

Original article

Influence of early day-care exposure on total IgE levels through age 3 years

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Background: Early day care is inversely associated with asthma and atopy in later childhood, but its association with early immunologic markers of asthma risk is not known.

Objective: We sought to assess the relation of day care by 3 months to total IgE levels through age 3 years.

Methods: Day care was assessed prospectively among 362 nonselected infants enrolled in the Infant Immune Study. Children were categorized based on day-care status by 3 months of age as follows: no day care, day care inside the home with other children, day care outside the home with no other children, or day care outside the home with other children. Total IgE levels were measured in blood obtained at 3, 12, 24, and 36 months. Relations between day care and IgE levels were assessed at each age and longitudinally, with stratification by maternal asthma and atopy.

Results: Day care by 3 months was associated with decreased IgE levels through age 3 years (coefficient: $-0.19 \log \text{ IU/mL}$, $P = .001$). The greatest effect was evident for children cared for outside the home. Stratified analyses indicated that the relation existed primarily among children who had atopic or asthmatic mothers. Day-care entry after 3 months showed no relation with IgE levels.

Conclusion: Day-care attendance by 3 months is associated with decreased total IgE levels in the first 3 years of life in children of mothers who are atopic, asthmatic, or both.

Clinical implications: Early day-care exposure can reduce IgE levels, which in turn might reflect a reduced risk of allergic disease in predisposed children. (J Allergy Clin Immunol 2007;119:1016-1024.)

Key words: IgE, day care, atopy, asthma, infancy, microbial exposure, hygiene hypothesis

Several studies in the industrialized world have shown that day-care exposure appears to protect against asthma. Ball et al¹ showed that children who attended large group

Abbreviations used

OR: Odds ratio

RR: Relative risk

day care in early life had higher rates of wheeze in early childhood but by age 8 years were significantly less likely to have physician-diagnosed asthma with current symptoms. In a selected population Celedon et al² found that day care in the first year was associated with lower asthma prevalence at age 6 years. Other researchers have documented similar protective effects of day care for the development of asthma³ and atopy.^{4,5}

Although it is generally thought that the pathway by which day care influences asthma risk might involve the immune system, relatively few studies have investigated relations of day-care exposure with immune system outcomes directly. Celedon et al⁶ found that total IgE levels were significantly lower at 4 years of age among children who had attended day care in the first 6 months. Although Hoffjan et al⁷ found that day care interacted with genotype to influence production of certain cytokines, data regarding the effect of day care alone on cytokine production was not provided. Furthermore, both of these studies were conducted in high-risk cohorts, and thus it is not known whether the same relations would apply to nonselected populations.

Several additional questions about the relation of day care to subsequent asthma remain. First, it is not known whether there is a critical window of vulnerability for entry into day care, after which day-care attendance will have little effect on allergic outcomes. Second, it is unknown what aspects of exposure to day care might influence risk for allergic outcomes. Ball et al¹ and others⁸⁻¹⁰ have postulated that the key characteristic is exposure to other children, but other elements of day care might be relevant. Third, most studies have been cross-sectional, and thus it is not known how early in life an effect on allergic outcomes might be evident. Finally, it is important to determine whether host susceptibility, as reflected by parental asthma and atopy, might influence the relation.

The current study assessed relations of early day care with total and allergen-specific IgE levels at 4 time points in the first 3 years of life in a nonselected population followed from birth. In addition, we investigated whether

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characteristics of day care and host susceptibility to allergic disease, as reflected in maternal atopy and asthma, influenced the relations observed.

METHODS

Study design and population

The Infant Immune Study is a prospective birth cohort study of immune system maturation and its relation to the development of asthma and allergic disease in childhood. Participants were healthy children born to pregnant women who planned to obtain care for their newborns from collaborating pediatricians. Women were contacted at 32 to 35 weeks' gestation to obtain informed consent and to begin data collection on maternal factors.

IgE measurements

Blood specimens were obtained at 3 months ($n = 363$; mean $\pm$ SD age, 2.87 ± 1.22 months), 1 year ($n = 362$; 12.97 ± 1.66 months), 2 years ($n = 295$; 25.37 ± 2.02 months), and 3 years ($n = 280$; 38.19 ± 3.87 months). Total and allergen-specific IgE levels were measured by using the Pharmacia AutoCAP assay (Pharmacia/Upjohn, Kalamazoo, Mich) before its discontinuation in 2006 and subsequently by using Immulite 2000 (Siemens Medical Solutions, Los Angeles, Calif). Samples analyzed on both instruments ($n = 25$) yielded a correlation coefficient of 0.995. Total IgE values were log transformed (log 10) and age adjusted for all analyses. Specific IgE levels were measured for 6 inhalant allergens (*Alternaria* species, *Dermatophagoides farinae*, Bermuda grass, careless weed, olive, and mulberry tree) at 1, 2, and 3 years of age and 2 foods (ovalbumin and β -lactoglobulin) at 3 months and 1, 2, and 3 years of age. Inhalant allergen-specific IgE levels were not measured at 3 months because of extremely low levels of detectability. Each allergen-specific IgE was first categorized as detectable or not detectable, from which dichotomous variables were created categorizing children as having any inhalant-specific IgE, any food-specific IgE, or any specific IgE (having either food- or inhalant-specific IgE).

Assessment of day care

Information on day-care attendance was obtained by means of telephone or personal interviews with parents at 1, 2, 3, 4, 6, and 9 months. For this analysis, we focused on day-care exposure by 3 months so that the exposure of primary interest preceded the first blood draw. Day-care status by 3 months of age was categorized as no day care (62.4%), day care inside the home with other children (7.5%; ie, nonsiblings), day care outside the home with no other children (8.0%), and day care outside the home with other children (22.1%). For some analyses, the last 3 categories were combined into a group receiving any day care. In other analyses the first 2 categories were combined to form a group not receiving day care outside the home, which was compared with the group receiving any day care outside the home formed by combining the last 2 categories. To assess whether there was a critical window of vulnerability for day-care exposure in relation to IgE, we created a day-care timing variable to determine whether associations with IgE differed depending on whether entry into outside day care was by 3 months or between 4 and 9 months.

Covariates

Information on infant feeding practices was obtained on the same questionnaire. Children were classified as to how they were fed at 3 months: exclusively breast-fed, formula fed only, or receiving both human milk and formula (mixed) at 3 months. Information on most other potential confounders was obtained by means of questionnaire before or soon after the infant's birth. These included ethnicity (2 non-Hispanic white parents vs 1 or more parent of another ethnicity),

parental age, parental education, parity, number of children in the household, maternal smoking at birth and 3 months, presence of dogs or cats, and whether the mother or father had ever been given a diagnosis of asthma. To assess parental atopy, skin prick tests were performed on parents shortly after the child's birth by using 17 allergen extracts reflecting a range of indoor and outdoor exposures common in the Tucson area, as described previously.¹¹ A parent was considered to be atopic if he or she had at least 1 positive skin test reaction (horizontal and vertical wheal sum of ≥ 3 mm after subtraction of the reaction to a negative glycerin control) to any of the allergens tested. Blood was drawn from parents at the same time for determination of total IgE levels.

Statistical analysis

Differences in parental and children's traits by day-care exposure (in 4 categories) were assessed by using χ^2 tests for categorical variables and by using 1-way ANOVA for continuous variables. Comparisons between raw total IgE levels at each age and day-care exposure were also assessed by using 1-way ANOVA.

Random effects models and generalized estimating equations were used to assess longitudinal differences in total IgE levels and detectable allergen-specific IgE levels, respectively, at ages 3, 12, 24, and 36 months in relation to day-care status by 3 months of age. These longitudinal models provide predictive effect measures while adjusting for within-subject correlations. Both fixed covariates (eg, sex and ethnicity) and age, a time-dependent covariate, were included in the models. Initially, marginal analyses were performed at each age to assess the significance of covariates believed to be associated with IgE or day care, either from the literature or from previous analyses. Each age-specific base model included day-care status and the potential covariate being assessed. Covariates that had a significance of less than or equal to 0.1 or that were considered important based on the literature were retained for the longitudinal random effects model. From this longitudinal model, covariates that had a significance level of less than 0.1 were again retained to create best-fitting longitudinal models for total IgE and detectable specific IgE as a function of day care. Age at the time of blood draw was included as a covariate in all longitudinal models.

Because the effect of day care on allergic outcomes might differ by parental risk factors, we repeated the longitudinal models for 3 groups: mothers with neither asthma nor atopy, mothers with atopy only, and mothers with asthma regardless of atopy. Finally, the relation of total IgE level at ages 1, 2, and 3 years by timing of outside day-care entry through 9 months was assessed by using random effects models, adjusting for the same covariates.

STATA version 8.2 (StataCorp, College Station, Tex) was used for statistical analyses. A 2-sided P value of .05 was considered significant for all tests. This research was approved by the Institutional Review Board of the University of Arizona. Parents signed consent forms for initial enrollment and separate forms for testing at each age.

RESULTS

IgE levels were assessed at least once in 434 children. Compared with subjects for whom IgE data were not obtained at any age ($n = 50$), these children were significantly more likely to have received some human milk at 3 months (68.6% vs 31.0%, $P < .001$), less likely to have smoking mothers at 3 months (7.8% vs 20.0%, $P = .022$), more likely to have 2 non-Hispanic white parents (60.5% vs 35.4%, $P = .001$), and more likely to have older parents (mothers' mean age, 29.2 vs 26.2 years [$P = .001$] fathers' mean age, 31.4 vs 27.7 [$P < .001$]) with more years of

TABLE I. Parental and child traits by day-care status by 3 months of age, unless otherwise noted

	No day care (% [n])	Day care inside the home with others (% [n])	Day care outside the home without others (% [n])	Day care outside the home with others (% [n])
Total	62.4 (226)	7.5 (27)	8.0 (29)	22.1 (80)
Sex				
Male	65.7 (109)	6.0 (10)	9.6 (16)	18.7 (31)
Female	59.7 (117)	8.7 (17)	6.6 (13)	25.0 (49)
Ethnicity				
Anglo-Anglo	66.1 (152)	5.2 (12)	7.4 (17)	21.3 (49)
Hispanic/other	56.5 (74)	11.4 (15)	9.2 (12)	22.9 (30)
Feeding at 3 mo†				
Exclusive BF	75.6 (68)	4.4 (4)	5.6 (5)	14.4 (13)
Mixed fed	65.8 (108)	7.9 (13)	7.3 (12)	18.9 (31)
Exclusive FF	45.8 (49)	9.4 (10)	11.2 (12)	33.6 (36)
Mother's education†				
Mean y (SD)	15.7 (2.9)	14.0 (2.5)	14.2 (2.5)	15.4 (2.6)
Mother's age‡				
Mean y (SD)	30.2 (5.7)	25.9 (4.6)	27.6 (6.6)	29.0 (5.8)
Maternal atopy				
No	60.9 (67)	10.0 (11)	8.2 (9)	20.9 (23)
Yes	64.0 (153)	5.9 (14)	8.0 (19)	22.2 (53)
Paternal atopy*				
No	53.5 (38)	12.7 (9)	8.4 (6)	25.4 (18)
Yes	69.0 (171)	4.4 (11)	7.7 (19)	19.0 (47)
Maternal asthma				
No	63.2 (179)	7.4 (21)	8.1 (23)	21.2 (60)
Yes	63.2 (43)	5.9 (4)	5.9 (4)	25.0 (17)
Paternal asthma				
No	64.6 (186)	6.9 (20)	7.3 (21)	21.2 (61)
Yes	61.4 (27)	2.3 (1)	13.6 (6)	22.7 (10)
Maternal IgE				
Mean log IU/mL (SD)	1.41 (0.70)	1.28 (0.68)	1.32 (0.70)	1.31 (0.70)

BF, Breast-feeding; FF, formula feeding.

* .01 < *P* < .05.† .001 < *P* < .01.‡ *P* < .001.

education (mothers' mean, 15.3 vs 13.8 years [*P* = .001]; fathers' mean, 15.1 vs 13.8 years [*P* = .005]). Children with IgE data did not differ significantly from those without IgE data in use of day care, number of children in the household, prenatal maternal smoking, dogs or cats in the household, sex, maternal parity or IgE, and parental asthma or atopy.

Data on day-care status by 3 months of age were available for 362 (83.4%) of the 434 children whose IgE levels had been measured. There was no significant difference in IgE level at any age between children who did or did not have information on day care. **Table I** displays the characteristics of these subjects with respect to day-care status in 4 categories. Day care was significantly associated with reduced intake of human milk (*P* = .002) and varied with maternal education (*P* = .003), maternal age (*P* < .001), and paternal atopy (*P* = .028).

Total IgE levels increased with age, with geometric mean levels being 1.3 IU/mL (95% CI, 1.2-1.5 IU/mL) at 3 months, 8.9 IU/mL (95% CI, 7.7-10.2 IU/mL) at 12 months, 15.0 IU/mL (95% CI, 12.6-17.9 IU/mL) at 24 months, and 16.4 IU/mL (95% CI, 13.8-19.5 IU/mL) at 36

months. These values were comparable with those shown elsewhere for children of these ages.¹² Percentages of detectable specific IgE at each age are presented in **Tables E1 to E3** (available in this article's Online Repository at www.jacionline.org).

Relation of day care by 3 months to IgE

As shown in **Fig 1**, total IgE levels differed significantly at each age across the 4 day-care exposure groups based on ANOVA multiple-comparison tests. When day-care exposure categories were combined, children attending day care of any kind by 3 months were found to have significantly lower total IgE levels at each of the 4 ages assessed than children who did not attend day care (0.02 vs 0.18 log IU/mL [*P* = .007] at 3 months; 0.81 vs 1.01 log IU/mL [*P* = .004] at 1 year; 1.04 vs 1.29 IU/mL [*P* = .005] at 2 years; and 1.07 vs 1.31 IU/mL [*P* = .009] at 3 years).

Table II shows the difference in total IgE levels at each age associated with day-care exposure ("base model"). The difference in total IgE levels at each age associated with each significant or marginally significant covariate

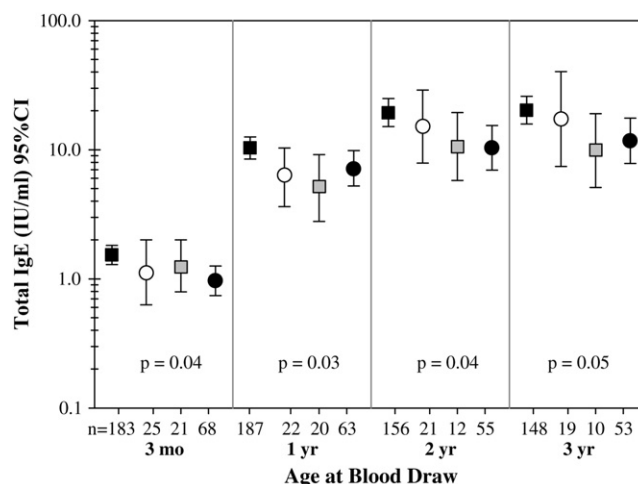


FIG 1. Total IgE at 3 months and 1, 2, and 3 years by day-care exposure at 3 months. *P* values were calculated by means of ANOVA. *Solid squares*, No day care; *open circles*, day care inside the home with others; *shaded squares*, day care outside the home without others; *solid circles*, day care outside the home with others.

TABLE II. Predicted difference in log IgE by day-care status and age at blood draw

	3 mo		12 mo		24 mo		36 mo	
	Coefficient	<i>P</i> value	Coefficient	<i>P</i> value	Coefficient	<i>P</i> value	Coefficient	<i>P</i> value
Base model								
No day care	Reference	—	Reference	—	Reference	—	Reference	—
Day care inside the home with others	−0.14	.228	−0.21	.114	−0.11	.589	−0.07	.746
Day care outside the home without others	−0.09	.396	−0.30	.019	−0.26	.087	−0.31	.051
Day care outside the home with others	−0.20	.006	−0.16	.050	−0.27	.009	−0.24	.021

individually is shown in Table E4 (available in this article's Online Repository at www.jacionline.org). Sex, ethnicity, maternal IgE level, maternal age, and maternal asthma all showed significant association ($P < .05$) with total IgE production at 1 or more time points when considered with day care. Exclusive formula feeding, the number of children in the household, paternal IgE, and maternal education were of marginal significance ($.05 < P < .10$) in relation to total IgE at 1 or more time points when considered with day care. Maternal smoking either during pregnancy or in the first months after the child's birth, dogs or cats in the home, and parental atopy showed no relation to total IgE (data not shown).

In the longitudinal model any day care by 3 months of age was associated with decreased total IgE levels through age 3 years (coefficient: -0.19 log IU/mL, $P = .001$). In our overall population day care outside the home with no other children was associated with significantly reduced total IgE levels through 36 months (coefficient: -0.23 log IU/mL, $P = .023$), as was day care outside the home with other children (coefficient: -0.19 log IU/mL, $P = .006$). Day care inside the home with other children by 3 months also appeared to reduce total IgE levels, but the effect was not statistically significant (coefficient: -0.15 log IU/mL, $P = .160$). Total IgE levels were also reduced in association with female sex (coefficient: -0.21 log IU/

mL, $P < .001$) and positively associated with maternal IgE (coefficient: 0.14 log IU/mL per log unit of maternal IgE, $P < .001$). The number of children within the day-care setting, whether considered as a continuous or categorical variable, showed no relation to total IgE (data not shown).

Similar relations to those observed between day care and total IgE levels were observed for allergen-specific IgE, despite smaller group sizes and low prevalence of specific IgE at these ages. In longitudinal analysis the risk of having any detectable specific IgE through age 3 years was decreased by any day care (relative risk [RR], 0.71; $P = .018$). This relation was significant for any food allergen-specific IgE (RR, 0.63; $P = .015$) but not for any inhalant allergen-specific IgE (RR, 0.80; $P = .312$). In comparing the 3 categories of day care with no day care, only children attending day care outside the home with others were significantly less likely to have any detectable specific IgE (RR, 0.69; $P = .043$). This group, day care outside with others, showed similar, although nonsignificant, relations to both any food-specific and any inhalant-specific IgE (RR of 0.64 [$P = .063$] and RR of 0.76 [$P = .299$], respectively). Tables E1 to E3 (available in this article's Online Repository at www.jacionline.org) provide a full breakdown of percentages of children with any food-specific, any inhalant-specific, and any detectable specific IgE by day-care status and age at blood draw.

TABLE III. Predicted difference in log IgE through age 3 years by day-care status with significant covariates and stratified by maternal asthma and maternal atopy, adjusted for age

	Nonasthmatic nonatopic mother (n = 93)*		Atopic nonasthmatic mother (n = 175)*		Asthmatic mother with or without atopy (n = 66)*	
	Coefficient	P value	Coefficient	P value	Coefficient	P value
No day care	Reference	—	Reference	—	Reference	—
Day care inside with others	0.01	.957	−0.14	.362	−0.35	.184
Day care outside without others	0.03	.921	−0.26	.032	−0.62	.011
Day care outside with others	−0.08	.619	−0.18	.050	−0.37	.008
Sex (female)	−0.41	.001	−0.09	.211	−0.11	.343
Maternal prenatal IgE (per log unit)	0.09	.342	0.12	.058	0.14	.123

*Children without data on maternal atopy, asthma, or both were excluded from these models.

TABLE IV. Predicted difference in log IgE through age 3 years by timing of entry into day care outside the home with significant covariates for study population and stratified by maternal asthma and maternal atopy, adjusted for age

	Nonasthmatic nonatopic mother (n = 85)*		Atopic nonasthmatic mother (n = 163)*		Asthmatic mother with or without atopy (n = 63)*	
	Coefficient	P value	Coefficient	P value	Coefficient	P value
No day care (outside the home)	Reference	—	Reference	—	Reference	—
Day care entry by 3 mo	−0.07	.652	−0.19	.040	−0.36	.021
Day care entry between 4 and 9 mo	−0.26	.123	0.03	.786	0.28	.118
Sex (female)	−0.50	<.001	−0.13	.109	−0.11	.421
Maternal prenatal IgE (per log unit)	0.09	.375	0.13	.061	0.21	.038

*Sample excludes children for whom day-care entry through 9 months could not be determined.

Stratification by maternal asthma and maternal atopy

The relation between day care and total IgE differed by maternal history of asthma and atopy (Table III). There was no significant relation between day care of any type and total IgE for children of nonasthmatic and nonatopic mothers. In contrast, children of nonasthmatic atopic mothers and children of asthmatic mothers both showed significant reductions in total IgE levels that were statistically significant for the 2 outside-the-home day-care categories when compared with the group not receiving day care.

This stratified analysis was not completed for allergen-specific IgE because convergence of generalized estimating equation models was not achieved in all cases.

Relation of timing of day-care exposure to total IgE

Initiation of day care outside the home at or before 3 months of age was associated with significantly reduced total IgE levels (coefficient: -0.20 log IU/mL, $P = .008$) for the group as a whole. In contrast, day-care entry between 4 and 9 months appeared to have no effect on total IgE levels (coefficient: -0.01 log IU/mL, $P = .854$). Table IV shows the relation between timing of day-care exposure and total IgE stratified by maternal history of atopy and asthma. For children whose mothers had neither atopy nor asthma, there was no significant decrease in total IgE levels with day-care entry either before 4 months or

between 4 and 9 months of age. Initiation of day care outside the home after 3 months of age also showed no significant relation with total IgE levels in children of asthmatic mothers or women with atopy only.

DISCUSSION

This study indicates that day-care exposure by 3 months of age is associated with decreased total and allergen-specific IgE levels through age 3 years. The relation between day care and total IgE level was consistent for each time point assessed (3 months and 1, 2, and 3 years), in longitudinal analysis including all time points, and after adjusting for potential confounders. Day-care attendance outside the home showed a stronger relation to decreased total IgE levels than did exposure to day care inside one's own home with other unrelated children. These relations were limited to children of atopic and asthmatic mothers. Finally, day-care entry before 3 months of age was critical for the effect.

Since Strachan¹³ observed that the risk of hay fever was inversely related to the number of children in the household, the postulate that exposure to other children in early life might reduce the risk for subsequent allergic disease has been tested. Indeed, several studies have shown an inverse association between the number of siblings and the prevalence of asthma,^{1,3,10} hay fever,^{8-10,14} and atopy.¹⁵ A focus on exposure to other children in day-care settings has also demonstrated similar protective effects for

asthma¹⁻³ and atopy^{4,5} and a decrease in IgE levels.⁶ If the critical component of day-care exposure is the number of other children, one might expect to see an inverse dose response between the outcome and number of children in the setting, as Strachen¹³ showed for hay fever and number of siblings. However, few studies have investigated the effects of differing numbers of children in day care on allergic outcomes. One exception is the study by Celedon et al,² who report no difference in effect between day-care settings of more than 10 or less than 10 other children. The current study found the relation of IgE level to day care to be unrelated to the number of children within the day-care setting or the child's own household.

In contrast to the number of children, we found that the most salient feature of day care with regard to IgE was whether the exposure took place outside the home. To our knowledge, no other study has assessed specifically whether relations with allergic outcomes differ depending on whether the child is cared for inside or outside the home. Celedon et al² reported no differences between residential or nonresidential day care but did not distinguish residential day care within the child's own home from day care taking place in a different home. In an Australian study Haby et al⁴ found that the odds of developing atopy by 3 to 5 years of age were much lower for children who received care by 3 years of age in a day-care center (odds ratio [OR], 0.26; 95% CI, 0.14-0.50) versus in another home (OR, 0.66; 95% CI, 0.41-1.04), both relative to no day-care exposure. Like the study by Celedon et al,² these authors did not differentiate the group of children cared for in their own home with other children present. The finding of Haby et al⁴ might be consistent with ours, however, if their family day-care group included the subset of children cared for at home with other children, which would reduce the protective effect for subsequent atopy in that group.

Previous studies of the relations between day care and allergic outcomes vary in terms of the defined age range at which day-care exposure began. In several studies, day-care entry by 1 year was associated with decreased risk for allergic outcomes but not day-care entry after that age.^{1-3,6} Using day care before 1 year as a reference group, Kramer et al¹⁶ found that risk of atopy increased when children entered day care between 1 and 2 years (OR, 1.99) or after 2 years (OR, 2.72) of age. Celedon et al² reported that day care in the first year reduced recurrent wheeze, eczema, and asthma, and the strength of the association was similar for children who attended day care in the second 6 months as for those who attended in the first 6 months of life. However, the window of vulnerability might be even earlier than 1 year. In 2000, we showed (in a different cohort) that day-care entry by 6 months was significantly inversely associated to later asthma (between 6 and 13 years) when compared with day-care entry after 6 months.¹ Our current study shows that day care outside the home by 3 months of age, but not day-care entry after 3 months, was associated with decreased IgE levels when compared with those of children with no day-care exposure in the first 9 months. It should be noted that only 6.9% of subjects attended day care outside the home in the first 6 months of life in

our previous study, which enrolled children from 1980 to 1984,¹ compared with 46.8% in the current study. Thus lower rates of exposure to outside day care in earlier studies might have resulted in group sizes that were too small to ascertain effects of very early day care on allergic outcomes.

Unlike studies conducted in selected populations, we were able to assess whether the day-care effect differed by host susceptibility defined with reference to maternal asthma and atopy. Stratified analyses indicated that day-care exposure by 3 months of age was unrelated to IgE levels for children whose mothers had neither asthma nor atopy. From these results, it appears that the inverse relation between IgE and day care by 3 months is unique to high-risk children based on the mother's atopic or asthmatic status and that these groups were essentially driving the decrease in IgE levels with day care that we found in the general population. That this relation exists only for high-risk children is consistent with studies in high-risk populations^{2,6} and with findings from the cross-sectional study of Svanes et al,⁸ which reported stratified analyses by parental allergy. This limitation of the effect might also explain the negative findings from the Oslo Birth Cohort,¹⁷ in which the rate of parental atopy was considerably lower than ours, thereby diluting any possible relationship.

Our findings are consistent with the hygiene hypothesis, which posits that early-life microbial exposure might be protective against allergic disease and that reduced exposure to microorganisms might be one cause of the worldwide increase in asthma. Epidemiologic studies from Europe have convincingly shown that prevalence of asthma and atopy are reduced among children raised on farms.¹⁸⁻²⁰ Although relatively few children in the United States live on farms, many are exposed to high levels of microbes through day care, where levels of endotoxin, a component of the cell wall of gram-negative bacteria, are 3 times higher than levels found in homes.²¹ Gereda et al²² have shown that levels of endotoxin in the home are positively associated with IFN- γ production in early childhood, providing a possible mechanism underlying the day-care/IgE findings. The finding that day care outside the home was more strongly associated with decreased IgE levels than exposure to other children suggests that regular exposure to microbes from 2 different environments might play a greater role in immune development than early exposure to other children. An alternative explanation is that the mechanism might be related to the infant having a second primary caretaker. Because the effect was found in children by 3 months of age, these infants undoubtedly have more interaction with the second caretaker than with other children within a day-care setting.

A natural follow-up to the current analysis would be to assess the relation between day-care duration and IgE levels. Unfortunately, we cannot address this question directly because parents were asked only about current day-care status at the time of each questionnaire. Although we were careful to distinguish between day care inside or outside of the home and the number of nonrelated children therein, we did not inquire about the amount of time the

child was in day care between questionnaires. A second limitation is that we were unable to separate the effect of a second primary caretaker from the effect of exposure to a second home environment. Finally, although the results of this study are remarkably consistent over time and after adjustment for covariates, we acknowledge that sample sizes for 2 of the day-care groups (day care inside with others and day care outside without others) are relatively small, yielding less precise estimates in their effect on IgE levels.

In conclusion, this study shows that day-care exposure by 3 months of age is associated with reduced total IgE levels in the first years of life and that this relation is strongest for those exposed to day care outside the home. Furthermore, the protective relation of day care to IgE appears to be limited to children of atopic or asthmatic mothers. Finally, only day care initiated within the first 3 months of life was associated with low IgE levels, which offers support for the idea that a critical window of vulnerability exists during which exposures, possibly of a microbial origin, might influence immune system maturation.

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TABLE E1. Detectable inhalant allergen-specific IgE by day-care status by age at blood draw

Day-care status	Any inhalant allergen					
	1 y		2 y		3 y	
	N	Percentage detectable	N	Percentage detectable	N	Percentage detectable
No day care	183	7.6	156	14.7	150	28.0
Day care inside with others	20	5.0	12	8.3	10	40.0
Day care outside without others	22	0.0	22	13.6	19	26.3
Day care outside with others	64	10.9	58	6.9	52	19.2
<i>P</i> value (χ^2)		.390		.458		.471

TABLE E2. Detectable food allergen-specific IgE by day-care status by age at blood draw

Day-care status	Any food allergen							
	3 mo		1 y		2 y		3 y	
	N	Percentage detectable	N	Percentage detectable	N	Percentage detectable	N	Percentage detectable
No day care	181	6.1	186	19.4	156	34.0	151	37.8
Day care inside with others	21	4.8	20	10.0	12	16.7	10	10.0
Day care outside without others	25	4.0	21	14.3	21	19.0	19	26.3
Day care outside with others	65	7.7	63	19.0	58	19.0	52	21.2
<i>P</i> value (χ^2)		.910		.727		.084		.054

TABLE E3. Any detectable allergen-specific IgE by day-care status by age at blood draw

Day-care status	Any specific allergen					
	1 y		2 y		3 y	
	N	Percentage detectable	N	Percentage detectable	N	Percentage detectable
No day care	182	24.7	156	40.4	151	50.3
Day care inside with others	20	15.0	12	25.0	10	40.0
Day care outside without others	21	14.3	21	28.6	19	36.8
Day care outside with others	63	28.6	58	22.4	52	28.8
<i>P</i> value (χ^2)		.434		.073		.051

TABLE E4. Predicted difference in log IgE for significant and marginally significant covariates when considered with day-care status by 3 months of age and by age at blood draw

	3 mo		12 mo		24 mo		36 mo	
	Coefficient	P value	Coefficient	P value	Coefficient	P value	Coefficient	P value
Marginal analyses of covariates with day care (in 4 categories)								
BF: exclusive BF	Reference	—	Reference	—	Reference	—	Reference	—
Mixed fed	0.08	.302	−0.00	.972	−0.04	.682	0.01	.883
Exclusive FF	0.15	.065	−0.09	.358	−0.01	.904	0.00	.986
Children in home (per child)	0.03	.311	0.05	.100	0.01	.795	0.03	.535
Sex (female)	−0.14	.015	−0.21	.002	−0.42	<.001	−0.26	.003
Hispanic or other parent	0.18	.003	0.08	.265	0.07	.428	0.03	.744
Maternal age (per y)	−0.01	.031	0.00	.693	−0.00	.698	−0.01	.396
Maternal education (per y)	−0.02	.087	−0.00	.849	−0.01	.673	−0.01	.363
Maternal prenatal IgE (per log unit)	0.10	.025	0.19	<.001	0.15	.013	0.20	.001
Paternal IgE (per log unit)	0.09	.070	0.11	.052	0.08	.293	0.06	.401
Maternal asthma	−0.00	.993	0.08	.362	0.12	.295	0.27	.011

BF, Breast-feeding; FF, formula feeding.