

# Development of nasal basophilic cells and nasal eosinophils from age 4 months through 4 years in children of atopic parents

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**Background:** The importance of the inflammatory response in allergic disease has warranted investigation of two of the more integral cells in the pathogenesis of allergic rhinitis, namely, nasal basophilic cells (NBCs) and nasal eosinophils (NEs). In the present study the natural history of NBCs and NEs was studied from birth through 4 years of age in infants of atopic parents to better understand their relationship to the development of various atopic disorders.

**Methods:** During a prospective, randomized, controlled trial of the effect of maternal and infant food avoidance on the development of atopic disorders in 288 infants of atopic parents, NBCs and NEs were determined semiquantitatively on Wright-Geimsa stained nasal mucosal scrapings and related to each other and to various atopic parameters.

**Results:** In these infants who are at high risk NBCs developed in both those becoming atopic and those remaining nonatopic, but they occurred with increased frequency and quantity in those children in whom most allergic disorders and food or inhalant sensitization developed. NEs, rare at 4 months in all infants, increased in atopic children from 1 to 4 years, and remained infrequent and nominal in nonatopic children. Levels of NBCs and NEs (1) correlated with each other strongly, directly, and with increasing magnitude in children from ages 4 months to 4 years and (2) correlated with log serum IgE levels weakly and moderately in children from ages 2 to 4 years. Stepwise linear regression analyses revealed that allergic rhinitis accounted for most of the variance seen in the level of NBCs and NEs during early childhood, reaching 50% by age 4 years. Asthma, atopic dermatitis, food allergy, food and inhalant sensitization, serum IgE level, and IgG beta-lactoglobulin level accounted for a small proportion of the variance in NBCs and NEs from ages 4 months to 4 years.

**Conclusions:** These findings help to elucidate the natural history of NBCs and NEs in infants at risk for atopic disorders and document the temporal association of these cells with the development of atopic disease, principally allergic rhinitis. (*J ALLERGY CLIN IMMUNOL* 1993;91:723-34.)

**Key words:** Basophils, eosinophils, atopy, IgE/IgG food antibodies

The nasal cellular inflammatory reaction represents the hallmark of the atopic response in allergic rhinitis. Primary effector cells in this response include nasal basophilic cells (NBCs) and nasal eosinophils (NEs).

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## Abbreviations used

NBC: Nasal basophilic cell

NE: Nasal eosinophil

Prevalence: Period prevalence of children with NBCs and NEs

SPT: Skin puncture test

IgG BLG: Immunoglobulin G beta-lactoglobulin antibody level

IgG OVA: Immunoglobulin G ovalbumen/ovomucoid antibody level

MC<sub>T</sub>: Tryptase-positive, chymase-negative mast cell subtype

MC<sub>TC</sub>: Tryptase-positive, chymase-positive subtype

S<sub>r</sub>: Spearman rank correlation

TABLE I. Development of NBCs and NEs in offspring of atopic parents

| Mo. | Offspring<br>(no.) | NBC             |                           | NE              |                           |
|-----|--------------------|-----------------|---------------------------|-----------------|---------------------------|
|     |                    | Prevalence (%)* | $\bar{x}$ Grade $\pm$ SEM | Prevalence (%)* | $\bar{x}$ Grade $\pm$ SEM |
| 4   | 282                | 21.3            | 0.25 $\pm$ 0.04           | 0.7             | 0.01 $\pm$ 0.01           |
| 12  | 275                | 41.1            | 0.47 $\pm$ 0.05           | 7.6             | 0.07 $\pm$ 0.02           |
| 24  | 260                | 49.6            | 0.64 $\pm$ 0.06           | 22.7            | 0.27 $\pm$ 0.04           |
| 36  | 238                | 45.4            | 0.70 $\pm$ 0.07           | 28.6            | 0.44 $\pm$ 0.06           |
| 48  | 220                | 38.2            | 0.69 $\pm$ 0.08           | 38.2            | 0.60 $\pm$ 0.07           |

\*Chi square for trend: NBCs and NEs,  $p < 0.0001$ .

NBCs stain basophilic with the basic aniline dyes and metachromatically with toluidine blue, leading to the synonymous respective terms "basophilic" or "metachromatic" in descriptions of these cells. NBCs obtained from human nasal scrapings include basophil leukocytes and two types of mast cells.<sup>1</sup> These nasal mast cells are classified by their content of neutral protease into tryptase-positive, chymase-negative ( $MC_T$ ) or tryptase-positive, chymase-positive ( $MC_{TC}$ ) subtypes.<sup>2</sup> These subtypes may correspond to the rodent mucosal and connective tissue mast cells, respectively.<sup>3</sup> Ultrastructural examination of the allergic nasal mucosa reveals three types of NBCs, which differ in their epithelial location: (1) basophil leukocytes predominate on the mucosal surface, (2)  $MC_T$  occur principally in the epithelium, and (3)  $MC_{TC}$  are found mainly in the deeper lamina propria.<sup>1,4</sup> Nasal mucosal NBCs obtained by gentle scraping of the inferior turbinates are predominately basophil leukocytes and  $MC_T$ .<sup>1,5</sup> The concentration of these nasal surface NBCs increases in (1) allergic disorders (with their numbers correlating with the severity of allergic rhinitis), pollenosis during seasonal exposure, and nasal allergen challenge<sup>5</sup> and in (2) nonallergic eosinophilic nasal conditions such as nonallergic eosinophilic rhinitis, nasal polyps, and aspirin sensitivity.<sup>4</sup>

Eosinophils within the human nasal mucosa are identified by their affinity to the acidic dye eosin or their content of major basic protein.<sup>6</sup> Activated nasal eosinophils contain the cleaved form of eosinophil cationic protein and are thought to be analogous to the hypodense activated eosinophil found in peripheral blood in subjects with allergic rhinitis.<sup>7</sup> Similar to the situation with NBCs, NE numbers increase with the severity of allergic rhinitis, and their concentrations appear to correlate with NBCs.<sup>4</sup> Recent findings with respect to NBCs and NEs in allergic rhinitis suggest that chemotactic factors stimulate both cell types to migrate from the circulation to the superficial nasal mucosa. Once in the nose, local growth factors, in-

cluding granulocyte macrophage-colony stimulatory factors, interleukins, and nerve growth factors, stimulate NBC and NE growth and differentiation.<sup>8</sup> Such differentiation probably is dependent on functioning T cells.<sup>9</sup>

The natural history of NBCs and NEs in human beings is poorly understood: past studies have been limited by sample size,<sup>10</sup> retrospective design,<sup>8</sup> or brief follow-up.<sup>11</sup> The present study examines the development of NBCs and NEs in a large cohort of offspring of atopic parents followed prospectively from birth to 4 years of age,<sup>12</sup> and evaluates the relationship of these cells to atopic disease including food allergy, food and inhalant sensitization, cord and serum IgE levels, and the IgG response to cow milk and egg.

## MATERIAL AND METHODS

### Study design

The study design, protocol, subject recruitment, dietary interventions, and definitions of atopic outcomes have been detailed in a previous report and will only be summarized here.<sup>12</sup> Families from the Kaiser Permanente Health Plan of San Diego with at least one immunologically determined atopic parent were randomized before the third trimester of pregnancy into either a maternal-infant food allergen avoidance group (prophylactic-treated group) that delayed introduction of the major food allergens (milk, egg, peanut, fish, soy, wheat, and nuts) until 1 year of age, or into a maternal-infant control group that adhered to routinely recommend infant feeding practices.<sup>12</sup> Children were evaluated at 4 months of age and then annually to age 4 years. Parents read and signed an informed consent approved by the Kaiser Foundation Hospitals Institutional Review Board.

### Definitions of atopic disease outcome measures

The following criteria, as summarized below and detailed elsewhere,<sup>12</sup> were used to define the presence of atopic disorders during the 4-year course of the study:

**Atopic dermatitis.** Atopic dermatitis was defined as an eczematous eruption associated with at least three of the following four criteria suggested by Hanifin and Lobitz<sup>13</sup>

**TABLE II.** Correlation of NBC and NE grade with each other and with serum log IgE from 4 months to 4 years of age

| Mo. | Spearman correlation coefficient (R) |                     |                     |
|-----|--------------------------------------|---------------------|---------------------|
|     | NBC vs NE                            | NBC vs log IgE      | NE vs log IgE       |
| 4   | 0.1979 (n = 282)**                   | 0.0128 (n = 279)    | 0.0571 (n = 279)    |
| 12  | 0.3487 (n = 275)**                   | 0.0586 (n = 274)    | 0.0835 (n = 274)    |
| 24  | 0.5998 (n = 260)***                  | 0.1421 (n = 257)*   | 0.1268 (n = 257)    |
| 36  | 0.6555 (n = 238)***                  | 0.2239 (n = 236)*** | 0.3328 (n = 236)*** |
| 48  | 0.8440 (n = 220)***                  | 0.3945 (n = 217)*** | 0.4560 (n = 217)*** |

\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

and modified for offspring of atopic parents: pruritis, typical morphology and distribution, a tendency toward chronicity and recurrence, and concurrent specific IgE.

**Urticaria and angioedema.** Urticaria was defined as a pruritic eruption with typical morphologic appearance reported by physician or parent with or without incriminating agent(s) identified.

**Allergic rhinitis.** Allergic rhinitis was considered a nasal condition with characteristic sneezing, itching, and/or rhinorrhea with concurrent specific IgE and nasal eosinophilia (see Methods section).

**Asthma.** Asthma was defined as a physician-documented lower respiratory condition with characteristic reversible bronchospasm, occurring at least twice and unassociated with other anatomic, congenital, or immunologic causes.

**Food allergy.** Food allergy was defined when food-specific IgE occurred concurrently with symptoms consistent with atopic dermatitis, urticaria and angioedema triggered at least twice by a specific food, or gastrointestinal allergy—vomiting and diarrhea induced on at least two occasions by a specific food—confirmed when possible by double-blind, placebo-controlled food challenges.<sup>12, 14</sup>

## Determination of immunologic outcome measures

**NBC and NE determination.** Nasal mucosa specimens were obtained, processed, and enumerated by previously reported methods,<sup>15</sup> which will only be summarized here. Mucosal specimens were obtained from the posterior surface of the inferior nasal turbinate with a disposable flexible plastic curette (Synbiotics Corp., San Diego, Calif.). They were transferred onto a glass slide, fixed in 95% ethyl alcohol, and stained with a modified Wright-Giemsa procedure. NBCs and NEs were enumerated at  $\times 1000$  magnification on a single specimen from subjects at each evaluation. The entire slide was examined and then graded semiquantitatively by one investigator (R.S.Z.) masked to the status of the subjects.<sup>13</sup> The relative quantity of NBCs and NEs was rated on a scale ranging from 0 to 4+ as follows 0 = no NBCs or NEs;  $\frac{1}{2}+$  = occasional NBCs or NEs; 1+ = scattered small numbers or clumps of NBCs or NEs; 2+ = moderate numbers or clumps of NBCs or

NEs; 3+ = large numbers or clumps of NBCs or NEs not covering an entire field; and 4+ = large numbers or clumps of NBCs or NEs covering entire fields. For the purpose of this study levels of NBCs and NEs within the nasal mucosa of  $\frac{1}{2}+$  or greater was used to determine the prevalence of these cells because the intent of the study was to understand the natural history of these cells. With use of cutoffs of  $\frac{1}{2}+$  to 2+ for both NBCs and NEs in their relationship to the period prevalence of any atopic disease at 4 years led to similar screening efficiencies of approximately 80% for both cell types at all three cutoff levels. To test the validity of the cellular grading, 50 mucosal specimens from subjects with allergic rhinitis determined by two different cytologists revealed coefficients of variation of 0.82 and 0.87 for NBCs and NEs, respectively.

**Skin testing.** Skin puncture testing (SPT) was performed on a subject's upper back with Multi-Test (Center Laboratories, Port Washington, N.Y.) units. Extracts tested were foods (milk, egg, peanut, wheat, corn, soy, cod, chicken, beef, and other foods incriminated in a historical adverse reaction) and inhalants (mite, *Alternaria*, *Cladosporium*, grass mix of 30% bermuda and 70% grass [combination], dog, and cat) (Hollister-Stier, Division of Cutter Laboratories, Spokane, Wash.). SPT allergen wheals 3 or more millimeters larger than the saline control were considered positive. Subjects with nasal eosinophilia and negative SPTs had intradermal aeroallergen testing at concentrations 100-fold less than SPTs as described previously.<sup>12</sup> Histamine phosphate (10 mg/ml for SPTs and 0.1 mg/ml for intradermal testing) (Center Laboratories) served as positive controls.

**Total serum IgE concentration.** Total serum IgE levels were determined by Phadebas PRIST calibrated for detection limits between 0.50 IU/ml and 1000 IU/ml for children, as described previously.<sup>12, 16</sup> Undetectable IgE levels were considered 0.1 IU/ml for continuous data analyses.

**Specific immunoglobulin G beta-lactoglobulin antibody level (IgG BLG) and immunoglobulin G ovalbumin/ovomucoid antibody level (IgG OVA).** IgG BLG and IgG OVA levels were determined by ELISA (enzyme-linked immunoassay) with a less than 10% intratest coefficient of variation by methods described in detail elsewhere.<sup>17</sup>

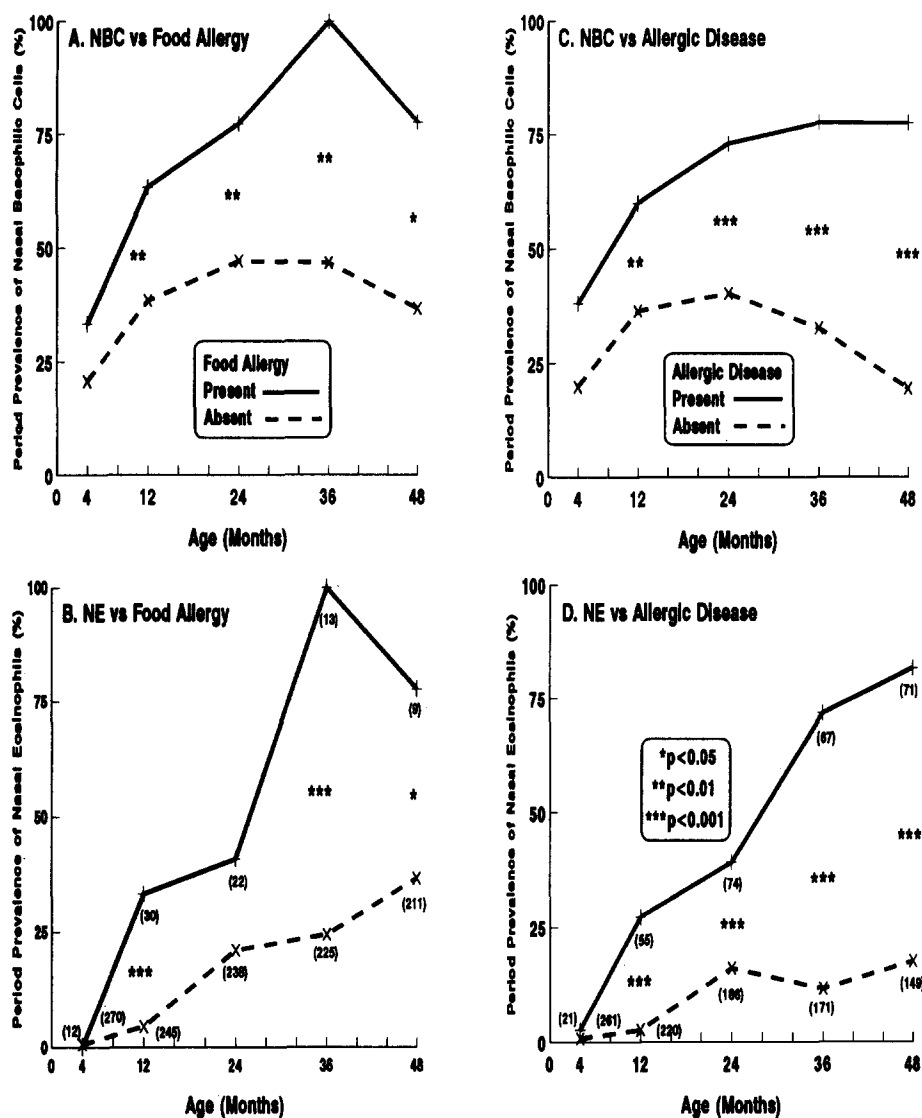


FIG. 1. Period prevalence of children with NBCs (A and C) and NEs (B and D) related to the presence or absence of food allergy (A and B) or any allergic disease (C and D). Number of subjects in this and subsequent figures shown between parentheses.

## Data analysis

Data were analyzed at the Biostatistical Division of the University of California-Los Angeles Health Services with the BMDP statistical software package version 1990 (BMDP Statistical Software, Inc., Los Angeles, Calif.). Period (current) prevalence was used to determine prevalence and was defined as the percent of subjects currently evidencing NBCs and NEs, atopic manifestations, or skin test sensitization at age 4 months and then annually to age 4 years.<sup>12</sup> Fisher's exact test or Pearson's chi square were used for categorical univariate analysis. Mann-Whitney U test was performed for comparisons of nonparametric continuous data. Correlations of nonparametric data were determined by Spearman rank correlation analyses ( $S_r$ ). Bonferroni corrections were

applied to multiple comparisons. Data from prophylactic-treated and control groups were combined during analyses when univariate analyses did not reveal group differences in NBC or NE determinations. Stepwise linear regression analyses determined the extent to which certain factors contributed to the variance in NBC and NE levels from ages 4 months to 4 years by use of the following covariates: group, sex, parental ethnicity (nonwhite vs white), parental smoking (either parent smokes vs neither parent smokes), the atopic disorders entered separately (currently present vs absent), food SPT and inhalant skin tests (currently positive vs negative), serum log IgE, IgG BLG, and IgG OVA levels at the corresponding ages, and maternal log IgE level.

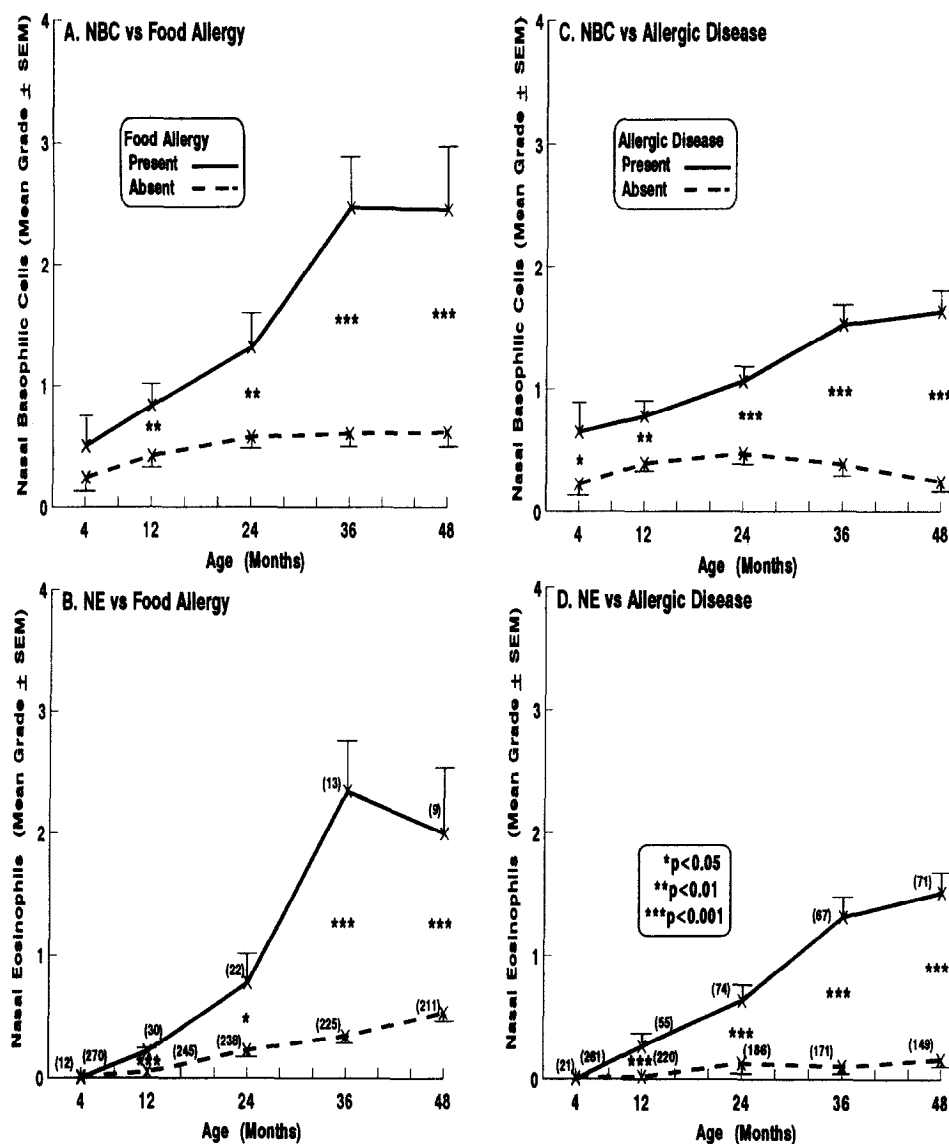


FIG. 2. Mean grade of NBCs (A and C) and NEs (B and D) related to the presence of food allergy (A and B) or any allergic disease (C and D).

## RESULTS

### Development of NBCs and NEs

Because no group differences in NBCs and NEs were found for nearly all univariate group comparisons, only combined group data will be reported, unless otherwise noted. In the entire cohort of subjects analyzed without regard to atopy, NBC prevalence and number increased from ages 4 months to 2 years, thereafter plateauing (Table I). NEs, in contrast, increased from 4 months to 4 years without reaching a plateau or peak (Table I). NBCs and NEs correlated positively and significantly with each other at each age, with

correlations increasing in magnitude from weak at age 4 months to strong at age 4 years (Table II).

**Relationship to atopic diseases.** NBCs and NEs occurred at most ages in higher prevalence (Fig. 1) and in greater quantity (Fig. 2) in the presence of at least one atopic disease and also food allergy. Approximately 80% of atopic children compared with less than 20% of nonatopic children had NBCs and NEs at 4 years of age ( $p < 0.001$ ) (Fig. 1, C and D). Similarly, NBCs and NEs were present in significantly greater numbers in children with atopic dermatitis (ages 1 to 4 years for NBCs and NEs),

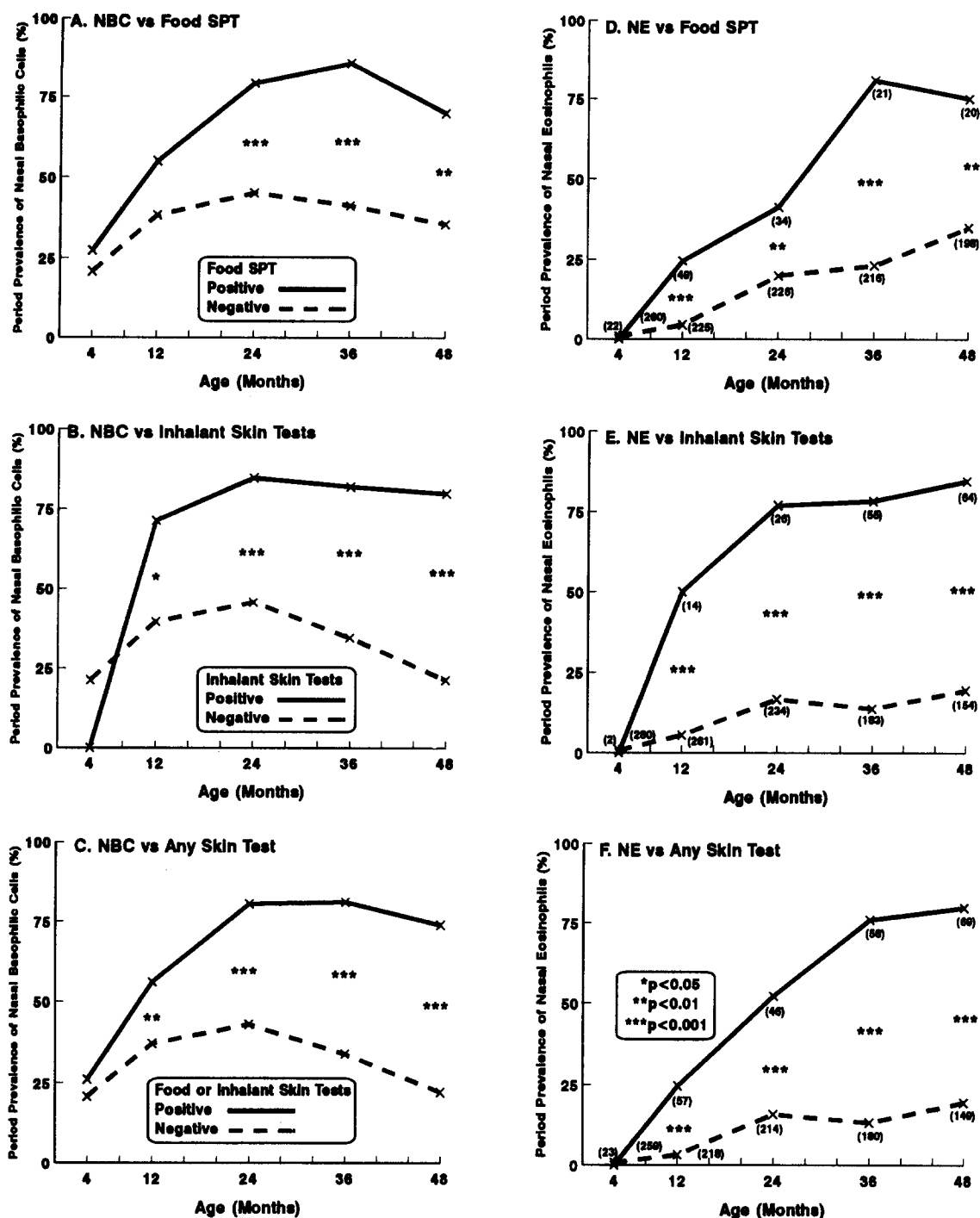


FIG. 3. Period prevalence of children with NBCs (A through C) and NEs (D through F) related to the presence of food SPT sensitization (A and D), inhalant skin sensitization (B and E), and food or inhalant sensitization (C and F).

urticaria (ages 2 to 4 years for NBCs and ages 1, 3, and 4 years for NEs), allergic rhinitis (ages 1 to 4 years for NBCs and NEs), and asthma (ages 4 months, 3 and 4 years for NBCs and ages 1, 3, and 4 years for NEs) when these disorders were evaluated indi-

vidually and compared with children without these disorders at the respective age (data not presented).

*Relationship to immediate skin test sensitization.* Subjects with positive food or inhalant skin tests demonstrated a significantly greater prevalence (Fig. 3)

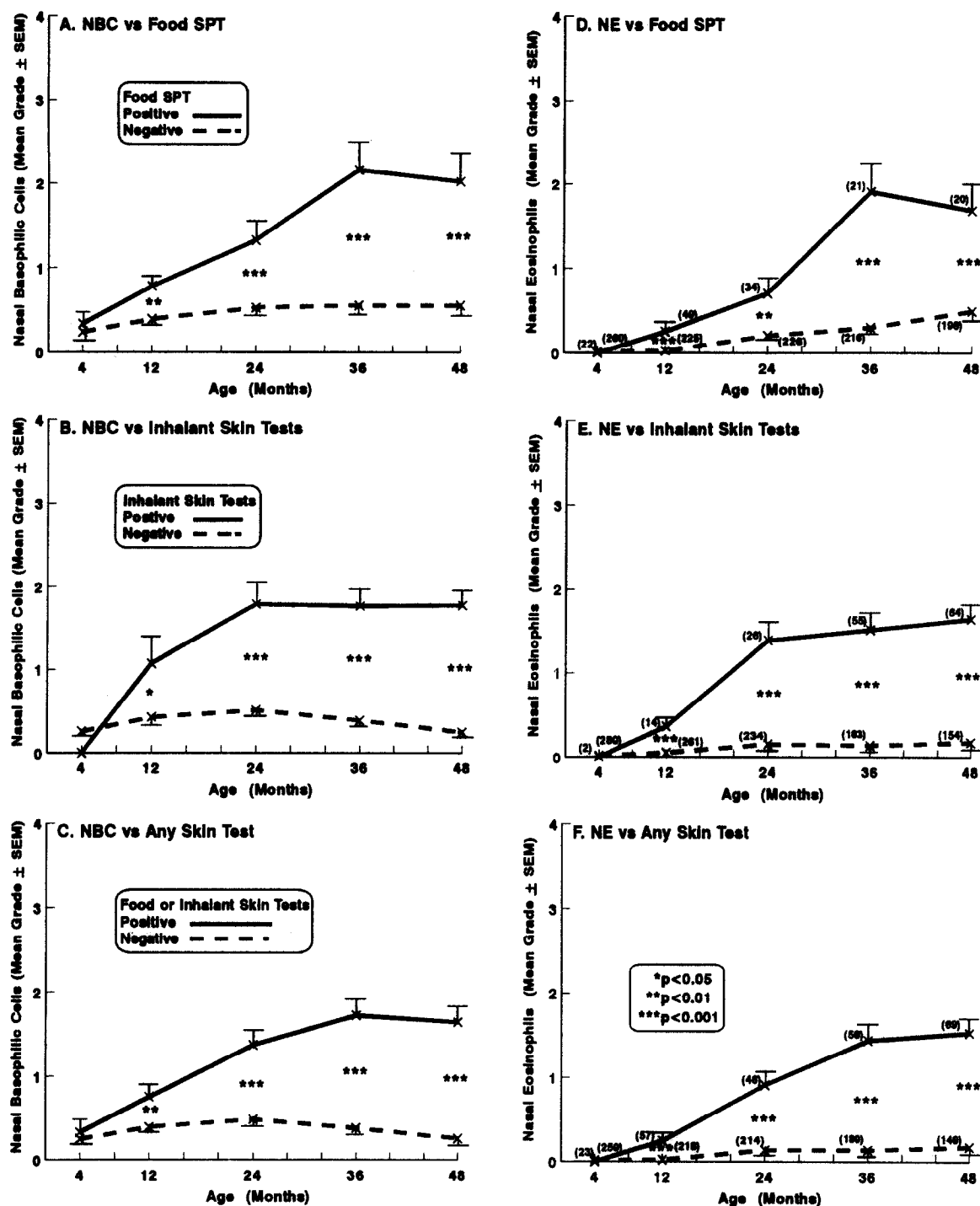


FIG. 4. Mean grade of NBCs (A through C) and NEs (D through F) related to the presence of food SPT sensitization (A and D), inhalant skin sensitization (B and E), and food or inhalant sensitization (C and F).

and quantity (Fig. 4) of NBCs and NEs from ages 1 to 4 years.

**Relationship to serum IgE levels.** No relationship was noted between cord blood IgE level and subsequent NBC or NE level developing at 4 months to 4

years of age. ( $p > 0.1$ ). Significantly positive correlations were noted between NBC and NE levels and log serum IgE levels from ages 2 to 4 years, attaining highest association at age 4 years (Table II). Consistent with the above, children with NBCs and NEs

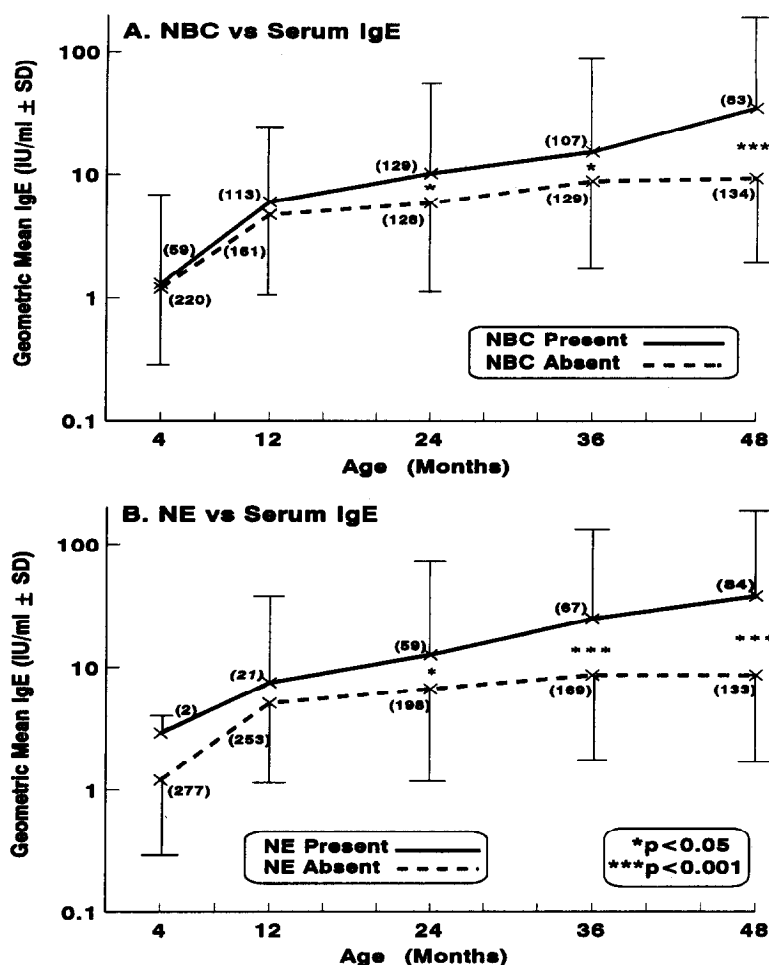


FIG. 5. Geometric mean serum IgE levels from ages 4 months to 4 years related to the presence or absence of NBCs (A) and NEs (B).

compared with those without NBCs and NEs demonstrated significantly higher geometric mean serum IgE levels from ages 2 to 4 years (Fig. 5).

**Relationship to serum IgG BLG and IgG OVA levels.** In the prophylactic-treated but not control group, significant but weak correlations were noted between log IgG BLG and NBCs at 2 years ( $r = 0.29$ ,  $n = 96$ ,  $p < 0.01$ ) and NEs at 4 months ( $r = 0.34$ ,  $n = 100$ ,  $p < 0.001$ ). No relationships occurred between NBCs or NEs and log IgG OVA in either group.

**Relationship to parental smoking.** NBCs were found in greater prevalence at age 2 years (33/52 [63.5%] vs 96/208 [46.2%],  $p < 0.05$ ) and in greater quantity at ages 3 and 4 years ( $p < 0.05$ ) (data not presented) in children of patients who smoked compared with children of parents who did not smoke. At ages 1 and 2 years, NEs occurred in higher prevalence and quantity ( $p \leq 0.01$ ) (data not presented) in chil-

dren of parents who smoked. In addition, a direct but weak correlation was noted between NEs and number of cigarettes smoked daily by the mother when the child was 2 to 4 years of age ( $S_r = 0.21 - 0.23$ ,  $p < 0.01$ ).

#### Factors contributing to the variance in NBC and NE levels

**NBCs.** Stepwise multiple linear regression analyses revealed that the variance in NBCs was affected to varying degrees by the following factors (Table III): (1) principally allergic rhinitis from ages 1 to 4 years, reaching 47% at the latter age, (2) asthma at age 4 months, (3) atopic dermatitis at ages 4 months and 4 years, (4) food SPTs from ages 2 to 4 years, (5) log IgG BLG at age 2 years, and (6) parental smoking when the child is 4 years old. At 4 years of age, 54% of the variance in NBCs was attributed to identified covariates (Table III).

*NEs.* Similar linear regression analyses of the variance in NEs revealed that allergic rhinitis accounted for 40% to 53% of the variance from ages 1 to 4 years (Table III). Other covariates with lesser effect on the variance in NEs included asthma at 3 and 4 years of age, atopic dermatitis and food allergy at 3 years of age, inhalant skin test positivity and control group at 1 year of age, and log serum IgE at 4 years of age. At ages 3 and 4 years these covariates together comprised nearly 60% of the variance in the level of NEs.

## DISCUSSION

This is the largest study to date to examine prospectively the development of NBCs and NEs in children of atopic parents, providing insight into the natural history of these cells in children from ages 4 months to 4 years. NBCs were observed in both atopic and nonatopic children in infancy and early childhood, but in nonatopic children they occurred in low prevalence and numbers, decreasing after 2 years (Table I and Figs. 1 and 2). NBCs occurred in a greater prevalence of subjects and numbers in the presence of atopic disease (Figs. 1 and 2) (principally allergic rhinitis [Table III] and food and inhalant sensitization [Figs. 3 and 4]) and correlated with serum IgE levels (Table II). The following findings were elucidated about NEs: they rarely developed in early infancy (Table I, Figs. 1 to 4); they occurred in low prevalence and numbers in nonatopic patients (Figs. 1 and 2); they correlated with NBCs in children from ages 4 months to 4 years but most strongly at 4 years of age (Table II); they increased in prevalence (Fig. 1) and in quantity (Fig. 2) with atopic disease (principally with allergic rhinitis [Table III]) and with skin test reactivity (Figs. 3 and 4); they correlated moderately with serum IgE levels at ages 3 and 4 years (Table II); and they increased in association with parental smoking. In addition, maternal-infant food allergen avoidance had little to no influence on NBCs and NEs because it failed to affect NBC development and only minimally affected NEs at 1 year as noted in linear regression (Table III) but not univariate analysis.

Although a more quantitative determination of NBCs and NEs would have been optimal, the semi-quantitative grading of NBCs and NEs in the present study showed acceptable reproducibility. A previous report<sup>15</sup> has related the grading scale used in this study to ranges of NBCs and NEs present per high-power field (HPF) spanning for NBCs from  $\frac{1}{2}+ = 0.1 - 0.3$  cells per HPF to  $4+ = >6.0$  cells per HPF and for NEs from  $\frac{1}{2}+ = 0.1 - 1.0$  cells per HPF to  $4+ = >20$  cells per HPF. Future

studies would benefit from a recently described, quantitative, yet more laborious, cytopsin technique for determining concentrations of NBCs and NEs within and on the nasal mucosal.<sup>11</sup> The present study did not determine the subtypes of NBCs or NEs within the nasal mucosa. With use of immunohistochemical techniques to determine subtypes of NBCs by the neutral protease content and NEs by the content of the cleaved form of eosinophil cationic protein,<sup>2</sup> additional studies should be able to identify the natural history of the subtype pattern of NBCs and NEs in nasal mucosa characteristic of nonatopic patients and various atopic disorders.

Before the present and other recent studies,<sup>10, 11</sup> the natural history of NBCs and NEs had been inadequately examined. Nasal mucosal scraping and biopsy techniques have demonstrated that normal nasal mucosal cytology from birth to age 18 months in healthy subjects consists of ciliated and nonciliated epithelium, goblet cells, and basal cells interspersed on occasion with small numbers of NBCs, NEs, and neutrophils.<sup>10, 11</sup> Recently Borres et al.<sup>11, 18</sup> studied the development of NBCs and NEs with a quantitative cytopsin technique in 64 infants from 3 months to 18 months of age, and noted that NBCs were found in the nasal mucosa of nonatopic infants of atopic parents but not in nonatopic infants of nonatopic parents. In agreement with findings in the present study, Borres et al.<sup>18</sup> also noted that NBCs were more prevalent in atopic than nonatopic infants, often appearing before the manifestation of both respiratory and skin (atopic dermatitis) allergy, and were associated with higher levels of serum IgE levels at 18 months.<sup>18</sup> Similar to findings in the present study, Kemp et al.<sup>19</sup> noted that the presence of any NBCs within the nasal mucosa was associated with a higher prevalence of positive skin tests (Fig. 3).

Past studies disagree with respect to the natural history of NEs in the perinatal period. Matheson et al.<sup>20</sup> found nasal secretion eosinophilia in nearly 40% of 129 newborns, which persisted through the first postnatal month, disappeared by ages 1½ to 3 months, only to reemerge in approximately 20% from ages 3 to 12 months. Crawford<sup>21</sup> noted 26 of 50 healthy newborns with some NEs in nasal secretions, but in contrast to Matheson et al.,<sup>20</sup> only 1 of 50 had NEs by 3 months. With use of a nasal scraping procedure combined with cytopsin preparation and cell enumeration, small numbers of NEs were also found by Borres et al.<sup>11</sup> in 12 of 25 nonatopic children 3 months to 18 months of age. Cohen et al.<sup>10</sup> found rare to occasional NEs in 3 of 22 (13.6%) healthy children at varying

**TABLE III.** Factors affecting the variance in NBCs and NEs from 4 months to 4 years in offspring of atopic parents determined by stepwise multiple linear regression

| Age   | Variance in NBCs and NEs explained by individual $r^2$ in percent* |      |        |     |                   |     |              |     |          |    |                    |     |
|-------|--------------------------------------------------------------------|------|--------|-----|-------------------|-----|--------------|-----|----------|----|--------------------|-----|
|       | Allergic rhinitis                                                  |      | Asthma |     | Atopic dermatitis |     | Food allergy |     | Food SPT |    | Inhalant skin test |     |
|       | NBC                                                                | NE   | NBC    | NE  | NBC               | NE  | NBC          | NE  | NBC      | NE | NBC                | NE  |
| 4 Mo. | —                                                                  | —    | 2.6    | —   | 2.3               | —   | —            | —   | —        | —  | —                  | —   |
| 1 Yr. | 14.2                                                               | 39.7 | —      | —   | —                 | —   | —            | —   | —        | —  | —                  | 3.3 |
| 2 Yr. | 23.4                                                               | 46.3 | —      | —   | —                 | —   | —            | —   | 1.7      | —  | —                  | —   |
| 3 Yr. | 40.0                                                               | 53.4 | —      | 4.2 | —                 | 0.8 | —            | 2.4 | 2.4      | —  | —                  | —   |
| 4 Yr. | 47.8                                                               | 52.6 | —      | 2.6 | 3.8               | —   | —            | —   | 1.0      | —  | —                  | —   |

\* $p < 0.05$  for all notations.

ages from birth to 1 year of age using a nasal scraping technique. Unlike most prior studies in which NE data were combined over varying age ranges, the current study presented NE data at specific ages. In the present study NEs were found in less than 1.0% and 3.0% of nonatopic subjects at ages 4 and 12 months, respectively (Fig. 1, *D*), and increased both in prevalence and quantity in atopic children from ages 1 to 4 years (Figs. 1, *D* and 2, *D*). The correlation between NBCs and NEs at 18 months ( $r = 0.40$ ) noted by Borres et al.<sup>18</sup> fell between the values found in the present study at ages 1 and 2 years ( $S_r = 0.35$  and  $0.60$ , respectively, Table II) and was considerably lower than the  $S_r = 0.84$  at age 4 years (Table II).

**Factors affecting NBC and NE variance (Table III).** The magnitude of the NBC and NE response in patients from 1 to 4 years of age was related to the development (presence) of allergic rhinitis, because nearly 50% of the variance in the quantity of these cells could be accounted for by allergic rhinitis by age 4 years (Table III). However, other atopic manifestations, immunologic responses, and demographic covariates also affected NBC and NE variance throughout the study, although, at much lower magnitudes and therefore lower importance. Nevertheless, the contribution of atopic dermatitis, food allergy, and food SPT to NBC and NE variance in the present study is consistent with the increased concentration of NBCs in children with atopic dermatitis without respiratory allergy noted before 18 months by Borres et al.,<sup>18</sup> and the observed increase in NBCs and NEs after food challenge in children with respiratory allergies.<sup>22</sup> The presence of NBCs and NEs in atopic dermatitis, urticaria, and food allergy may point to the future development of respiratory allergy.

Nearly all of the association of NBCs and NEs to serum IgE levels (Table II and Fig. 5) is best explained

by the development of allergic rhinitis because less than 1% of the variance of NEs could be attributed by linear regression to the serum IgE level and then only at age 4 years (Table III). Parental smoking was associated with higher levels of NBCs and NEs at several ages during the study as determined in univariate analyses. Because parental smoking accounted for approximately 2% of the variance in NBCs by age 4 years, second-hand smoke may represent an added risk for the future development of respiratory allergies, which has been noted previously for asthma.<sup>12, 23, 24</sup>

The IgG BLG response in infancy has been shown to relate to the following: the quantity introduced and age of introduction and weaning of cow milk before 1 year, variance in serum IgE levels at 4 months, food allergy at 4 months and 1 year, and inhalant sensitization at 2 years.<sup>25</sup> The greater IgG BLG response at 2 years of age in children in whom inhalant sensitization subsequently developed by 4 years may help explain the interrelationship between the IgG BLG response and NBCs (Table III).<sup>25</sup>

### Summary and perspective

The present study delineates prospectively the development of NBCs and NEs up to 4 years of age in children of atopic parents and demonstrates the strong association of these cells with the development of atopic disorders (most prominently allergic rhinitis), immediate skin test sensitization, and elevated serum IgE levels. Moreover, in contrast to the nonatopic patients in whom NBCs and NEs remained low or declined during early childhood, in the atopic subject NBCs and NEs increased in number and prevalence from ages 4 months to 4 years. Such findings highlight the association of NBCs and NEs with atopy, confirm their usefulness in monitoring the development of atopy, and demonstrate their modulation by environ-

Variance in NBCs and NEs explained by individual  $r^2$  in percent\*

| Log serum IgE level |     | Log IgG BLG |    | Parents smoke |    | Control group |     | Multiple $r^2 \times 100$ |      |
|---------------------|-----|-------------|----|---------------|----|---------------|-----|---------------------------|------|
| NBC                 | NE  | NBC         | NE | NBC           | NE | NBC           | NE  | NBC                       | NE   |
| —                   | —   | —           | —  | —             | —  | —             | —   | 4.9                       | —    |
| —                   | —   | —           | —  | —             | —  | —             | 1.4 | 14.2                      | 44.4 |
| —                   | —   | 2.4         | —  | —             | —  | —             | —   | 27.5                      | 46.3 |
| —                   | —   | —           | —  | —             | —  | —             | —   | 42.4                      | 60.8 |
| —                   | 0.9 | —           | —  | 1.8           | —  | —             | —   | 54.4                      | 56.1 |

mental factors such as exposure to parents who smoke. Future studies should elucidate the natural history of the local growth factors that stimulate NBC and NE growth and differentiation.

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## Determinants of patient compliance with allergen immunotherapy

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**Background:** Allergen immunotherapy (AIM) has been demonstrated to be safe and effective in the treatment of allergic respiratory disease. Although it requires considerable patient commitment, little attention has been paid to the factors that contribute to patient compliance.

**Methods:** Patients in a practice based at an urban university hospital, who discontinued AIM, were contacted to determine their reasons for stopping treatment. Results were correlated with demographic and disease data obtained from the patient charts.

**Results:** Review of practice records identified 217 patients on AIM. One hundred forty-eight had allergic rhinitis (R), 66 had allergic rhinitis and asthma (RA), and 3 had allergic asthma without evidence of rhinitis (A). Seventy-four (50%) of the patients in the R group, 32 (48%) of the patients in the RA group, and three (100%) of those in the A group discontinued treatment. The groups differed in that 55% of the patients in the R group discontinued treatment because of inconvenience, compared with only 22% of the patients in the RA group ( $p < 0.01$ ). By contrast, 25% of the patients in the RA group stopped because they thought that they were "better" with medication, compared with none of the patients in the R group ( $p < 0.001$ ). All of the patients in the RA group who stopped because they were better completed at least the first four-vial treatment series.

**Conclusions:** Inconvenience is the major contributing factor in noncompliance with AIM. Regulations designed to protect patients from adverse reactions should balance the potential risk of reactions against the benefits of treatment and the effect of more stringent guidelines on patient compliance. (*J ALLERGY CLIN IMMUNOL* 1993;91:734-7.)

**Key words:** Allergens administration and dosage, asthma, allergic rhinitis, desensitization immunologic adverse effect, hay fever therapy, pollen immunology, asthma therapy, patient compliance

There has been considerable interest in recent years in allergen immunotherapy (AIM) for the treatment of allergic rhinitis and asthma. Particular attention has been paid to the risks associated with allergen im-

### Abbreviation used

AIM: Allergen immunotherapy

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munotherapy, and various safety criteria have been proposed to minimize life-threatening complications.<sup>1-3</sup> By contrast, no published studies exist addressing the factors that determine patient compliance with immunotherapy prescribed by their physician. These factors have potentially important implications at a time when there is increasing concern about deaths from asthma<sup>4</sup> as well as increasing evidence that AIM offers an effective additional avenue of treatment for that disorder.<sup>5-9</sup>