

ORIGINAL ARTICLE

Chickenpox in childhood is associated with decreased atopic disorders, IgE, allergic sensitization, and leukocyte subsets

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Abstract

Background: Wild-type varicella zoster infection (WTVZV) up to 8 yr of age has been shown to protect against atopic dermatitis (AD) and asthma. We sought to determine whether WTVZV in childhood protects against atopic disorders, allergic sensitization or decreases serum Immunoglobulin E (IgE) levels.

Methods: We conducted a retrospective, practice-based study of outpatient pediatric practices in NY. One hundred children with WTVZV up to 8 yr of age and 323 children who received varicella vaccine (VV) were randomly selected.

Results: WTVZV up to 8 yr of age is associated with decreased odds of subsequent asthma (exact logistic regression; OR = 0.12, 95% CI = 0.03–0.57, $p = 0.003$), allergic rhinoconjunctivitis (OR = 0.16, 95% CI = 0.05–0.49, $p = 0.0003$), and AD (OR = 0.57, 95% CI = 0.33–0.96, $p = 0.02$), but not food allergies ($p = 0.78$); decreased total serum IgE levels [mixed linear model, LSM (95% CI): 129.09 (33.22–501.63) vs. 334.21 (102.38–1091.04) IU/ml; $p = 0.02$] remained significant at all time intervals after WTVZV (<5, 5–10, and >10) compared with VV ($p = 0.003$ –0.03). WTVZV was associated with decreased allergic sensitization (logistic regression, OR = 0.11, 95% CI = 0.03–0.38, $p = 0.0004$). WTVZV is also associated with persistently decreased numbers of peripheral blood lymphocytes ($p < 0.0001$) for up to 12 yr ($p = 0.0003$ –0.047), monocytes ($p = 0.002$) for up to 16 yr ($p < 0.001$) and basophils at ages 4–6, 10–12, and 14–16 ($p < 0.03$).

Conclusion: WTVZV up to 8 yr of age protects against atopic disorders, which is likely mediated by suppression of IgE production and allergic sensitization, as well as altered leukocyte distributions.

Abbreviations

WTVZV, wild-type varicella zoster virus infection; VV, varicella vaccine; IgE, Immunoglobulin E; OR, odds ratio; CI, confidence interval; AD, atopic dermatitis; AR, allergic rhinoconjunctivitis; CBC, complete blood count; LSM, Least squares mean(s) ; CI95, 95% confidence interval.

Atopic disorders are among the most common disorders of childhood, including atopic dermatitis (AD) and asthma. The prevalence of AD and asthma in children in the United States are 17% (1) and 9.1% (2). The etiology of these disorders is multifactorial, including genetic predisposition, socioeconomic status, and environmental factors [reviewed in (3–5)].

Recent studies from our group found that a single WTVZV up to 8 yr old protected against subsequent development of AD and asthma (6). These findings are consistent with the broader hygiene hypothesis, which suggests that infections and unhygienic exposures in childhood may confer protection against atopic disorders (7).

It is unknown whether WTVZV, in early or late childhood, is similarly protective against allergic sensitization. Further, few studies have examined the long-term effects of WTVZV on the immune system. The identification of specific cellular changes after WTVZV could help elucidate the mechanisms for its suppression of AD and asthma.

The effects of the attenuated Oka VZV exposure on cell-mediated immunity have been studied extensively (8–12). These studies found that long-term memory T-cell responses to WTVZV are produced after vaccination. However, the effects of WTVZV on cell-mediated immunity, including distribution and function of leukocyte subsets, as well as serum Immunoglobulin E (IgE) levels and allergic sensitization are not well defined.

Prior studies found conflicting results regarding the effects of WTVZV on production of specific IgE (13, 14). Nilsson et al. (13) found no significant association between WTVZV and IgE sensitization to specific allergens, while Mommers et al. (14) reported increased IgE sensitization to specific allergens after WTVZV. Other authors analyzed the acute and subacute effects of WTVZV on peripheral leukocyte distributions (15, 16). However, this study is the first, to our knowledge, to assess the effects of WTVZV on leukocyte distributions beyond 2-yr post-infection.

This study was designed to examine the relationship between exposure to WTVZV in early and late childhood and atopic disorders and atopy, including serum IgE levels, allergic sensitization, and leukocyte distributions.

Methods

The study was approved by the institutional review board for human subjects of the SUNY Downstate Medical Center, Brooklyn, NY. This retrospective study examined the effects of exposure to WTVZV (ICD9: 052.0) infection occurring up to 8 yr of age. The control group included children who received varicella vaccine (VV) and did not have WTVZV. Data were collected from electronic medical records of outpatient pediatric practices in Brooklyn, New York, between January 2000 and December 2007. Selection was performed using a computerized random-number generator list provided by a statistician who was not involved in the data collection. Inclusion criteria were children who received primary care at these practices, male or female, and age 1–18 yr. Exclusion criteria included the following medical condition(s): WTVZV after 8 yr of age, history of atopic disorders prior to WTVZV or VV, history of autoimmune disorders or immunodeficiency, hyper-IgE syndrome, hyper-eosinophilic and lymphoproliferative disorders, usage of corticosteroids or immunotherapy, personal or family history of cigarette smoking, and incomplete follow-up.

Wild-type varicella zoster virus infection

History and date of onset of clinical chickenpox were routinely screened by a physician to determine eligibility for VV, by asking parents at well visits and physical exam at all visits. Date of WTVZV was documented as the date of initial presentation of symptoms. Previous studies of Silverberg et al. (6) demonstrated that WTVZV up to 8 yr of age has protective effects against AD and asthma. Thus, only children with WTVZV up to 8 yr of age were included.

Varicella vaccine

All patients who did not have history of WTVZV were given the live attenuated VV (Varivax®; Merck and Company, Inc, West Point, PA, USA) according to the recommended schedule of the American Academy of Pediatrics (AAP) (17). From 1995 to 2006, patients received a single-dose vaccine; in 2006, the AAP's two-dose vaccine schedule was initiated. The first dose was given at 12–15 months of age. The second dose was given at 4–6 yr of age, but may have been given earlier provided that it was at least 3 months after the first dose. Children 13 yr or older received two doses at least 28 days apart according to the catch-up immunization schedule of the AAP (17). Compliance rates with VV were nearly 100% after 1998 because of mandatory immunization requirements for entry into New York City and State schools aside from parents' beliefs about vaccination.

Atopic history

At each well visit and sick visit for allergic disease, parents were routinely asked, as part of review of systems, about symptoms of allergy affecting the skin, eyes, nose/oropharynx, lower airway and gastrointestinal and any relationship to environmental and dietary triggers. The children were routinely examined by a pediatrician, and physical signs of AD, allergic rhinoconjunctivitis (AR), and wheeze were documented. Diagnoses of AD, AR, and asthma/wheeze were initially found by international classification of diseases version 9 codes (691.8, 477, and 493, respectively) and validated by chart review of signs and symptoms. Diagnostic criteria used for AD and asthma were based on Hanifin and Rajka (18) and the National Heart, Lung, and Blood Institute guidelines (19), respectively.

Laboratory measures

Specific-allergen IgE and total serum IgE (normal 20–100 IU/ml) were analyzed in patients with suspected atopy and/or signs and symptoms of allergic exacerbation of atopic disorders, including allergic asthma, AR, and AD (Quest Diagnostics). Complete blood count (CBC, Quest Diagnostics) with differential was ordered as part of routine age-specific pediatric screening practice (age-specific normal reference ranges provided in Table S1). Total and specific serum IgE levels were collected for evaluation of symptomatic

children with suspected allergies or atopic disorders. Laboratory measures ordered during sick visits or 4 wk after WTVZV, other viral or bacterial infection were excluded from analysis as acute infection may have affected these values.

Statistical analyses

The baseline characteristics of those patients with and without WTVZV were examined. Comparisons for categorical variables were made by chi-square tests. All laboratory values were not normally distributed as judged by analysis of normality using Shapiro–Wilk and Kolmogorov–Smirnov tests ($p < 0.01$) (20, 21), but were normally distributed after log transformation. Data are presented as medians, interquartile ranges (IQRs), and ranges, after exponentiation of estimates. All tests of significance were two-sided. Statistical analyses were performed using SAS 9.2 Software (SAS Institute Inc., Cary, NC, USA).

Logistic regression

The relationships between WTVZV exposure and subsequent atopic disorders were assessed using logistic regression. For disorders with few subjects per group (< 5), exact logistic regression was used to calculate p-values. All models included year of birth (YOB) (continuous), gender (male vs. female), race/ethnicity (caucasian vs. other), and YOB (continuous). Adjusted odds ratios (OR), 95% confidence intervals (95% CI), and p-values for prior WTVZV exposure were calculated.

As patterns of WTVZV exposure and vaccination were different before and after the introduction of the AAP vaccination schedule, subset analyses were also performed and stratified by YOB divided into tertiles.

Mixed linear models

The relationship between repeated laboratory measures and WTVZV was assessed using mixed linear models (22). Models were constructed with each log laboratory value as dependent variables and included the entire cohort. Comparisons of baseline laboratory measures between WTVZV and VV exposure groups were performed by separate models that were limited to pre-exposure data.

A categorical variable was encoded for age of laboratory testing with 2-yr intervals from birth to 16 yr of age. If a subject had > 1 test in a given interval, the test with the closest date to the midpoint of that interval was selected. Thus, no subject had > 1 test per interval; however, some subjects has no tests per interval, which were treated as missing values. YOB (continuous) was included as a potential confounder because most cases of WTVZV occurred before 1998 and all VV occurred after 1998.

A binary variable was encoded in some models to represent whether prior WTVZV occurred. A categorical variable was encoded in other models to represent the interval from

prior WTVZV to laboratory testing (0–5, 5–10, > 10 yr). Gender, race/ethnicity, and YOB were included as potential confounders in adjusted models.

Selection of the best-fitting intra-subject correlation structure was made by residual analysis and minimizing the Akaike's information criterion. Outliers were excluded using studentized residual and Cook's distance measures. LSM and CI95 were generated, which control for other covariates, including age, gender, race/ethnicity, and YOB. *Post hoc* comparisons were made using Tukey's pair-wise comparisons.

Correction for multiple tests ($k = 65$) with the approaches of Bonferroni, Benjamini and Hochberg (23), and Benjamini and Yekutieli (24) yielded critical p-values for statistical significance of 0.0008, 0.031, and 0.011, respectively.

Results

Population characteristics

One hundred and sixteen patients with WTVZV exposure were randomly selected for the study; nine were excluded because of incomplete follow-up, and seven were excluded based on exclusion criteria. Three hundred and sixty-one patients who received VV and without history of WTVZV were randomly selected as non-exposure controls; Twenty-one were excluded because of incomplete follow-up, and SEVENTEEN were excluded based on exclusion criteria. WTVZV patients were similar to VV patients with respect to gender (54.0% vs. 52.6%, $p = 0.81$), race/ethnicity (100.0% vs. 98.5%, $p = 0.21$), season of birth ($p = 0.42$), and other immunizations being up-to-date (100.0% vs. 98.4%, $p = 0.22$). Median $\pm$ IQR age at WTVZV exposure was 2.5 ± 1.8 yr, and age at VV was 1.6 ± 1.6 yr (Table 1). This difference of age is because the majority of WTVZV exposures occurred prior to the AAP guidelines for VV. Therefore, age at follow-up and YOB were included in statistical models and/or models were stratified by YOB-tertile to account for these historical differences.

Association of prior WTVZV and atopic disorders

WTVZV was associated with decreased odds of asthma (exact logistic regression; aOR = 0.12, 95% CI = 0.03–0.57, $p = 0.003$), AR (aOR = 0.16, 95% CI = 0.05–0.49, $p = 0.0003$), and AD (logistic regression; aOR = 0.57, 95% CI = 0.33–0.96, $p = 0.02$), but not food allergies ($p = 0.78$) (Table 2).

In subset analyses stratified by YOB-tertile, estimates for WTVZV were not estimable for YOB-tertile 1 (2003–2008) because of the low prevalence of WTVZV infection after widespread vaccination. However, WTVZV was significantly associated with decreased AR (aOR = 0.12, 95% CI = 0.02–0.93, $p = 0.04$), AD (aOR = 0.38, 95% CI = 0.22–0.67, $p = 0.0009$), and food allergies (aOR = 0.16, 95% CI = 0.04–0.73, $p = 0.02$), but not asthma (not estimable, $p = 0.95$) for YOB-tertile 2 (1999–2002) and AR (aOR = 0.03, 95% CI = 0.01–0.09, $p < 0.0001$), asthma (aOR = 0.009,

Table 1 Comparison of baseline variables between wild-type varicella zoster virus infection (WTVZV) and varicella vaccine (VV) subjects

Variable	WTVZV (n = 100)	VV (n = 323)	p-value*
Male sex, n (%)	54 (54.0)	170 (52.6)	0.81
Season of birth, n (%)†			
Winter	22 (22.0)	79 (24.5)	0.42
Spring	25 (25.0)	102 (31.6)	
Summer	27 (27.0)	77 (23.8)	
Autumn	26 (26.0)	65 (20.1)	
Age at WT VZV infection (yr)‡	2.5 (1.8, 6.2)	n/a	–
Age at first varicella vaccine (yr)‡	n/a	1.6 (1.6, 15.0)	–
Caucasian race, n (%)	100 (100.0)	318 (98.5)	0.21
IUTD, n (%)§	100 (100.0)	311 (98.4)	0.22
Leukocytes ($\times 10^3$ cells/mm ³)**	(n = 86)¶	(n = 321)¶	0.25
Neutrophils**	7.87 (7.13–8.68)	8.33 (8.04–8.63)	0.74
Lymphocytes**	3.91 (3.28–4.67)	3.80 (3.57–4.04)	
Monocytes**	3.41 (3.02–3.84)	3.38 (3.23–3.53)	
Monocytes**	0.58 (0.46–0.69)	0.64 (0.61–0.67)	
Eosinophils**	0.15 (0.10–0.25)	0.19 (0.17–0.22)	0.39
Basophils**	0.02 (0.02–0.04)	0.04 (0.03–0.04)	0.09

*p-values for the comparison between the WTVZV and VV groups were calculated by chi-square test, as appropriate. †Season of birth: Winter = December through February; Spring = March through May; Summer = June through August; Autumn = September through November. ‡Values are median [interquartile range (IQR), range]. §IUTD – all other immunizations up to date, including hepatitis A virus, hepatitis B virus, influenza, inactivated polio vaccine, and measles–mumps–rubella, according to the recommended vaccination schedule of the American Academy of Pediatrics. ¶n represents the number of subjects with one or more serum IgE or complete blood count with differential *before* exposure to either WTVZV or varicella vaccine. **p-values for the comparison of pre-exposure laboratory values between the WTVZV and VV groups were calculated by mixed linear models, including age at testing as a fixed covariate. Values are least squares means (95% confidence interval) adjusted for age at testing.

Table 2 Logistic regression analysis of the effects of prior wild-type varicella zoster virus infection (WTVZV) infection on development of asthma, atopic dermatitis (AD), allergic rhinoconjunctivitis (AR) and food allergies

Disease	Prior WTVZV	No prior WTVZV	aOR (95% CI)*	p-value
Asthma	2 (2.0)	29 (9.0)	0.12 (0.03–0.57)	0.003†
AR	28 (28.3)	133 (41.1)	0.16 (0.05–0.49)	0.0003‡
AD	4 (4.0)	38 (11.7)	0.57 (0.33–0.96)	0.02†
Food allergies	5 (5.1)	18 (5.6)	0.80 (0.25–2.59)	0.78†

*All models include age (encoded as ordinal variable with 5-yr intervals), gender, race/ethnicity, and year of birth. †p-values for the comparison of prior WTVZV and VV groups were calculated by exact logistic regression models with diagnosis of asthma, AD, AR, or food allergies as the dependent variables. ‡p-values for the comparison of prior WTVZV and VV groups were calculated by logistic regression models with diagnosis of asthma, AD, AR, or food allergies as the dependent variables.

95% CI = 0.001–0.06, $p < 0.0001$), AD (aOR = 0.44, 95% CI = 0.30–0.64, $p < 0.0001$), and food allergies (aOR = 0.09, 95% CI = 0.04–0.19, $p < 0.0001$) for YOB-tertile 3 (1989–1998).

Baseline laboratory measures

Mixed linear models were constructed including gender and race/ethnicity as fixed covariates. The covariance structure was modeled most efficiently using the compound symmetry approach for virtually all models, and thus was consistently used for all models. There were no significant differences in distributions of peripheral leukocytes (mixed linear model,

$p = 0.09$ –0.88) prior to exposure to WTVZV vs. VV (Table 1).

WTVZV is associated with decreased total serum IgE levels

WTVZV, compared with no WTVZV exposure, was associated with subsequently decreased levels of total serum IgE [LSM (CI95): 129.09 (33.22–501.63) vs. 334.21 (102.38–1091.04) IU/ml; $p = 0.02$] (Table 3). Duration of decreased IgE was assessed by constructing mixed linear models including the interval from WTVZV exposure to IgE measurement as a fixed covariate. Serum IgE levels were significantly decreased at all time intervals after WTVZV (<5, 5–10, and >10) compared

Table 3 Mixed linear model analysis of the effects of prior wild-type varicella zoster virus infection (WTVZV) infection on serum IgE and leukocyte distributions

Variable	Prior WTVZV*	No prior WTVZV	p-value†
Immunoglobulin E (IU/ml)	(n = 10)‡ 129.09 (33.22–501.63)	(n = 46)‡ 334.21 (102.38–1091.04)	0.02
Leukocytes ($\times 10^3$ cells/mm ³)	(n = 96)‡ 7.00 (5.96–8.23)	(n = 323)‡ 8.23 (7.08–9.57)	<0.0001
Neutrophils	3.72 (2.88–4.80)	3.76 (2.96–4.76)	0.85
Lymphocytes	2.65 (2.17–3.23)	3.37 (2.80–4.06)	<0.0001
Monocytes	0.36 (0.25–0.51)	0.50 (0.36–0.69)	0.002
Eosinophils	0.15 (0.07–0.31)	0.17 (0.09–0.33)	0.36
Basophils at specific ages (yr)¶			
0–2	71.38 (31.63–161.05)	73.73 (39.66–137.07)	0.91
2–4	92.76 (43.33–198.58)	59.52 (30.99–114.33)	0.07
4–6	38.12 (18.95–76.66)	84.04 (42.74–165.22)	0.0003
6–8	54.58 (27.80–107.18)	57.58 (29.65–112.06)	0.77
8–10	59.84 (30.69–116.69)	69.34 (36.47–131.85)	0.33
10–12	46.89 (24.27–90.58)	66.16 (33.85–129.30)	0.04
12–14	52.46 (26.37–103.33)	58.80 (29.53–117.07)	0.58
14–16	53.31 (26.90–105.65)	84.14 (41.92–168.90)	0.03

*Models were constructed with prior WTVZV as a time-dependent covariate, which included subjects that were vaccinated and never had WTVZV and unvaccinated subjects that were tested prior to exposure to WTVZV. Values are least squares means (95% confidence interval) adjusted for age at testing, gender, race/ethnicity, and year of birth. †p-values for the comparison of pre-exposure laboratory values between the WTVZV and varicella vaccine groups were calculated by mixed linear models, including age at testing, gender, race/ethnicity, and year of birth as fixed covariates. Outliers were excluded by studentized residuals, cook's distances, and other influence statistics. ‡n represents the number of subjects with one or more serum IgE or complete blood count with differential after exposure to either WTVZV or varicella vaccine. ¶Models had significant interaction terms between age of laboratory testing and previous WTVZV, indicating varying effects of WTVZV on the dependent variable(s) at different time intervals. Therefore, slice analysis is presented for each age interval.

with VV ($p = 0.003$ – 0.03) (Fig. 1). All models included age, gender, race/ethnicity, and YOB as fixed covariates.

WTVZV is associated with decreased IgE sensitization

WTVZV, compared with no WTVZV, was negatively associated with allergic sensitization, as judged by at least one posi-

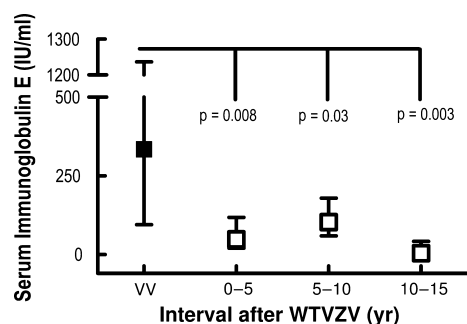


Figure 1 Association of total serum IgE and interval after wild-type varicella zoster virus infection (WTVZV). Mixed linear models were constructed with total serum IgE as the dependent variable and interval after WTVZV as an ordinal variable (0–5, 5–10 and 10–15 yr) and age of testing, gender, race/ethnicity, and year of birth as fixed covariates. Least squares means and 95% confidence intervals were adjusted with the Tukey's approach. p-values represent comparisons between varicella vaccine and the respective interval after WTVZV.

tive specific IgE value (logistic regression, OR = 0.11, 95% CI = 0.03–0.38, $p = 0.0004$) (Table 4).

WTVZV decreases numbers of circulating lymphocytes, monocytes and basophils

WTVZV, compared with no WTVZV exposure, was associated with subsequently decreased numbers of peripheral leukocytes [LSM (CI95): 7.00 (5.96–8.23) vs. 8.23 (7.08–9.57) $\times 10^3/\text{mm}^3$; $p < 0.0001$], specifically lymphocytes [2.65 (2.17–3.23) vs. 3.37 (2.80–4.06) $\times 10^3/\text{mm}^3$; $p < 0.0001$], and monocytes [0.36 (0.25–0.51) vs. 0.50 (0.36–0.69) $\times 10^3/\text{mm}^3$; $p = 0.002$] (Table 3). In the case of peripheral basophils, there was a significant interaction between age of testing and prior WTVZV, with significantly decreased numbers at ages 4–6, 10–12, and 14–16 ($p = 0.0003$ – 0.03), but not 0–2, 2–4, 6–8, 8–10, or 12–14 ($p = 0.07$ – 0.91) (Table 3). However, there were no significant associations between WTVZV, compared with no WTVZV exposure, and numbers of peripheral neutrophils ($p = 0.85$) or eosinophils ($p = 0.36$) (Table 3).

Duration of decreased numbers of lymphocytes, monocytes, and basophils was assessed by constructing mixed linear models including the interval from WTVZV exposure to laboratory measurement as a fixed covariate. The association between WTVZV and subsequently decreased numbers of leukocytes was significant at ages 0–5 ($p = 0.0004$) and 5–10 ($p = 0.001$), but not >10 yr post-infection ($p = 0.09$) (Fig. 2 a). Decreased numbers of lymphocytes remained significant

Table 4 Ordinal logistic regression analysis of the effects of prior wild-type varicella zoster virus infection (WTVZV) infection on allergic sensitization

Specific IgE positivity	Prior WTVZV*	No prior WTVZV	aOR (95% CI)	p-value†
Frequency of ≥ 1 positive test, n (%)	2 (20.0)	31 (73.8)	0.11 (0.03–0.38)	0.0004
No. of positive tests – mean $\pm$ s.d.	0.6 $\pm$ 1.6	3.4 $\pm$ 3.3		

*Models were constructed with prior WTVZV as a fixed covariate and included age, gender, race/ethnicity, and year of birth. †p-values for the comparison of pre-exposure laboratory values between the WTVZV and varicella vaccine groups were calculated by ordinal logistic regression models with number of positive specific IgE tests as the dependent variable.

for all ages ($p = 0.0003$ – 0.047) except 12–14 yr ($p = 0.39$ – 0.43) (Fig. 2e). Decreased numbers of monocytes were significant across all time points ($p < 0.0001$ – 0.001) (Fig. 2b). Numbers of basophils were significantly decreased at 4–6 yr of age 0–5 yr ($p < 0.0001$), and at 14–16 yr of age > 10 yr ($p = 0.04$), post-WTVZV (Fig. 2f). In contrast, there were no significant associations between interval after WTVZV and numbers of neutrophils ($p = 0.30$ – 0.53 ; Fig. 2c) or eosinophils ($p = 0.54$ – 0.75 ; Fig. 2d). All models included age, gender, race/ethnicity, and YOB as fixed covariates.

Discussion

This study demonstrates a strong association between WTVZV and increased immune tolerance. This study is consistent with, and supported by, a recent case-control study by Silverberg et al. (6) who reported that WTVZV in children up to 8–10 yr of age was associated with decreased odds ratio of developing AD and asthma, delayed onset of AD symptomatology, decreased AD severity, and fewer AD-related office visits. Similarly, studies of Illi et al. (25) showed that children with WTVZV by age 3 were less likely to develop asthma by age 7. This study indicates that WTVZV results in decreased allergic sensitization, lower serum IgE levels, and decreased numbers of peripheral blood lymphocytes, monocytes, and basophils.

The results of this study differ from previously published studies regarding an association between WTVZV and serum IgE levels. A prospective study of the association between Epstein–Barr virus and IgE sensitization in Swedish children at age 24 months found no significant association between VZV seropositivity (secondary outcome) and IgE sensitization (13). A nested case-control study found increased IgE sensitization to specific allergens after parental reports of WTVZV in German children aged 7–8 yr (14). While the former study was prospective, it did not distinguish between WTVZV and subclinical infection or very mild disease (typically found in infants) and WTVZV was not the primary outcome studied; the latter was based on parental questionnaire. The questionnaire approach has limitations given that there are clinical mimics of WTVZV, e.g., hand–foot–mouth disease. Neither study examined the effects of WTVZV for the decade after infection, comparative age categories of WTVZV, or whether WTVZV preceded IgE sensitization.

The goal of this study was to elucidate whether WTVZV can prevent AD and what mechanisms might be implicated. Thus, subjects with a diagnosis of atopic disorders prior to

WTVZV or VV exposure were excluded as they already had the outcome of interest. Future studies are warranted to determine whether WTVZV can suppress AD or other atopic disorders in children with already diagnosed disease.

Previous studies in our laboratory reported $> 30\%$ decrease in serum IgE levels 5 months after shingles infection, which was accompanied by the presence of specific IgE anti-VZV antibodies (26). Interestingly, the shingles infection occurred at age 16, well beyond the window for WTVZV up to 8–10 yr of age. We posit that the occurrence of shingles, after WTVZV occurred within the critical 8–10 yr window, may also allow for another episode of suppression of IgE production. However, the length of suppression of IgE production after WTVZV recurrence has yet to be determined.

This study found persistent decreases in numbers of peripheral lymphocytes, monocytes, and sometimes basophils. The decreased numbers of lymphocytes and monocytes are potentially involved in suppression of serum IgE. Given the findings of this study, it is possible that these cells are a potential factor by which WTVZV protects against AD and asthma. In contrast with the findings of this study, a previous study of peripheral blood in West African subjects study found significantly increased numbers of CD4+ and CD8+ T cells during the acute phase of WTVZV (27). This study only examined leukocyte subsets for up to 2 months after WTVZV, but found that there were no persistent changes 1 month after WTVZV. Interestingly, their study also found persistently increased numbers of eosinophils 1 month after infection. A possible explanation for the difference between that study and the present study is older age at WTVZV, ranging from 3 months to 30.3 yr (28) vs. 2 months to 6.3 yr, respectively. Age of initial infectious and unhygienic exposures has been proposed as a crucial factor to explain the hygiene hypothesis (7). In line with this hypothesis, we previously reported that WTVZV in children after age 8–10 yr did not confer any protection against AD or asthma (6). Given the exclusion criteria of WTVZV > 8 yr of age, the present study may better detect WTVZV-mediated suppression of IgE.

The potential mechanism for induction of immune tolerance by WTVZV, specifically suppression of IgE production and decreased numbers of lymphocytes, monocytes, and basophils remains unknown. However, one possible explanation is that WTVZV directly infects peripheral T cells and monocytes [see review (29)], resulting in apoptosis of specific pro-allergic cell subsets (e.g., IL-4 producing Th2 cells). Other studies in peripheral blood CD8+ T cells from

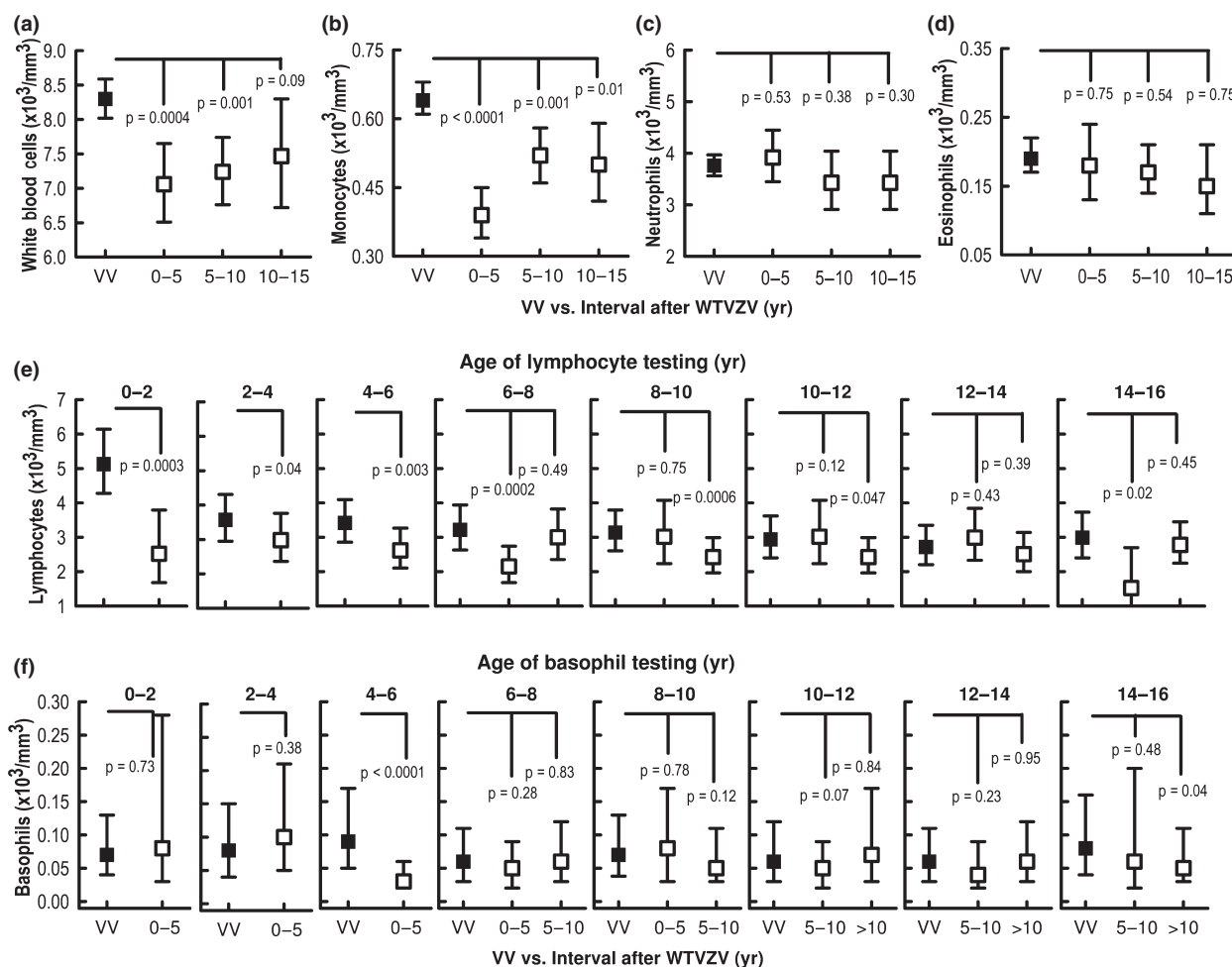


Figure 2 Association of leukocyte distributions and interval after wild-type varicella zoster virus infection (WTVZV). Mixed linear models were constructed with cell numbers as the dependent variable and interval after WTVZV as an ordinal variable (0–5, 5–10 and 10–15 yr) and age of testing, gender, race/ethnicity and year of birth as fixed covariates. Least squares means and 95% confidence intervals were adjusted with the Tukey's approach. Estimates for

(a) white blood cells, (b) monocytes, (c) neutrophils, and (d) eosinophils across all age intervals are presented, as there were no significant interaction terms in the model. (e) Lymphocytes and (f) basophils are presented at each 2-yr age interval, as there was a significant interaction between these cell types and age of testing. p-values represent comparisons between varicella vaccine and the respective interval after WTVZV.

subjects with acute herpesvirus infections have demonstrated a decrease in expression of costimulatory molecules CD28, CD5, and CD6 and were highly susceptible to apoptosis (30). A study of patients with herpes zoster found increased CD4⁺ T cells at 14–28 days post-infection (16). Alternatively, WTVZV may result in expansion of regulatory and suppressor T-cell subsets (e.g., IL-10 producing CD4⁺CD25⁺ Tregs and/or Tr1 cells). Previous studies of the effects WTVZV on cytokine production found increased levels of IL-4, IL-10, and IFN- γ *in vitro* (31), and IL-10, IL-12, and IFN- γ *in vivo* (32). IL-10 has been shown to induce anergy of allergen-specific peripheral T cells and to suppress IgE production (33). While the mechanisms involved are not completely understood, it is likely that induction of immune tolerance after WTVZV is multifactorial, including a combination of these proposed mechanisms.

Of notable interest, were the persistent baseline changes of leukocyte distributions after WTVZV compared with VV cohorts, suggesting that such changes may be related to the severity of clinical infection, including fever, viral load, and skin damage with secondary bacterial infections. Another possible explanation may be virulence factors present in the WTVZV strain, which are not present in the attenuated Oka vaccine strain. This observation has been documented by others who similarly demonstrated that Th1 responses induced by microbial infections may counterbalance allergen-induced Th2 responses, which may be a potential mechanism for the hygiene hypothesis (34–36). However, we were unable to assess the long-term effects of VV on leukocyte distributions because there were no patients who were naïve to VZV antigens, i.e., neither WTVZV nor VV.

This study found persistent decreases of numbers of basophils at some age intervals after WTVZV. It is unclear what physiologic role basophils play in atopic disorders, such as AD or asthma. However, basophils have been shown to release histamine and leukotrienes, as well as IL4 and IL13, which may contribute to Th2 responses and IgE production [recently reviewed in (37)]. A possible explanation is that WTVZV directly affects basophils, resulting in decreased numbers or function. These cells, combined with increased inflammatory cytokine production and possible increased IgE receptor surface expression, may represent a novel pathway by which WTVZV protects against atopic disorders. Alternatively, it is possible that WTVZV indirectly affects basophils by downregulating cytokines that contribute to their activation, e.g., IL3, IL5, and GM-CSF.

This study has several strengths, including clinical screening by a physician for occurrence of WTVZV that is less likely to be affected by recall bias. Moreover, given the major decline of WTVZV in the United States, a retrospective study was necessary for recruiting adequate numbers of WTVZV cases. Conducting a prospective study withholding VV from children against the AAP vaccination schedule would be unethical. A possible alternative design might be to conduct a prospective study by following children whose parent(s) have already refused vaccination, and future studies may employ this approach.

A number of limitations in our study have to be considered. The study's primary limitations are inherent to the retrospective design. IgE levels may have a selection bias, because they were only collected as part of an evaluation of

patients with signs and symptoms of asthma or atopy. However, this potential bias would likely have been similar for both the WTVZV and the VV groups. As the majority of subjects in the study were Caucasian, the results may not be generalizable to other racial/ethnic populations. Another limitation is that this study was retrospective; thus, several important laboratory tests were not assessed, including varicella antibody concentrations, characterization of lymphocyte and monocyte subsets (e.g., CD4+ vs. CD8+ and CD4+CD25+ T regulatory cells). Further studies aimed at such characterization are warranted.

The results of this study suggest that children with WTVZV are less likely to have allergic sensitization, have persistently lower total serum IgE levels, and decreased numbers of circulating lymphocytes, monocytes, and basophils. These findings may help explain the protective role of WTVZV against AD and asthma. WTVZV-mediated suppression of IgE production may be translatable into novel therapeutic strategies for atopic disorders.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Normal Reference ranges for leukocyte differentials.

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References

- Hanifin JM, Reed ML. A population-based survey of eczema prevalence in the United States. *Dermatitis* 2007; **18**: 82–91.
- Summary Health Statistics for U.S. Children: National Health Interview Survey, 2009. Vital and Health Statistics, **Vol. Series 10**. Hyattsville, MD, 2010.
- Kiken DA, Silverberg NB. Atopic dermatitis in children, part 1: epidemiology, clinical features, and complications. *Cutis* 2006; **78**: 241–7.
- Lemanske RF Jr, Busse WW. 6. Asthma: factors underlying inception, exacerbation, and disease progression. *J Allergy Clin Immunol* 2006; **117**: S456–61.
- Silverberg JI, Norowitz KB, Kleiman E, et al. Association between varicella zoster virus infection and atopic dermatitis in early and late childhood: a case-control study. *J Allergy Clin Immunol* 2010; **126**: 300–5.
- Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989; **299**: 1259–60.
- Habermehl P, Lignitz A, Knuf M, Schmitt HJ, Slaoui M, Zepp F. Cellular immune response of a varicella vaccine following simultaneous DTaP and VZV vaccination. *Vaccine* 1999; **17**: 669–74.
- Arvin AM. Cell-mediated immunity to varicella-zoster virus. *J Infect Dis* 1992; **166** (Suppl. 1): S35–41.
- Cauda R, Grossi CE, Whitley RJ, Tilden AB. Analysis of immune function in herpes zoster patients: demonstration and characterization of suppressor cells. *J Immunol* 1987; **138**: 1229–33.
- Jordan GW, Merigan TC. Cell-mediated immunity to varicella-zoster virus: in vitro lymphocyte responses. *J Infect Dis* 1974; **130**: 495–501.
- Weinberg A, Lazar AA, Zerbe GO, et al. Influence of age and nature of primary infection on varicella-zoster virus-specific cell-mediated immune responses. *J Infect Dis* 2010; **201**: 1024–30.
- Nilsson C, Linde A, Montgomery SM, et al. Does early EBV infection protect against IgE sensitization? *J Allergy Clin Immunol* 2005; **116**: 438–44.
- Mommers M, Swaen GM, Weishoff-Houben M, et al. Childhood infections and risk of wheezing and allergic sensitisation at age 7–8 years. *Eur J Epidemiol* 2004; **19**: 945–51.
- Terada K, Kawano S, Yagi Y, Shimada Y, Kataoka N. Alteration of T cells and natural killer cells during chickenpox in infancy. *J Clin Immunol* 1996; **16**: 55–9.
- Cauda R, Prasthofer EF, Tilden AB, Whitley RJ, Grossi CE. T-cell imbalances and NK activity in varicella-zoster virus infections. *Viral Immunol* 1987; **1**: 145–52.
- American Academy of Pediatrics. Committee on Infectious Diseases. Varicella vaccine update. *Pediatrics* 2000; **105**: 136–41.
- Hanifin J, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol Suppl (Stockh)* 1980; **92**: 44–7.
- New NHLBI guidelines for the diagnosis and management of asthma. National Heart, Lung and Blood Institute. *Lippincott Health Promot Lett* 1997; **2**: 1.
- Illi S, von Mutius E, Lau S, et al. Early childhood infectious diseases and the development of asthma up to school age: a birth cohort study. *BMJ* 2001; **322**: 390–5.
- Lev Tov HJJ, Kohlhoff S, Norowitz KB, et al. IgE anti-varicella virus (VZV) and other

- immune responses before, during, and after shingles. *J Allergy Clin Immunol* 2008; **121**: S207.
21. Lisse IM, Qureshi K, Poulsen A, et al. T-lymphocyte subsets and eosinophil counts in acute and convalescence chickenpox infection: a household study in Guinea-Bissau. *J Infect* 2005; **50**: 125–9.
 22. Poulsen A, Qureshi K, Lisse I, et al. A household study of chickenpox in Guinea-Bissau: intensity of exposure is a determinant of severity. *J Infect* 2002; **45**: 237–42.
 23. Hambleton S. Chickenpox. *Curr Opin Infect Dis* 2005; **18**: 235–40.
 24. Borthwick NJ, Bofill M, Hassan I, et al. Factors that influence activated CD8⁺ T-cell apoptosis in patients with acute herpesvirus infections: loss of costimulatory molecules CD28, CD5 and CD6 but relative maintenance of Bax and Bcl-X expression. *Immunology* 1996; **88**: 508–15.
 25. Hayward AR, Cosyns M, Jones M, et al. Cytokine production in varicella-zoster virus-stimulated cultures of human blood lymphocytes. *J Infect Dis* 1998; **178** (Suppl. 1): S95–8.
 26. Jenkins DE, Redman RL, Lam EM, Liu C, Lin I, Arvin AM. Interleukin (IL)-10, IL-12, and interferon-gamma production in primary and memory immune responses to varicella-zoster virus. *J Infect Dis* 1998; **178**: 940–8.
 27. Akdis CA, Blesken T, Akdis M, Wuthrich B, Blaser K. Role of interleukin 10 in specific immunotherapy. *J Clin Invest* 1998; **102**: 98–106.
 28. Garn H, Renz H. Epidemiological and immunological evidence for the hygiene hypothesis. *Immunobiology* 2007; **212**: 441–52.
 29. von Mutius E. Allergies, infections and the hygiene hypothesis—the epidemiological evidence. *Immunobiology* 2007; **212**: 433–9.
 30. Stone KD, Prussin C, Metcalfe DD. IgE, mast cells, basophils, and eosinophils. *J Allergy Clin Immunol* 2010; **125**: S73–80.

Supplemental Table 1. Normal Reference ranges for leukocyte differentials.						
Variable*	2 wks	2–3 mos	2 yr	6 yr	12 yr	18 yr
Leukocytes (x 10 ³ cells/mm ³)	9.0 – 30.0	5.0 – 19.5	6.0 – 17.0	5.0 – 16.0	4.5 – 13.5	4.5 – 13.0
Neutrophils	1.5 – 10.0	1.0 – 8.8	1.0 – 8.5	1.5 – 8.5	1.5 – 8.0	1.8 – 8.0
Lymphocytes	2.0 – 17.0	3.3 – 15.0	4.0 – 10.5	2.0 – 8.0	1.5 – 6.5	1.2 – 5.2
Monocytes	0.3 – 2.4	0.3 – 2.4	0.2 – 1.0	0.2 – 1.0	0.2 – 1.0	0.2 – 0.9
Eosinophils	0 – 900	15 – 800	15 – 700	15 – 600	15 – 600	15 – 500
Basophils	0.0 – 0.3	0.0 – 0.3	0.0 – 0.3	0.0 – 0.3	0.0 – 0.3	0.0 – 0.3

* Normal reference ranges are from Quest Diagnostics.