomized, placebo-controlled study was designed to test the effects of oral steroid therapy alone on kinin production and symptoms during experimental rhinovirus colds.

METHODS

Volunteers

Young men (aged 18 years and older) with reciprocal serum neutralizing antibody titers to the challenge virus of less than or equal to 2 were recruited from the Charlottesville/University of Virginia community. Volunteers were excluded from the study if they had a history of asthma, sinusitis, chronic rhinitis, or allergies or if they had symptoms of an upper respiratory tract infection or fever within 1 week before the study. Subjects gave written consent to participate, and the protocol was approved by the Human Investigation Committee of the University of Virginia.

Infection of volunteers

Viral challenges were performed as previously described[4, 13] with an inoculum of rhinovirus type 39, a clinical isolate passed twice in WI-38 human embryonic lung fibroblasts (HEM Research, Rockville, Md.). The volunteers were isolated at the time of inoculation and housed in separate hotel rooms for the course of the study. The volunteers were each inoculated intranasally on two occasions 10 to 20 minutes apart with 0.25 ml drop per nostril at 9:00 AM on day 0. Each subject received a total inoculum of approximately 300 tissue culture infective dose.

Medication

Crushed 20 mg prednisone tablets and a placebo were prepared and packaged in identical opaque capsules by the University of Virginia Hospital Pharmacy to maintain double-blind conditions. Patients were randomly assigned to receive either the prednisone or the placebo. Patients began treatment at 10:00 PM, 11 hours before viral inoculation. Capsules containing 20 mg of prednisone or placebo were then administered three times a day (at 8:00 AM, 2:00 PM, and 8:00 PM) for 5 days (days 0 to 4). All treatments were directly observed by a study nurse to ensure compliance.

Surveillance of illness

Patients were interviewed on the morning of day 0 before viral challenge and on the mornings of days 1 to 5. They were asked to describe the severity of eight symptoms including sneezing, nasal discharge, nasal obstruction, sore throat, cough, headache, malaise, and chilliness. Symptoms were rated on a scale of 0 (no symptoms) to 4 (very severe symptoms). A modified version[14] of the criteria of Jackson et al.[13] for definition of a cold was used. This version required a total symptom score of greater than 5 over 5 days after challenge plus either nasal discharge for 3 days or the subject's belief that he had had a cold during the study. Patients were asked each evening if they had any problems that may be related to the medications. All paper tissues used by the subjects were collected in airtight containers placed in the subjects' rooms. Used tissues were counted each day, and nasal mucus weights were determined by a previously described method.[15]

Nasal lavage

Nasal lavages with isotonic saline solution were performed on seated subjects at 4:00 AM and 8:00 AM on study days 0 to 5. With the subject's head tilted forward, the washes were collected in a small dish and transferred to 15 ml tubes. Ethylenediaminetetraacetic acid was added to achieve a final concentration of 40 mmol/L in order to prevent kinin degradation by kininases in the nasal fluid.[16] The lavage fluid was then placed on ice until the specimens could each be divided into two aliquots. One aliquot was used for viral cultures, and the other was stored at −70° C for assays of kinin and albumin concentrations.

Virology

Nasal lavage aliquots were combined with veal infusion broth, stored on wet ice, and used to inoculate duplicate monolayers of human embryonic lung fibroblasts; strains MRC-5 and WI-38 were used on alternating days (Whittaker M. A. Bioproducts, Walkersville, Md.). Viral titrations were performed on 10-fold dilutions of once-frozen and thawed samples. One isolate from each volunteer was identified as a rhinovirus type 39 serotype by a neutralization test.[17] Blood was collected from each subject before and approximately 21 days after inoculation for determination of homotypic neutralizing antibody titers.[17] Infection was defined by the shedding of the challenge virus on 1 or more days or a fourfold or greater increase in the serum antibody titer.

Kinins

Kinins were measured with a radioimmunoassay able to detect 20 pg/ml of bradykinin.[16] There was no distinc-

tion made between bradykinin, lysyl bradykinin, and methionyllysyl bradykinin.

Albumin

Human serum albumin concentrations were measured with a radioimmunoassay sensitive to 1 ng/ml[18] (used as an assessment of vascular permeability).

Statistical analysis

The frequency of categorical variables in the two groups was compared by using the chi square test or two-tailed Fisher's exact test. Continuous variables were compared by using Student's *t* test. Data from uninfected subjects were excluded from our analysis. Probability values less than 0.05 were considered significant.

RESULTS

Subjects

Twenty-three subjects were randomized to the steroid group, and 24 subjects were randomized to the placebo group. All of the subjects were male. The mean age in the steroid group was 21.1 years, and the mean age in the placebo group was 20.3 years. Of the 24 subjects in the placebo group, two had a neutralizing antibody titer of 1:4 or greater in response to the challenge virus as determined by immediate prechallenge serology, three did not become infected, and two shed a wild rhinovirus. Thus 17 infected, placebo-treated subjects with initial antibody titers of less than or equal to 1:2 were available for evaluation. Of the 23 initial subjects in the steroid group, one was not infected, and one shed a wild virus at the initiation of the study. Thus 21 steroid-treated subjects were available for evaluation.

Infection and viral shedding

All infected subjects shed virus during the study. Subjects in the steroid group shed virus for an average of 4.8 days (range, 3 to 5 days), and those in the placebo group shed virus for an average of 4.6 days (range, 3 to 5 days). Eight of 17 (47%) infected placebo recipients had fourfold or greater homotypic serum neutralizing antibody responses, as compared with seven of 21 (33%) infected steroid recipients ($p = 0.59$).

The frequency of virus shedding did not differ between the groups on any postchallenge day. Viral titers (Fig. 1) in both groups peaked on day 2 and gradually fell over the next 3 days. However, during the 5-day postchallenge period, the mean (±SEM) titer in the placebo group was lower than that in the steroid group (0.79 ± 0.47 vs 1.13 ± 0.49, $p = 0.03$). The titers in the steroid group were significantly higher on days 3 ($p < 0.05$) and 4 ($p < 0.01$) after challenge (Fig. 1).

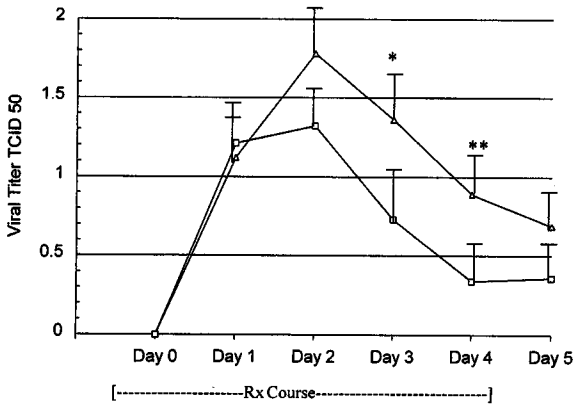

FIG. 1. Viral titers. *Triangles,* Prednisone recipients; *squares,* placebo recipients. *Indicates a significant difference ($p < 0.05$). **Indicates a significant difference ($p < 0.01$). Mean titers plus 1 SEM are plotted for data collected from nasal lavages on days 0 through 5. Viral challenge occurred on day 0. *TCID 50,* Tissue culture infective dose.

Illness measures

Twelve of 17 (71%) infected volunteers met the modified criteria for the definition of a cold in the placebo group, as did 15 of 21 (71%) infected volunteers in the steroid group. Total symptom scores and total nasal symptom scores were not significantly different between steroid and placebo recipients. There were no important differences in the individual respiratory or systemic symptom scores between the two groups (Fig. 2). Symptom profiles for rhinorrhea, nasal obstruction, sore throat, and cough were very similar. Chilliness, though rarely reported, was more prevalent throughout the days of observation in the placebo group but did not reach statistical significance on any of the days (data not shown). The symptom score for headache also tended to be lower in steroid recipients but also did not reach statistical significance. Sneezing was more common in the steroid group on days 1 and 2. Only the difference on day 1 was significant ($p = 0.01$).

The mean mucus weight produced during the study in steroid recipients (20.7 ± 5.1 gm per 5 days) was comparable to that in the placebo group (20.1 ± 4.9 gm per 5 days) (Fig. 3). The mean total number of tissues used by the steroid recipients (43 ± 8.6 per 5 days) was similar to that of the placebo group (46 ± 10.2 per 5 days).

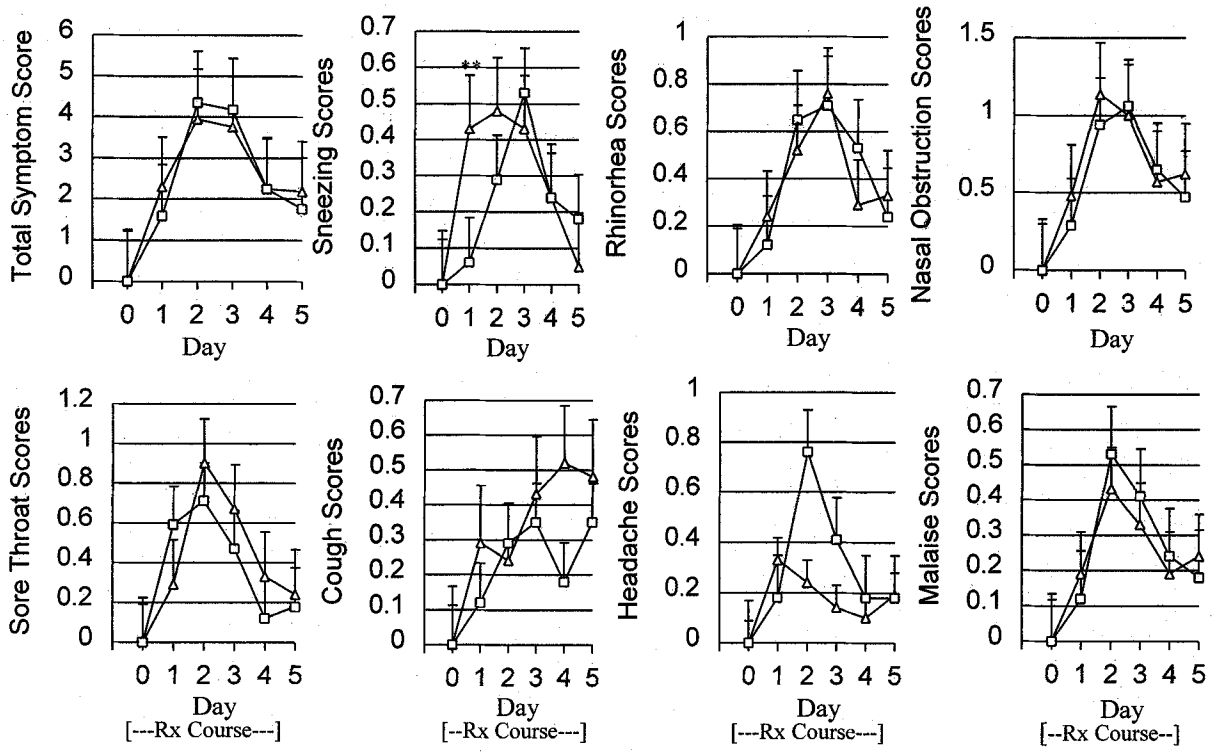

FIG. 2. Symptom scores. *Triangles,* Prednisone recipients; *squares,* placebo recipients. Patients were asked to score their symptoms from 0 to 4, with 0 being no symptoms. Total symptom scores were calculated by taking the sum of all of the other symptom scores. Means plus 1 SEM are plotted. Viral challenge occurred on day 0. **Indicates a significant difference ($p < 0.01$).

Nasal lavage kinin and albumin levels

Mean kinin concentrations (Fig. 4) for the entire study group were lower in the steroid recipients (287 ± 39 pg/ml) than in the placebo recipients (449 ± 55 pg/ml) ($p = 0.005$). The difference between daily mean kinin levels was significant on day 3 and during the first lavage on day 4 ($p < 0.01$). Mean kinin values were higher in subjects who were ill than in those who did not become ill (401 ± 27 pg/ml vs 302 ± 37 pg/ml) ($p = 0.03$). In the steroid treatment group, there was a significant difference in kinin levels: 356 ± 34 pg/ml in subjects who were ill versus 165 ± 22 pg/ml in subjects who did not become ill ($p < 0.005$). In the placebo group, there was no difference between mean kinin levels of subjects who became ill and those who did not (449 ± 43 pg/ml vs 468 ± 73 pg/ml).

Mean albumin concentrations (Fig. 4) over the duration of the study were significantly lower in the steroid recipients (32 ± 5 μg/ml vs 50 ± 6 μg/ml ($p < 0.05$). The mean albumin concentrations were lower in the steroid group in first lavages on days 2, 3, and 4 ($p < 0.05$) and in second lavages on days 3 ($p = 0.001$) and 5 ($p = 0.01$). There was a significant difference between the mean albumin levels of subjects who became ill and those who did not (43.2 ± 2 vs 32.9 ± 3.1, $p = 0.03$).

DISCUSSION

The purpose of this study was to determine the effect of oral steroid therapy on the clinical course of rhinovirus infection and to use the treatment as a tool for studying the pathogenesis of rhinovirus colds. An earlier investigation by Farr et al.,[12] in which oral and topical steroid administration were combined, showed a trend toward fewer cold symptoms and lower nasal mucus weights and kinin concentrations in steroid recipients. Our study provides further evidence that oral steroid administration can reduce nasal secretory kinin levels during experimental rhinovirus infections. However, in this study steroid administration was not associated with a reduction in symptoms or nasal mucus weights. Nor was there any difference in the percentage of volunteers who became ill between the steroid and placebo groups.

The failure of steroids to have an impact on the

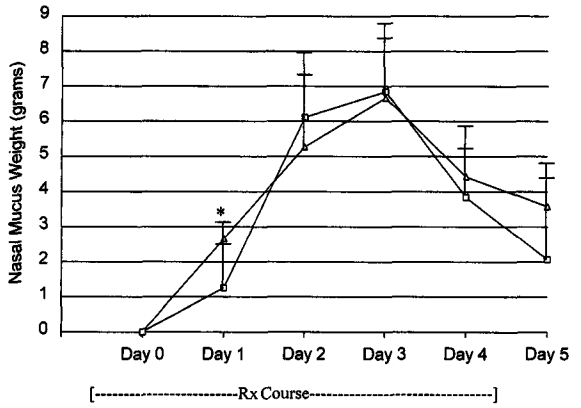

FIG. 3. Nasal mucus weights. *Triangles,* Prednisone recipients; *squares,* placebo recipients. Nasal mucus weights were calculated as the difference in weight between used tissues and an equal number of unused tissues at the end of each day. Viral challenge occurred on day 0. *Indicates a significant difference ($p < 0.05$).

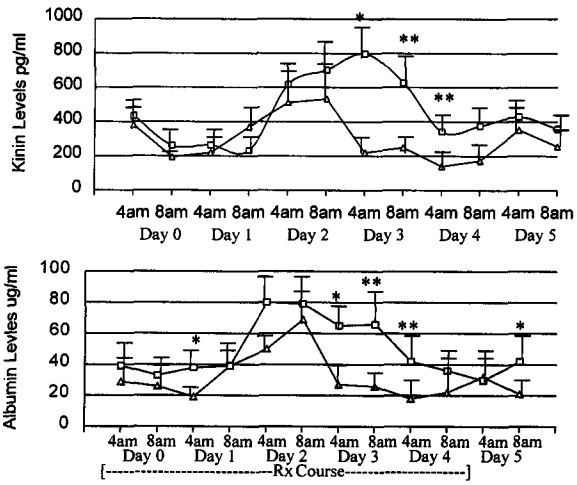

FIG. 4. Kinin and albumin concentrations. *Triangles,* Prednisone recipients; *squares,* placebo recipients. Means plus 1 SEM are plotted for kinin and albumin data collected from nasal lavages at 4:00 AM and 8:00 AM on days 0 through 5. Viral challenge occurred on day 0. *Indicates a significant difference ($p < 0.05$). **Indicates a significant difference ($p < 0.01$).

symptoms of rhinoviral infection is surprising, given their diverse antiinflammatory effects. Steroids are an accepted means of controlling the rhinorrhea and nasal obstruction of allergic rhinitis. The ineffectiveness of steroids for treatment of similar symptoms during rhinoviral infections most likely represents differences in the mediators of these illnesses. This study focused on kinins as potential mediators for rhinoviral cold symptoms.

Previous investigations have shown a correlation between kinin levels and symptom scores during rhinoviral infections[4] and have induced nasal obstruction, rhinorrhea, and sore throat in volunteers by exposing their nasal mucosa to kinins.[9] To fully determine the role that kinins play as mediators in rhinoviral infections, however, clinical trials with specific kinin inhibitors that examine the relationship between kinin inhibition and symptoms are necessary. In this study steroids significantly reduced kinin levels but failed to have any symptomatic benefits over a placebo. Unlike previous investigations,[4, 7, 12] our study showed no correlation between kinins and symptoms in the placebo group. The failure to find such a correlation makes it difficult to interpret what the inhibitory effect of steroids on kinin levels meant in terms of symptom scores.

The reasons that we did not find a correlation between kinins and symptom scores in the placebo group are unknown. The higher levels of kinins in patients who were ill may be an epiphenomenon reflecting increased levels of other mediators temporally related to increased kinin levels. Lysyl bradykinin can be produced from kininogen by enzymes located in the nasal mucosa. Thus if kinin is generated after the transudation of serum containing kininogen, other mediators may be responsible for the initial inflammatory response and increase in vascular permeability.

The effects of other mediators, such as the arachidonic acid derivatives, tumor necrosis factors, and interferons, on rhinoviral symptoms are poorly known. In particular, the contribution of proteases, oxygen radicals, and lipid mediators produced by the influx of neutrophils during rhinoviral infections is not fully understood. The failure to observe a reduction in symptoms, despite a significant reduction in kinin levels, may reflect the continued presence of these and other mediators. Our study did not examine these other mediators, and so their contributions in this clinical trial cannot be determined.

Farr et al.[12] examined the effects of combined oral and topical steroids. The differences between our two studies may reflect the action of topical steroid sprays on the local nasal mucosa. As previously mentioned, kinin production from kininogen during rhinoviral infections may occur in cells of the nasal mucosa. Other mediators may appear already formed in extravasated serum, may arise from inflammatory cells coming in response to chemotaxis, or may be produced locally by cells in the nasal mucosa. It is possible that topical application of steroids through nasal sprays enhances the effect of the steroid on the local mucosal

environment and thus on any locally produced or acting mediators.

The reason for the significant difference in viral shedding between the steroid and control groups was not determined. Possible causes include increased viral replication, increased nasal secretions (although this was not seen) and other unknown mechanisms. There may have been enhanced growth of the virus caused by steroidal suppression of the immune response.[19] In particular, steroids have been shown to inhibit the activity of interferon.[20, 21] Farr et al.[12] reported a trend toward more days of rhinoviral shedding in patients receiving steroid therapy than in control subjects, but viral titers were not reported.

The effects of increased viral levels on the pathogenesis of rhinoviral colds are unknown. Rhinovirus has been shown to be cytotoxic in human embryonic lung cell cultures, but no evidence of cell damage has been found in vivo during rhinoviral infections.[2, 3] In addition, the relationship between viral titers and the levels or activity of mediators involved in rhinovirus cold symptoms has not been investigated. Thus the effects of increased viral levels on reported symptoms in patients taking steroids is unknown.

The results of this study provide evidence for a suppressive effect of oral steroid administration on kinin generation. Oral steroid therapy reduced the level of kinins in experimental rhinoviral infections but did not provide relief from the symptoms associated with the infection. According to the results of this study, steroids do not appear to be an effective means of reducing rhinoviral cold symptoms, and they significantly increase viral titers during such infections. The role of kinins and other mediators in the pathogenesis of rhinoviral infections is not fully understood, and further investigations are needed to answer this question and to focus efforts on more specific treatment.

REFERENCES

1. Douglas RG, Alford BR, Couch RB. Atraumatic nasal biopsy for studies of respiratory virus infections in volunteers. In: Hobby GL, ed. Antimicrobial agents and chemotherapy. Baltimore: William & Wilkins, 1968:340-3.
2. Hamory BH, Hendley JO, Gwaltney JM Jr. Rhinovirus growth in nasal polyp organ culture. Proc Soc Exp Biol Med 1977;155:577-82.
3. Winther B. Histopathology of naturally occurring colds. Presented at the Symposium on Cellular and Neurogenic Mechanisms in Nose and Bronchi; May 27, 1982; Copenhagen, Denmark.
4. Naclerio RM, Proud D, Lichtenstein LM, et al. Kinins are generated during experimental rhinoviral colds. J Infect Dis 1988;157:132-42.
5. Schleimer RP. Glucocorticosteroids: their mechanisms of action and use in allergic diseases. In: Middleton E Jr, Reed CE, Ellis CF, Adkinson NF Jr, Yunginger JW, eds. Allergy: principles and practice. 3rd ed. St. Louis: CV Mosby, 1988:739-65.
6. Pipkorn U, Proud D, Lichtenstein LM, Kagey-Sobotka A, Norman PS, Naclerio RM. Inhibition of mediator release in allergic rhinitis by pretreatment with topical glucocorticosteroids. N Engl J Med 1987;316:1506-10.
7. Proud D, Naclerio RM, Hendley JO, Gwaltney JM Jr. Kinins are generated in nasal secretions during natural rhinovirus colds. J Infect Dis 1990;161:120-3.
8. Gwaltney JM Jr, Park J, Paul RA, et al. A controlled trial of clemastine fumarate in experimental rhinovirus colds. Presented at Thirty-fourth Interscience Conference Antimicrobial Agents and Chemotherapy; October 4-7, 1994; Orlando, Florida. Abstract no. H41.
9. Proud D, Reynolds CJ, LaCapra S, Kagey-Sobotka A, Lichtenstein LM, Naclerio RM. Nasal provocation with bradykinin induces symptoms of rhinitis and a sore throat. Am Rev Respir Dis 1988;137:613-6.
10. Cline MR, Melmon KL. Plasma kinins and cortisol: a possible explanation of the antiinflammatory action of cortisol. Science 1966;153:1135-8.
11. Pipkorn U, Proud D, Lichtenstein LM, et al. Effect of systemic glucocorticoid treatment on human nasal mediator release after antigen challenge [Abstract]. J ALLERGY CLIN IMMUNOL 1986;77:180.
12. Farr BM, Gwaltney JM Jr, Hendley JO, et al. A randomized controlled trial of glucocorticoid prophylaxis against experimental rhinovirus infection. J Infect Dis 1990;162:1173-7.
13. Jackson GG, Dowling HF, Spiesman IG, Boand AV. Transmission of the common cold to volunteers under controlled conditions. I. The common cold as a clinical entity. Arch Intern Med 1958;101:267-78.
14. Gwaltney JM Jr, Moskalski PB, Hendley JO. Interruption of experimental rhinovirus transmission. J Infect Dis 1980; 142:811-5.
15. Hayden FG, Gwaltney JM Jr. Prophylactic activity of intranasal enviroxime against experimentally induced rhinovirus type 39 infection. Antimicrob Agents Chemother 1982;21:892-7.
16. Proud D, Togias A, Naclerio RM, Crush SA, Norman PS, Lichtenstein LM. Kinins are generated in vivo following nasal airway challenge of allergic individuals with allergen. J Clin Invest 1983;72:1678-85.
17. Hamparian VV. Rhinoviruses. In: Lennete EH, Schmidt NJ, eds. Diagnostic procedures for viral and rickettsial infections. Washington, DC: American Public Health Association, 1979:535-75.
18. Baumgarten CR, Togia AG, Naclerio RM, Lichtenstein LM, Norman PS, Proud D. Influx of kininogens into nasal secretions after antigen challenge of allergic individuals. J Clin Invest 1985;76:191-7.
19. Beisel WR, Rappoport MI. Interrelations between adrenocortical functions and infectious illness (concluded). N Engl J Med 1969;280:596-604.
20. Kilbourne ED, Smart KM, Porkorny A. Inhibition by cortisone of the synthesis and action of interferon. Nature 1961;190:650-1.
21. Ho M. Factors influencing the interferon response. Arch Intern Med 1970;126:135-46.