Oral pathogens and allergic disease: Results from the Third National Health and Nutrition Examination Survey

Samuel J. Arbes, Jr, DDS, MPH, PhD,[a] Michelle L. Sever, BS,[a] Ben Vaughn, MS,[b] Eric A. Cohen, BS,[a] and Darryl C. Zeldin, MD[a] *Research Triangle Park and Durham, NC*

Background: The hygiene hypothesis contends that fewer opportunities for infection have led to increases in the prevalences of asthma and other allergic diseases.
Objective: This study evaluated the association between asthma, wheeze, and hay fever and antibodies to 2 oral bacteria associated with periodontal disease.
Methods: Data were obtained from the Third National Health and Nutrition Examination Survey. Serum levels of IgG antibodies to *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* were quantified by enzyme-linked immunoassays in 9385 subjects age 12 years and older. The outcomes were current asthma, wheeze, and hay fever. Odds ratios (ORs) representing a 1–log-unit increase in IgG concentrations were estimated with logistic regression. ORs were adjusted for 8 confounders and weighted to represent the US population.
Results: For each disease outcome, geometric mean antibody concentrations were higher in persons without the disease outcome than with the disease outcome. For a 1–log-unit increase in *P gingivalis* antibody concentration, adjusted ORs were 0.41 (95% CI, 0.20-0.87) for asthma, 0.43 (0.23-0.78) for wheeze, and 0.45 (0.23-0.93) for hay fever. For *A actinomycetemcomitans*, those ORs were 0.56 (0.19-1.72), 0.39 (0.17-0.86), and 0.48 (0.23-1.03), respectively.
Conclusion: Consistent with the hygiene hypothesis, higher concentrations of IgG antibodies to *P gingivalis* were significantly associated with lower prevalences of asthma, wheeze, and hay fever, and higher concentrations of IgG antibodies to *A actinomycetemcomitans* were significantly associated with a lower prevalence of wheeze.
Clinical implications: Colonization of the oral cavity by bacteria and other microbes might play a protective role in the etiology of allergic disease. (J Allergy Clin Immunol 2006;118:1169-75.)

Key words: *Asthma, wheeze, hay fever, hygiene hypothesis, periodontal disease, epidemiology, survey, antibodies, oral pathogens,* Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis

Abbreviations used
EU: ELISA unit
NHANES: National Health and Nutrition Examination Survey
OR: Odds ratio

In the United States, the prevalence of self-reported, current asthma increased 74% between the years 1980 and 1996.[1] In addition, data from the second and third National Health and Nutrition Examination Surveys (NHANES) suggest that the prevalence of allergic sensitization to common allergens also increased during those years.[2] One of the leading explanations for increases in allergy and asthma rates in the United States and other industrialized countries is the hygiene hypothesis. The hypothesis contends that infections and microbial exposures, which are thought to be less prevalent now, ''might essentially immunize against the development of asthma and allergic and autoimmune diseases.''[3] The origin of the hypothesis is usually credited to D. P. Strachan and his 1989 article, ''Hay fever, hygiene, and household size.''[4] Observing that family size and number of older children in the household were inversely associated with current hay fever among a large British cohort, Strachan stated that such findings could be explained ''if allergic diseases were prevented by infection in early childhood, transmitted by unhygienic contact by older siblings, or acquired prenatally from a mother infected by contact with her older children.''[4] Strachan went on to state that ''later infection or reinfection by younger siblings might confer additional protection against hay fever.''[4] Strachan speculated that smaller family sizes and higher standards of personal cleanliness have reduced the opportunities for infections, resulting in more widespread atopic disease.[4] Several years later, a plausible biological mechanism—that infection shifts the balance of cytokine release away from a pattern responsible for IgE-mediated allergy—emerged.[5]

Although investigations of the hygiene hypothesis have not always provided consistent results, reports of inverse associations between allergic disease and various infections and exposures to farms, pets, endotoxin, and day care have provided support for the hypothesis.[3,5,6] As an extension of the hypothesis, researchers began examining the

From [a]the National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park; and [b]Rho, Inc, Durham.
Supported by the Intramural Research Program of the National Institutes of Health, the National Institute of Environmental Health Sciences.
Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.
Received for publication April 18, 2006; revised June 29, 2006; accepted for publication July 17, 2006.
Available online September 15, 2006.
Reprint requests: Darryl C. Zeldin, MD, NIEHS/NIH, PO Box 12233, Mail Drop D2-01, Research Triangle Park, NC 27709. E-mail: zeldin@niehs.nih.gov.
0091-6749/$32.00

doi:10.1016/j.jaci.2006.07.029

role of gastrointestinal microflora in the etiology of allergy and asthma. Several investigations have shown that the composition of gut microflora, which can be altered by antibiotics, diet, and infant feeding regimens, differs between individuals with and without allergy.[7] In addition, the supplementation of the diet with beneficial bacteria, known as probiotics, has shown promise in the prevention of atopic dermatitis.[8]

Similar to the gastrointestinal tract, the oral cavity harbors hundreds of species of microorganisms that colonize after birth and are influenced by environmental and host factors.[9] Because oral bacteria have been associated with local and systemic inflammation and with diseases outside the oral cavity, such as cardiovascular disease,[10-15] we hypothesized that oral bacteria may play a role in the etiology of allergic diseases. In 2002, a supplemental NHANES III dataset containing IgG antibody concentrations to 2 oral bacteria measured in stored serum samples was released. The 2 bacteria, *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*, are part of the normal oral flora; however, their proportions relative to other microflora are higher in persons with periodontal disease.[9] Periodontal disease is considered one of the most common chronic inflammatory disorders in adults, although it also occurs in children.[9] The objective of this study was to investigate the associations between IgG antibodies to those 2 oral bacteria and subject-reported asthma, wheeze, and hay fever.

METHODS

Data

Data were obtained from NHANES III, in which a complex survey design was used to sample the civilian, noninstitutionalized population of the United States. The NHANES III, conducted from 1988 to 1994, was designed so that its first 3 years (phase 1), its last 3 years (phase 2), and the entire 6 years were each national probability samples. Over the 6 years, 31,311 individuals age 2 months to 90 years were administered questionnaires and given medical examinations. Details of the NHANES III design may be found elsewhere.[16]

Oral pathogens

In the absence of direct measurements of the oral pathogens, serum IgG antibody concentrations were used as a marker of exposure. Stored sera from phase 2 subjects age 12 years and older were analyzed with enzyme-linked immunoassays for IgG antibodies to the oral pathogens *A actinomycetemcomitans* and *P gingivalis*. IgG antibody concentrations for *A actinomycetemcomitans* and *P gingivalis* were reported for 9372 and 9371 subjects, respectively. All IgG concentrations reported in this article are in ELISA units (EU). In a recently published article, Dye et al,[13] using the oral pathogen data from NHANES III, defined elevated levels of IgG antibodies to *A actinomycetemcomitans* and *P gingivalis* as concentrations greater than 156 EU and 168 EU, respectively. They determined those cut points by selecting the concentration at the 90th percentile among the population without periodontal disease, after excluding the highest and lowest 1% of the IgG distribution.

Disease outcomes

Information on asthma, hay fever, and wheeze was obtained by questionnaire, with parents or guardians providing information for child subjects. Patients with asthma were individuals who answered in the affirmative to the questions, ''Has a doctor ever told you that you had asthma?'' and ''Do you still have asthma?'' Likewise, patients with hay fever were those who answered in the affirmative to the questions, ''Has a doctor ever told you that you had hay fever?'' and ''Do you still have hay fever?''

Patients with wheezing were persons who answered in the affirmative to the questions, ''Have you had wheezing or whistling in your chest at any time in the past 12 months?'' and ''Apart from when you have a cold, does your chest ever sound wheezy or whistling?'' The second question was asked of all participants regardless of their answers to the first, and the second question was not framed by a time period. Thus, an affirmative answer to both questions does not necessarily mean that the wheezing in the past 12 months was apart from a cold.

Although allergy skin testing to 10 common indoor allergens was performed in NHANES III, allergy skin test positivity is not presented in this article as a primary outcome because the subpopulations for allergy skin testing (all subjects age 6-19 years and a random half-sample of subjects age 20-59 years) and oral pathogen measurements (age 12-90 years in Phase 2 only) had very little overlap, and each subpopulation has its own weighting variables. Of the 10,863 subjects with allergy skin test data and the 9385 subjects with oral pathogen data, only 3702 subjects had data on both. In an exploratory analysis, associations between IgG concentrations and allergy skin test positivity were tested, and those results were reported under the heading Additional analyses. Details of the allergy skin test procedures and the definitions of a positive test used in this analysis may be found elsewhere.[2] Briefly, an allergen-specific skin test result was considered positive if the difference in wheal diameters between the allergen-specific test and negative control was at least 3 mm. Allergy skin test positivity was defined as a positive test result to at least 1 of the 10 allergens.

Statistical analyses

Geometric mean antibody concentrations by disease status and by levels of the covariates were reported. Overall differences in those means were tested in unadjusted linear regression models with antibody concentrations logarithmically (base 10) transformed. Differences in prevalences of disease by elevated versus nonelevated IgG antibody concentrations were tested with χ^2 statistics. Odds ratios (ORs) for the associations between antibody concentrations and each disease outcome were estimated with logistic regression. ORs were adjusted for confounding by all variables listed in Table I. Income-related variables were not used in the analysis because a significant number of subjects (n = 749) had missing values for family income. In addition, inhaled corticosteroid use was not included as a potential confounder in the primary analysis because only 98 subjects reported inhaled corticosteroid use in the past month, and there were subjects who reported taking prescription medicines but did not answer some or all of the questions about the prescription medicines.[17] Differences in adjusted ORs by age, sex, and race were tested by the addition of 2-way interaction terms. For the assessment of interaction by race, individuals categorized as ''other'' were excluded from the analysis.

With the exception of the allergy skin test ORs, all reported statistics other than numbers of subjects were weighted with the Phase 2 variable WTPFHX2 to represent the civilian, noninstitutionalized population of the United States. SEs, with the exception noted, were adjusted for the survey design using SUDAAN statistical software (Release 9.0; Research Triangle Institute, Research Triangle Park, NC) and the phase 2 survey design variables SDPSTRA2 and SDPPSU2. Statistical significance was set at $P \leq .05$.