Antiviral Research, 13 (1990) 103–110
Elsevier

AVR 00402

Influenza-specific ELISA IgA and IgG predict severity of influenza disease in subjects prescreened with hemagglutination inhibition

Peter D. Reuman, David I. Bernstein, Scott P. Keely, James R. Sherwood, Elizabeth C. Young and Gilbert M. Schiff

James N. Gamble Institute of Medical Research, Cincinnati, OH 45219, U.S.A.

(Received 12 May; accepted 13 November 1989)

Summary

Four influenza A challenge studies were performed over a period of three years using the same dose of one virus pool. The first three studies were conducted two influenza seasons apart from the last study. In all four studies only subjects with screening hemagglutination inhibition (HAI) antibody titers ≤ 1:8 in sera were accepted as study subjects. The rate of infection after influenza challenge was 96% (25 of 26) in the first three studies, and only 73% (8 of 11) in the last study ($P < 0.04$). The rate of influenza illness in the first three studies was 62% (16 of 26), and only 9% (1 of 11) in the last study (all subjects: $P = 0.003$; infected subjects: $P = 0.01$). Even though screening HAI titers were comparable between groups, prechallenge serum influenza-specific IgG titers correlated inversely with respiratory symptoms ($P = 0.04$). Prechallenge nasal wash influenza-specific IgA titers were lower in subjects who developed influenza illness ($P = 0.03$). Prechallenge influenza-specific nasal wash ELISA-IgA titers and serum ELISA-IgG titers predicted influenza severity in patients prescreened by HAI during influenza challenge studies.

Influenza; Nasal IgA

Introduction

The relative contribution of local and systemic immunity to influenza virus infection has not been clearly defined in humans. Couch et al. (1981) reported that

Correspondence to: P.D. Reuman, James N. Gamble Institute of Medical Research, 2141 Auburn Avenue, Cincinnati OH 45219, U.S.A.

serum IgG levels to influenza correlated best with resistance to challenge in human volunteer studies. Local neutralizing antibody in the absence of detectable serum antibody has been associated with resistance to influenza infection and illness (Murphy et al., 1973). The contribution of serum antibody alone in humans has been inferred from studies correlating resistance to influenza A illness with the level of maternally transferred antibody in newborns (Puck et al., 1980). Studies in animals suggest that serum antibody protects the lower airway against influenza pneumonitis but not infection (Ramphal et al., 1979; Waldman et al., 1973) and that local antibody protects the upper respiratory tract against infection (Barber et al., 1978; Kris et al., 1985). We compared the ability of influenza-specific serum IgG and nasal wash IgA to predict the incidence and severity of respiratory and systemic influenza disease in subjects screened for low HAI antibody and challenged with the same strain of influenza A in four separate challenge studies performed over three years.

Materials and Methods

Study populations

Subjects selected for all four studies were between 18 and 40 years of age and in good health as assessed by a physician and by blood counts and clinical chemistries. Subjects exhibiting a history of any clinically significant disease were excluded from participation (Reuman et al., 1988). Written informed consent was obtained from all participants. Subjects were admitted only with a screening serum HAI antibody titer to the challenge virus of $\leq$ 1:8. Use of any medication during these studies was not allowed.

A total of 26 subjects were challenged with influenza in the first three studies conducted from 20 July 1986 to 15 August 1986, from 29 September 1986 to 30 October 1986 and from 10 November 1986 to 8 December 1986. In the last study, conducted from 16 May 1988 to 13 June 1988, 11 subjects were challenged.

Study design

For each of the four studies, subjects were selected as outlined above (examination and screening HAI titers $\leq$ 1:8) and were sequestered for at least one day prior to influenza challenge to obtain baseline data and repeat prechallenge serology. During each nine days of isolation, vital signs were obtained every four to six hours and clinical evaluations were performed twice daily. The same physician performed clinical evaluations in all four studies. During isolation nasal washes were performed once daily for the first two studies and three times daily for the second two studies. Once discharged from isolation, subjects were examined as outpatients at 14 days and 28 days after challenge. At each of the follow-up examinations, nasal lavage was performed and at day 28 a blood sample was obtained for serology. HAI titers for study analysis and correlations were performed on prechallenge and not on the screening serology.

The same virus pool of influenza A/Bethesda/1/85 (H3N2) (provided by Dr Carol Heilman (NIH)) was used for challenge in all four studies. This virus pool was initially cultured four times in primary chicken kidney cells and then twice in egg allantoic fluid. Subjects received 0.25 ml per nostril of $10^{7.1}$ 50% tissue culture infectious doses ($TCID_{50}$) of virus. The virus aliquot used for challenge was titered on the day of administration ($10^{7.5 \pm 0.5}$ $TCID_{50}$). These titers showed no significant variation from the original titer.

Symptoms were assessed twice daily during isolation and scored as present (score: 1) or absent (score: 0). Respiratory symptoms were calculated by adding the individual scores for rhinitis, pharyngitis and cough. Systemic symptoms were calculated by adding the individual scores for fever ($\geq$ 100°F), myalgia and chills and sweats. A study subject was considered to have illness if fever ($\geq$ 100°F) and two of these influenza symptoms were present at a single evaluation and the challenge influenza A virus strain was isolated. All fevers were confirmed within 1 h and all symptoms of influenza had to be present on two successive clinical evaluations.

Laboratory procedures

Viral titers

Nasal wash specimens were inoculated onto MDCK continuous cell line and hemadsorbed with guinea pig erythrocytes at 3 to 4, 10 and 14 days post-inoculation for detection of influenza virus (Palmer et al., 1975). One hemadsorption positive specimen from each patient was confirmed as influenza A by IFA according to the protocol of the Influenza Branch of the Centers for Disease Control, Atlanta, Georgia. End point titrations of positive specimens were performed on MDCK cells as previously described (Hsiung et al., 1982; Linnette, 1980; Monto et al., 1979).

Hemagglutination inhibition

Sera for antibody were evaluated using a standard hemagglutination inhibition (HAI) assay (Palmer et al., 1975) and were assayed against the challenge strain of influenza A/Bethesda/1/85 (H3N2). Statistical comparisons of titers were made by comparing log transformations of titers. Titers of <1:8 were treated as 1:4 for statistical calculations.

ELISA influenza-specific Ig

Enzyme-linked immunosorbent assays (ELISA) were performed using partially purified influenza A/Bethesda/1/85 (H3N2) prepared as previously published (Reuman et al., 1989a). Quantitative determination of influenza-specific IgG and IgA was determined by a previously published ELISA method (Reuman et al., 1989a). Each unknown sample and reference standard were diluted in a manner to assure the generation of a sigmoidal curve that passed through an optical density of 0.9 for serum IgG and 0.6 for nasal wash IgA. The ELISA titer of each unknown sample was obtained from this point and expressed as the log of the reciprocal dilution at this point. Values obtained in this manner for the reference

standards were used to adjust sample values for plate to plate variability. The background optical density reading of the ELISA sandwich when all components were used except serum or nasal wash was always below 0.1. Background values were subtracted from plate readings. Statistical comparisons of sample ELISA titers were made by comparing values expressed as log of reciprocal dilution. One infected subject from the first three studies had a nasal wash antibody titer less than three standard deviations below the mean titer for all challenged subjects. This subject's nasal wash titers were deleted from our analysis.

Statistics

Statistical comparisons were performed using MICROSTAT (ECOSOFT. Inc., Indianapolis, Indiana). Between group comparisons were done using one way analysis of variance (ANOVA) for continuous variables and chi squared test for discrete variables. Correlations were assessed by regression analysis. Significant differences were accepted at the 0.05 level.

Results

Comparisons between studies

Demographic features: No significant differences between the first three studies and the last study were found for sex, age, height, weight, medical history, abnormal physical findings or screening HAI on entry into the study.

Rates of infection and illness: The first three studies were conducted two years (two influenza seasons) prior to the last study. The rate of infection in the first three studies was 96% (25 of 26 subjects) and in the last study only 73% (8 of 11 subjects) ($P = 0.036$). Infected subjects had a peak viral titer (first three: 4.7 ± 1.6; last: 3.7 ± 1.6 PFU/ml) and duration of viral shedding (first three: 6.1 ± 2.0; last: 4.9 ± 2.0 days) that did not differ between the two groups of studies ($P = 0.12$ and 0.15 respectively). Control subjects (with high HAI titers) were not infected. The rate of influenza illness in the first three studies was 62% (16 of 26 subjects), and in the last study, 9% (one of 11 subjects) (all subjects: $P = 0.003$; infected subjects: $P = 0.01$). Illness was found only in infected subjects and began an average of 1.7 days after challenge.

TABLE 1

Comparison of prechallenge serum and nasal wash antibody titers between the first three studies completed in 1986 and the fourth study completed in 1988 ($\log_{10}$ means and standard deviations)

Study	No.	Serum ELISA-IgG	Nasal wash ELISA-IgA
1, 2 and 3	26	3.36 ± 0.45	0.44 ± 0.30
4	11	3.28 ± 0.12	0.71 ± 0.26
P value		0.55	0.02

TABLE 2

Comparison of prechallenge serum and nasal wash antibody titers between infected subjects who were ill and not ill with influenza A (log_{10} means and standard deviations)

Diagnosis	No.	Serum ELISA-IgG	Nasal wash ELISA-IgA
Ill	17	3.36 ± 0.53	0.41 ± 0.29
Not ill	20	3.30 ± 0.20	0.66 ± 0.33
P value		0.68	0.03

Effect of prechallenge antibody titers

Prechallenge antibody titers were compared for differences between studies. Subjects admitted to all four studies had *screening* HAI titers ≤ 1:8 four weeks prior to challenge but *prechallenge* HAI serology revealed a proportion of subjects with HAI titers > 1:8. In the first three studies 8 of 26 subjects had HAI titers > 1:8 and in the last study 2 of 11 subjects had prechallenge HAI titers > 1:8 (P = 0.43).

Comparisons between prechallenge antibody titers of the three different antibody assays revealed a significant correlation (P = 0.002) between the two serum titers (HAI and ELISA-IgG) but no correlation between either serum assay and nasal wash titers. Although serum antibody screening methods led to the participation of subjects who had comparable serum influenza-specific IgG levels, nasal wash influenza-specific IgA levels (Table 1) were significantly higher in the last study (P = 0.02).

The significant difference in nasal wash influenza-specific IgA levels found between the first three studies and the last study, where infection and illness were significantly less common was notable. Because of this we examined the relationship between prechallenge antibody titers and virologic and clinical parameters of

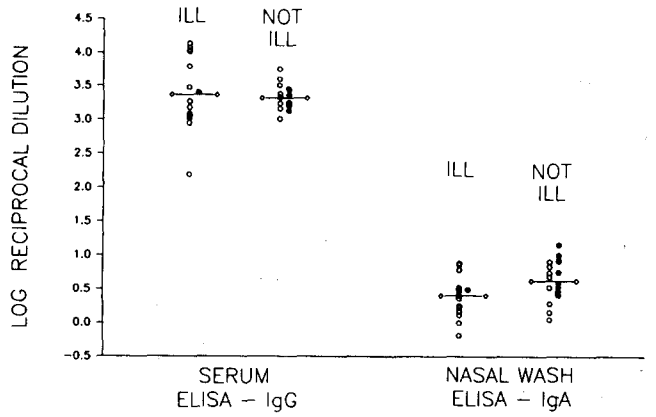

Fig. 1. Serum ELISA-IgG and nasal wash ELISA-IgA titers of study subjects. Data from the first three study groups are represented by open circles and data for the last study group are represented by closed circles.

influenza infection in all 37 inoculated subjects. No significant differences were found in prechallenge antibody titers between infected and uninfected subjects. This was not surprising considering that only four of 37 subjects were not infected. We then evaluated the effect of prechallenge antibody on influenza illness (Table 2 and Figure 1). There were no significant differences in mean prechallenge serum influenza-specific IgG titers between the 17 subjects who became ill and the 20 subjects who did not become ill ($P = 0.68$). However, mean prechallenge nasal wash influenza-specific IgA antibody titers were significantly higher in subjects who were not ill ($P = 0.03$). The geometric mean IgA titers of infected not-ill subjects was similar for the two groups of studies (first three studies: 0.52 ± 0.32; last study: 0.82 ± 0.26; $P = 0.08$).

When analyzed by regression, there was a significant negative correlation between prechallenge, influenza-specific serum IgG and respiratory symptom scores ($P = 0.04$). There were no significant correlations between prechallenge nasal wash antibody titers and symptom scores. Correlations between antibody titers and virus shedding were not significant (duration of virus shedding: nasal wash IgA: $P = 0.09$; serum IgG: $P = 0.89$; peak virus: nasal wash IgA: $P = 0.50$; serum IgG: $P = 0.64$).

Discussion

Significant differences were found in rates of infection and illness between influenza challenge studies. These differences were not attributable to differences in dose or strain of challenge virus, to differences in demographic factors or to differences in screening HAI serum antibody titers. The first 3 studies were conducted two influenza seasons apart from the last study. During the 1986–87 season, influenza A (H1N1) viruses similar to A/Taiwan/1/86 (H1N1) accounted for 99.3% of isolates (Morbid. Mortal. Weekly Rep., August 12, 1988). During the 1987–88 season, influenza A (H3N2) similar to A/Sichuan/2/87 and Shanghai/11/87 accounted for 75% of isolates and 8% were similar to influenza A/Taiwan/1/86 (H1N1) (Morbid. Mortal. Weekly Rep., August 19, 1988). It is probable that the circulation of H3N2 strains decreased the pool of susceptible subjects while the circulation of H1N1 strains might have boosted heterotypic immunity in the intervening two years.

Serum influenza-specific IgG titers were found to correlate inversely with respiratory symptoms. Subjects with high prechallenge influenza-specific IgG titers in their serum had fewer respiratory symptoms after influenza challenge. This was a surprising finding since all subjects entering these four studies were screened for HAI $\leq$ 1:8 before admission and were found to have prechallenge ELISA-IgG titers on admission that were comparable between studies. This suggests that ELISA-IgG specific for the challenge influenza A strain has sufficient variability even in subjects with low to absent strain specific HAI titers to be useful in screening to select subjects susceptible to more severe respiratory disease. The association of a high serum influenza-specific IgG antibody titer with less respiratory symptoms is

consistent with the findings of others (Ramphal et al., 1979; Francis et al., 1943; Potter et al., 1979; Clements et al., 1986).

Nasal wash influenza-specific IgA titers were significantly higher in the last study when fewer subjects became infected or ill following influenza challenge. This suggested that the presence of nasal influenza-specific IgA might be responsible for less severe influenza disease. Pre-challenge influenza-specific ELISA-IgA titers were tested for their effect on influenza infection and illness over all four studies. High screening nasal wash IgA titers were significantly associated with less influenza illness. Local antibody has been shown by others to be associated with resistance to infection and illness (Murphy et al., 1978) and is important in protecting the upper airway (Barber et al., 1978; Clements et al., 1986). This report cannot directly compare the ability of serum and nasal wash antibody assays to detect susceptible subjects. Subjects entering these four studies were excluded by screening only for serum HAI. However, when influenza-specific IgA titers performed on prechallenge nasal wash specimens from subjects already screened for HAI $\leq$ 1:8 were low, they appeared to select for subjects who became ill after challenge. Therefore, we suggest that measurement of influenza-specific IgA antibody in nasal wash might aid in the selection of susceptible subjects for challenge studies. Subjects selected with low titers should be more likely to develop influenza illness on challenge.

References

Barber, W.H. and Small, P.A. (1978) Local and systemic immunity to influenza infection in ferrets. Infect. Immun. 21, 222–228.

Clements, M.L., Betts, R.F., Tierney, E.L. and Murphy, B.R. (1986) Serum and nasal wash antibodies associated with resistance to experimental challenge with influenza A wild-type virus. J. Clin. Microbiol. 24, 157–160.

Couch, R.B., Kasel, J.A., Six, H.R. and Cate, T.R. (1981) The basis for immunity to influenza in man. In: D.P. Nayak and C.F. Fox (Eds.), Genetic Variation among Influenza Viruses, pp. 535–546. ICN-UCLA Symposia on Molecular and Cellular Biology XXI.

Francis, T. Jr., Pearson, H.E., Sullivan, E.R. and Brown, P.N. (1943) The effect of subcutaneous vaccination with influenza virus upon the virus inactivating capacity of nasal secretions. Am. J. Hyg. 37, 294–300.

Hsiung, G.D. and Fong, C.K.Y. (1982) Diagnostic Virology. Yale University Press, New Haven and London.

Influenza: United States, 1986–87 season. Morbid. Mortal. Weekly Rep., August 12, 1988 37(31), 466–470.

Influenza: United States, 1987–88 season. Morbid. Mortal. Weekly Rep. August 19, 1988 37(32), 497–503.

Johnson, P.R. Jr., Feldman, S., Thompson, J.M., Mahoney, J.D. and Wright, P.F. (1985) Comparison of long-term systemic and secretory antibody responses in children given live, attenuated, or inactivated influenza A vaccine. J. Med. Virol. 17, 325–335.

Kris, R.M., Asofsky, R., Evans, C.B. and Small, P.A., Jr. (1985) Protection and recovery in influenza virus-infected mice immunosuppressed with anti-IgM. J. Immunol. 134, 1230–1235.

Lennette, E.H. (1980) In: A. Balows, W.H. Hausler, Jr. and J.P. Truant (Eds.), Manual of Clinical Microbiology. American Society for Microbiology, Washington, D.C.

Monto, A.S., Gunn, R.A., Bandyk, M.G. and King, C.L. (1979) Prevention of Russian influenza by amantadine. J. Am. Med. Assoc. 241, 1003–1007.

Murphy, B.R., Chalhub, E.G., Nusinoff, S.R., Kasel, J. and Chanock, R.M. (1973) Temperature-sensitive mutants of influenza virus. III. Further characterization of the ts-1[E] influenza A recombinant. J. Infect. Dis. 128, 479–487.

Palmer, D.F., Dowdle, W.R., Coleman, M.T. and Schild, G.C. (1975) Advanced Laboratory Techniques for Influenza Diagnosis. Immunology Series No. 6, Procedural Guide. U.S. Department of Health, Education and Welfare, Atlanta, Georgia.

Potter, C.W. and Oxford, J.S. (1979) Determinants of immunity to influenza in man. Br. Med. Bull. 35, 69–75.

Puck, J.M., Glezen, W.P., Frank, A.L. and Six, H.R. (1980) Protection of infants from infection with influenza A virus by transplacentally acquired antibody. J. Infect. Dis. 142, 844–849.

Ramphal, R.R., Cogliano, R.C., Shands, J.W. Jr. and Small, P.A., Jr. (1979) Serum antibody prevents lethal murine influenza pneumonitis but not tracheitis. Infect. Immun. 25, 992–997.

Reuman, P.D., Bernstein, D.I., Keely, S.P., Young, E.C., Sherwood, J.R. and Schiff, G.M. (1989a) Differential effect of amantadine hydrochloride on the systemic and local immune response to influenza A. J. Med. Virol. 27, 137–141.

Reuman, P.D., Bernstein, D.I., Keefer, M.C., Young, E.C., Sherwood, J.R. and Schiff, G.M. (1989b) Efficacy and safety of low dosage amantadine hydrochloride as prophylaxis for influenza A. Antiviral Res. 11, 27–40.

Waldman, R.H., Jurgensen, P.F., Olsen, G.N., Ganguly, R. and Johnson, J.C. (1973) Immune response of the human respiratory tract. I. Immunoglobulin levels and influenza virus vaccine antibody response. J. Immunol. 111, 38–41.