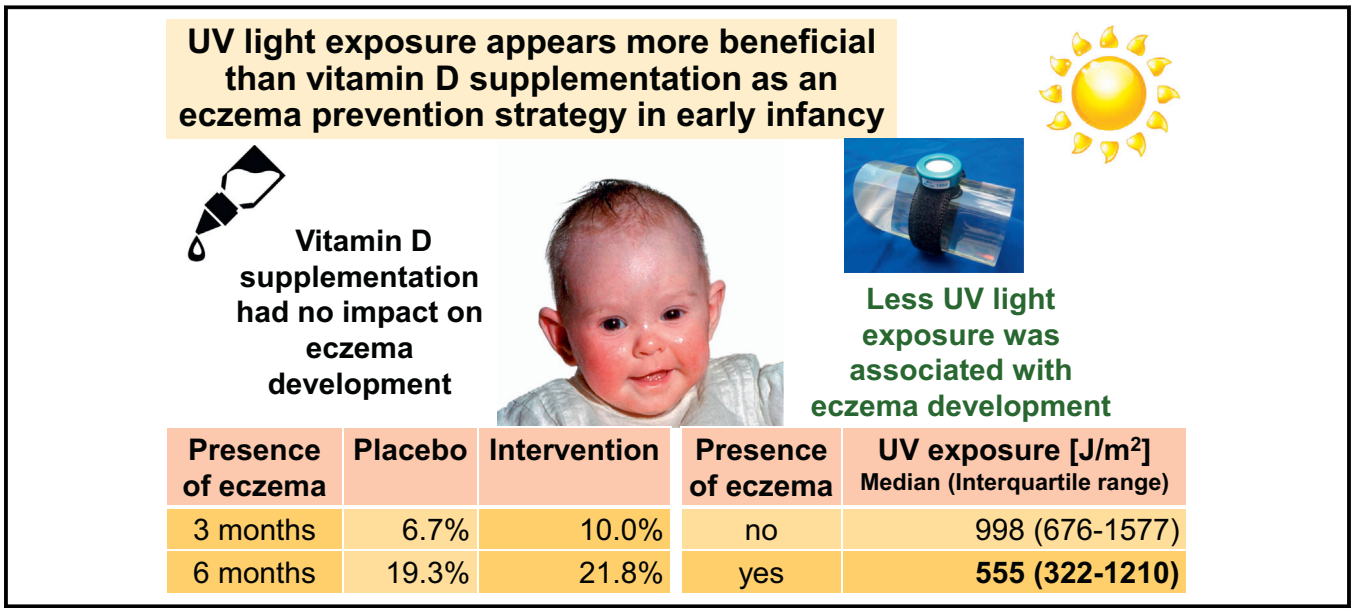
Direct infant UV light exposure is associated with eczema and immune development

Kristina Rueter, MD,[a,b,c]* Anderson P. Jones, PhD,[d]* Aris Siafarikas, MD,[a,b,d] Ee-Mun Lim, MD, PhD,[e,f] Natasha Bear, MBiostats,[g] Paul S. Noakes, PhD,[a,d] Susan L. Prescott, MD, PhD,[a,b,c,d] and Debra J. Palmer, PhD[a,d]

Crawley, Subiaco, Nedlands, and Perth, Australia, and West New York, NJ

GRAPHICAL ABSTRACT



Background: Suboptimal vitamin D levels during critical periods of immune development have emerged as an explanation for higher rates of allergic diseases associated with industrialization and residing at higher latitudes.

Objective: We sought to determine the effects of early postnatal vitamin D supplementation on infant eczema and immune development.

Methods: By using a double-blind randomized controlled trial, newborn infants were randomized to receive vitamin D supplementation (400 IU/d) or a placebo until 6 months of age. Some infants also wore personal UV dosimeters to measure direct UV light (290-380 nm) exposure. Infant vitamin D levels were measured at 3 and 6 months of age. Eczema, wheeze, and immune function outcomes were assessed at 6 months of age.

Results: At 3 ($P < .01$) and 6 ($P = .02$) months of age, vitamin D levels were greater for the vitamin D–supplemented group than the placebo group, but there was no difference in eczema incidence between groups. Infants with eczema were found to have had less UV light exposure (median, 555 Joules per square meter [J/m^2; interquartile range, 322-1210 J/m^2]) compared with those without eczema (median, 998 J/m^2 [interquartile range, 676-1577 J/m^2]; $P = .02$). UV light exposure was also inversely correlated with IL-2, GM-CSF, and eotaxin production to Toll-like receptor ligands.

Conclusion: This study is the first to demonstrate an association between greater direct UV light exposures in early infancy with lower incidence of eczema and proinflammatory immune markers by 6 months of age. Our findings indicate that UV light exposure appears more beneficial than vitamin D supplementation as an allergy prevention strategy in early life. (J Allergy Clin Immunol 2018;■■■:■■■-■■■.)

From [a]the School of Medicine, University of Western Australia, Crawley; [b]Princess Margaret Hospital, Subiaco; [c]inVIVO Planetary Health, Group of the Worldwide Universities Network (WUN), West New York; [d]Telethon Kids Institute, University of Western Australia, Subiaco; [e]the Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands; [f]the Department of Clinical Biochemistry, PathWest Laboratory Medicine, Queen Elizabeth II Medical Centre, Nedlands; and [g]the Department of Clinical Research and Education, Child Adolescent Health Service, Perth.

*These authors contributed equally to this work.

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Corresponding author: Debra J. Palmer, PhD, Telethon Kids Institute, Nedlands, Western Australia, 6008, Australia. E-mail: debbie.palmer@telethonkids.org.au.

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The pattern of infant allergic disease has changed dramatically in the last 20 years, suggesting new and changing environmental risk factors affecting young infants.[1] Although the prevalence of asthma and allergic rhinitis remains high, there has been a substantial increase in both eczema and food allergy in what has been described as the "second wave" of the allergy epidemic.[1] This has called for a more detailed investigation of the factors most likely affect the developing immune system in early life.

A "vitamin D hypothesis" emerged to explain associations found between regions of higher latitudes and increased risk of development of allergic diseases in children.[2-5] Vitamin D insufficiency became a key candidate associated with early-life allergic diseases based on both population[6-8] and mechanistic[9,10] studies that provided biological pathways of influence. However, intervention trials examining the effect of vitamin D supplementation during infancy as an allergy prevention strategy were lacking. Thus we designed this randomized controlled trial (RCT) to determine the effect of infant oral vitamin D supplementation in the early postnatal period on the developing immune phenotype.

In addition to investigating the effect of infant vitamin D supplementation, this trial has also quantitatively measured infant UV light exposure using personal UV dosimetry during the first 3 months of the postnatal period. UV light exposure is the predominant source of vitamin D in human subjects and has also been shown to independently modulate immune function.[11] The use of UV dosimeters is unique to this study investigating early-life vitamin D supplementation with early allergic disease symptoms and immune function outcomes. Use of UV dosimeters in this study has enabled us to also examine whether there could be an effect of UV light exposure that is independent of the parallel effect that UV light has on vitamin D status.

Abbreviations used
IQR: Interquartile range
J/m^2: Joules per square meter
25(OH)D: 25-Hydroxyvitamin D
OVA: Ovalbumin
RCT: Randomized controlled trial
TLR: Toll-like receptor

METHODS

Study design

This study was a double-blind, placebo-controlled RCT. Healthy term (delivered >37 weeks' gestation) singleton infants were recruited before 28 days of age. All the infants had a first-degree relative (mother, father, or sibling) with a history of allergic disease (asthma, eczema, and allergic rhinitis) to reduce heterogeneity in immune function outcomes. This study excluded infants whose mothers had smoked during pregnancy or had an underlying immunodeficiency/autoimmune disease or those with maternal 25-hydroxyvitamin D (25[OH]D) level serum concentrations of less than 50 nmol/L or greater than 100 nmol/L between 36 and 40 weeks' gestation, which was intended to reduce the risk of vitamin D deficiency or toxicity in the infant participants.

Written informed consent was obtained before trial participation. Human research ethics committee approvals were granted by the Princess Margaret Hospital for Children, Joondalup Health Campus, St John of God Subiaco, Southern Metropolitan Health Service, and the University of Western Australia. The trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12606000281594).

Randomization and allocation concealment

Randomization was conducted by the Princess Margaret Hospital for Children Clinical Trials Pharmacy and stratified according to a history of maternal allergic disease and the participant's sex. The pharmacy created a randomization plan from an online source (www.randomization.com) for each of the 4 stratification groups. Both the intervention (vitamin D) and control (placebo) oils were packaged to appear identical and to maintain the blind. Pharmacy staff had no contact with participants, and all research staff remained blind to the allocations until analyses were completed.

Trial intervention

The intervention group received 400 IU/d vitamin D3, whereas the placebo control group received an identical product of coconut and palm kernel oil but containing no vitamin D. These were given to the infants orally as 1 drop of liquid (0.03 mL) per day. Both products were supplied by Ddrops (Woodbridge, Ontario, Canada). Randomization occurred within 28 days after birth, and supplementation was stopped at 6 months of age. Following recommendations for infant vitamin D supplementation,[12] caregivers were advised to cease administration of the trial product if the infant was consuming 1000 mL/d or more vitamin D–fortified infant formula.

Blood collection

Peripheral infant blood samples were collected at 3 and 6 months of age by means of venipuncture, placed in lithium heparin tubes (Vacuette; Greiner Bio-One GmbH, Kremsmünster, Austria), and processed immediately after the clinic visit for plasma and PBMC analysis. Mononuclear cells were separated by means of density gradient centrifugation (Lymphoprep; Axis-Shield PoC AS, Oslo, Norway). Purified mononuclear cells were cryopreserved in RPMI 1640 media (Life Technologies, Grand Island, NY) and FCS with 7.5% dimethyl sulfoxide. Plasma samples were stored at −80°C.

Measurement of 25(OH)D levels

25(OH)D levels were quantified by using a competitive chemiluminescent immunoassay, automated on the Abbott Architect i2000 (Abbott Laboratories, Abbott Park, Ill [operated by PathWest Laboratory Medicine, Nedlands, Western Australia]) using a 2-step incubation process with human serum calibrators. Internal quality control data indicate the coefficient of variation for 25(OH)D as follows: 11.4% at 22 nmol/L, 5.2% at 48 nmol/L, 4.5% at 68 nmol/L, and 4.0% at 90 nmol/L. The Abbott Architect i2000 is accredited by the National Association of Testing Authorities for measurement of 25(OH)D levels.

Monitoring intervention safety

Plasma collected at 3 months of age was analyzed for 25(OH)D, calcium, phosphate, and alkaline phosphatase to ensure participants did not have vitamin D deficiency or toxicity during the trial. Test results were sent to a pediatric endocrinologist (AS) who informed caregivers and the participants' nominated general practitioner if any parameters were out of the expected normal range. The researchers involved in data collection and analysis remained blind to these test results throughout the trial.

UV dosimetry

This trial was conducted in Perth, Australia, with a latitude of 32° South. Perth has an average of 3200 hours of sunshine per year. Daily values range from 5 hours (June-August) to 11 hours (December and January;

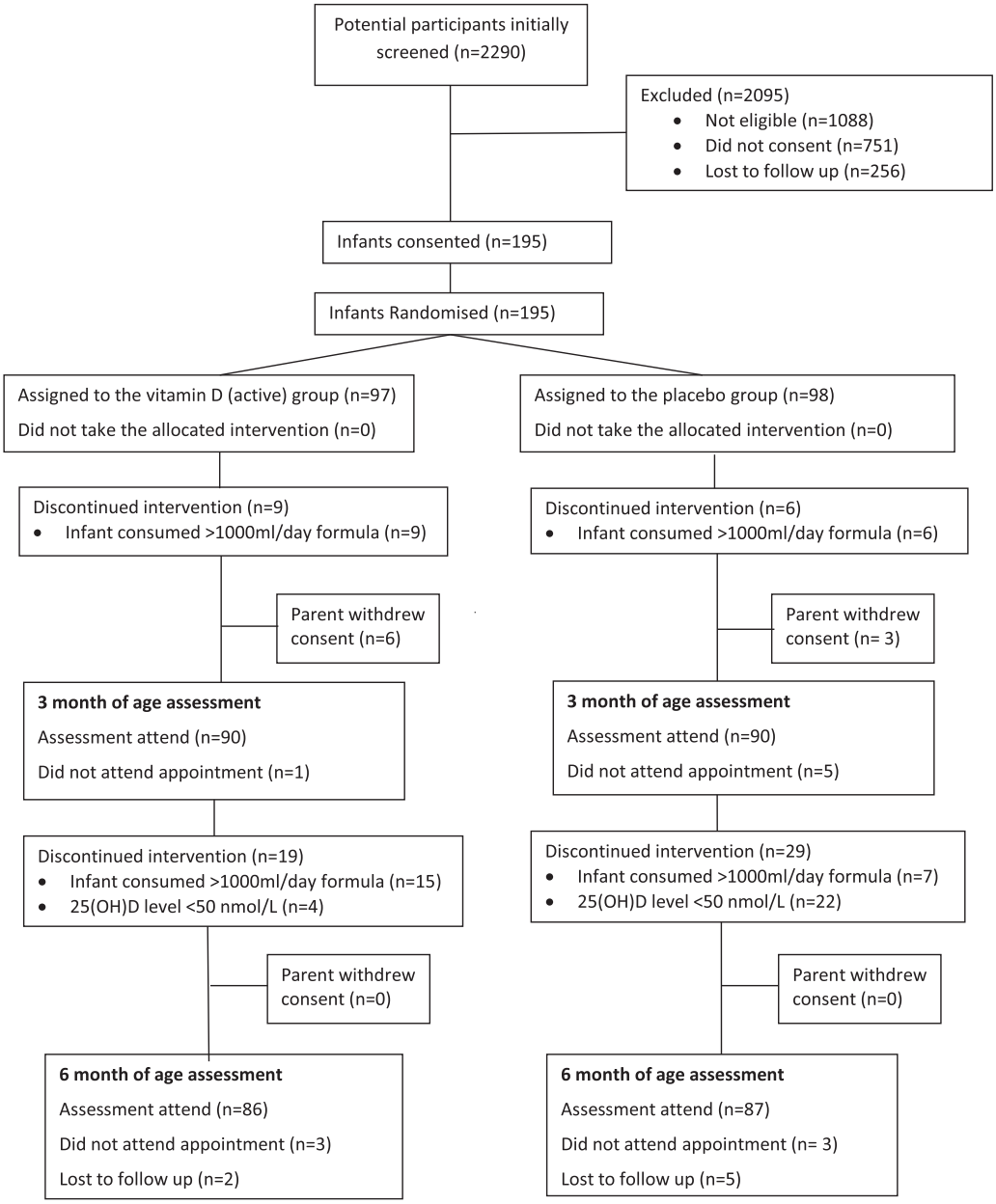

FIG 1. Study flow of participants.

https://www.livingin-australia.com/sunshine-hours-australia/). UV dosimeters (VioSpor blue line Type II; Biosense, Bornheim, Germany) were used to obtain objective quantitative data on infant UV exposure from 0 to 3 months of age. These dosimeters provide data on the total UV light (290-380 nm) exposure dose (in joules per square meter [J/m^2]) obtained over the period of use. Dosimeters consisted of a biological UV-sensitive film, a special filter-optic system, and the protective dosimeter casing. The highly sensitive DNA molecules of immobilized spores of *Bacillus subtilis* produce a responsivity profile that corresponds to that of human skin. After irradiation, the spore film is incubated in growth medium, and the proteins synthesized during spore germination are stained and evaluated by means of photometry. Bacterial activity is correlated with UV exposure.[13] These personal UV dosimeters had been used successfully in an infant study conducted in Germany by Siafarikas et al.[14] Because UV exposure measurement was not the primary outcome of the RCT, dosimeters were only issued to a subset of participants (3-4 per month to distribute over the course of 2 years because of the possible seasonal influence on UV exposure).

Instructions were given to attach dosimeters at the level of the infant's head or shoulders and positioned so that the UV-sensitive detector was unobscured to light.[15] This positioning was specified on the assumption that the face would be the area most reliably exposed to UV light not being covered by blankets or clothing. It was recommended that caregivers attach the dosimeter directly to the infant's clothing.

Infant allergic disease and infection data collection

Medical diagnosis of eczema was defined according to the guidelines of the American Academy of Dermatology Association by typical skin lesions, and severity was rated based on the SCORAD index.[16] Diagnosis of wheeze (virus induced and nonviral) was made by a medical doctor. Parents were also asked general infant health questions during the trial, including questions about use of antibiotics as a proxy assessment for infection given the requirement for a medical assessment and prescription. Data were collected at the age 3- and 6-month appointments.

TABLE I. Participants' baseline characteristics

	Placebo group (n = 98)	Vitamin D group (n = 97)
	Mean (SD)	Mean (SD)
Maternal 25(OH)D level, late gestation	75.9 (13.1)	76.8 (14.0)
Gestational age at birth (wk)	39.2 (1.0)	39.2 (1.0)
Age at randomization (d)	12.8 (4.8)	13.2 (5.2)
	No. (%)	No. (%)
Infant male sex	53 (54.1)	51 (52.6)
Caesarian section	38 (38.8)	37 (38.1)
Mother completed secondary school	93 (94.9)	90 (92.8)
Mother completed tertiary education	68 (69.4)	73 (75.3)
Maternal white race	79 (80.6)	83 (85.6)
Maternal history of allergic disease	75 (77.3)	75 (77.3)
Paternal history of allergic disease	71 (74.0)	75 (77.3)
Sibling history of allergic disease	21 (61.8)	20 (54.0)
Maternal Vitamin D supplementation in pregnancy	33 (34.0)	34 (35.8)
Infant season of birth		
Summer	13 (13.3)	17 (17.5)
Autumn	28 (28.6)	16 (16.5)
Winter	27 (27.6)	30 (30.9)
Spring	30 (30.6)	34 (35.1)

TABLE II. Participants' 25(OH)D levels

	Placebo group, mean (SD)	Vitamin D group, mean (S)D	*P* value
Infant age			
Three mo	59.2 (22.7)	83.2 (27.8)	<.01
Six mo	82.0 (27.9)	93.1 (28.7)	.02

Response to Toll-like receptor ligands, polyclonal mitogen, and allergens

Cell culture was conducted in a subset of infants from whom sufficient PBMCs were collected. PBMCs (1×10^6/mL) were cultured alone or with Toll-like receptor (TLR) 2/6 ligand (FSL-1; 100 ng/mL), TLR4 ligand (LPS-EK Ultrapure; 10 ng/mL), and TLR7/8 ligand (R848; 10 μg/mL), all of which were purchased from InvivoGen (San Diego, Calif), to assess innate immune functional responses. All cultures were plated in 96-well round-bottom plates in 250 μL of RPMI-1640 (Life Technologies) plus 10% non–heat-inactivated FCS (Australian Biosearch, Karrinyup, Australia) and incubated at 37°C in a 5% CO_2 atmosphere for 24 hours.

Polyclonal T-cell responses were assessed by culturing PBMCs in AIM-V media supplemented with 2-mercaptoethanol in the presence of PHA mitogen (1 μg/mL; Murex Biotech, Dartford, United Kingdom) for 48 hours at 37°C in a 5% CO_2 atmosphere. Cytokine production to house dust mite extract (10 mg/mL; gifted by the Department of Cell Biology, Telethon Kids Institute, Subiaco, Australia) and egg ovalbumin (OVA; 100 mg/mL; InvivoGen) were also determined after 48 hours of incubation at 37°C in a 5% CO_2 atmosphere. After the described cell-culture period, plates were centrifuged, and supernatants were placed in aliquots and stored at −20°C until cytokine analysis.

Cytokine protein detection

Cytokines in cell-culture supernatants were measured with the Human Cytokine Magnetic 25-plex Panel (Life Technologies, Frederick, Md) and Luminex 200 multiplexing technology (Luminex, Austin, Tex). A value equivalent to the upper limit of the assay was assigned to measurements exceeding this limit of detection. All data are shown as increases above cytokine levels in unstimulated control wells.

Statistical methods

Analyses were performed according to the intention-to-treat principle. Between-group differences were assessed by using unpaired Student *t* tests, assuming equal variances for parametric data. Continuous data were analyzed by using Spearman rank correlation (rho). Mean (SD) and median (interquartile range [IQR]) values are reported for parametric and nonparametric data, respectively. The association between intervention group and clinical outcomes was assessed by using the Pearson χ^2 test. All tests were 2-sided, with a *P* value of less than .05 considered significant. Statistical analysis was performed with IBM SPSS Statistics software for Windows (version 25; IBM, Armonk, NY).

RESULTS

Infant characteristics

Enrollment began on October 9, 2012, and ended on January 23, 2017. A total of 195 infants were randomized into the trial, 97 to the intervention vitamin D group and 98 to the placebo group. Fig 1 shows the participant flow diagram. Baseline characteristics of the 2 groups are described in Table I. Allocations in the vitamin D group compared with those in the placebo group were not different across seasons ($P = .24$). Data collection was completed on July 4, 2017. Ninety-two percent (180/195) of infant participants attended their appointment at 3 months of age, and 89% (173/195) of infants attended their appointment at 6 months of age. Nine (n = 6 from the vitamin D group) parents withdrew consent to participate during the intervention period.

25(OH)D levels

Blood samples were collected from 140 infants (n = 68 from the vitamin D group) at 3 months of age and 141 infants (n = 73 from the vitamin D group) at 6 months of age. At both 3 ($P < .01$) and 6 ($P = .02$) months of age, 25(OH)D levels were greater for the vitamin D group than the placebo group, as reported in Table II. At 3 months of age, 26 infants (n = 22 from the placebo group) were found to have a 25(OH)D level of less than 50 nmol/L. The parents of these 26 infants were advised to cease study product use and were referred to their local general practitioner for independent advice regarding vitamin D supplementation. These infants remained in the study for follow-up at 6 months of age.

Between 0 and 3 months of age, 15 infants (n = 6 from the placebo group) and an additional 7 infants (n = 1 from the placebo group) between 3 and 6 months of age consumed 1000 mL/d or more of vitamin D–supplemented infant formula and were advised to cease the study product use but remained in the study for follow-up at 6 months of age. In addition, by 3 months of age, 41% of infants in the placebo group had consumed some vitamin D–supplemented infant formula, and this increased to 56% by 6 months of age.

UV light exposure from 0 to 3 months of age

A total of 86 infants were given a personal UV dosimeter to wear until 3 months of age. Noncompliance with use of the UV dosimeter occurred with 4 infants, leaving 82 infants with useable UV light exposure data (n = 34 from the vitamin D group).

TABLE III. UV light exposure from 0 to 3 months of age and 25(OH)D levels

	Placebo group	Vitamin D group	All infants
	Correlation coefficient, no. of infants	Correlation coefficient, no. of infants	Correlation coefficient, no. of infants
Age 3 mo	0.108 (P = .52), n = 38	0.291 (P = .18), n = 23	0.250 (P = .05), n = 61
Age 6 mo	0.068 (P = .70), n = 34	0.326 (P = .09), n = 28	0.245 (P = .06), n = 62

The total median UV light exposure measured in these 82 infants was 952 J/m^2 (IQR, 557-1577 J/m^2) from 0 to 3 months of age, typically with faces, hands, and arms exposed. There was a difference (P = .047) in the measured UV light exposure between the vitamin D group (median, 1204 J/m^2 [IQR, 709-1955 J/m^2]) and the control group (median, 815 J/m^2 [IQR, 508-1394 J/m^2]). There were no correlations between UV light exposure and 25(OH)D levels at 3 or 6 months of age, as described in Table III. UV light exposure was not associated with season of birth (P = .33): winter-born infants had a median UV exposure of 1018 J/m^2 (IQR, 678-1955 J/m^2), and summer-born infants had a median UV exposure of 1203 J/m^2 (IQR, 717-1825 J/m^2).

Clinical allergic disease and infection outcomes

By 6 months of age, 35 infants had doctor-diagnosed eczema, and 30 had at least 1 episode of doctor-diagnosed wheeze. The frequency of either outcome did not differ significantly between the vitamin D and placebo groups, as reported in Table IV. Eczema severity (objective SCORAD score) also did not differ between the vitamin D and placebo groups at either 3 (P = .81) or 6 (P = .94) months of age. There was also no difference (P = .51) in maternal late gestation vitamin D levels between infants with doctor-diagnosed eczema (25[OH]D mean, 78.4 nmol/L [SD, 14.2 nmol/L]) and those without eczema (25(OH)D mean, 76.7 nmol/L [SD, 13.3 nmol/L]). The majority of infant wheeze occurred with a viral illness; only 4 infants (all from the placebo group) had nonviral wheezing during the intervention period.

However, infants with doctor-diagnosed eczema in the first 6 months of life had significantly less UV light exposure (n = 16: median, 555 J/m^2 [IQR, 322-1210 J/m^2]; n = 64: median, 998 J/m^2 [IQR, 676-1577 J/m^2]; P = .02) by 3 months of age. No significant difference in UV exposure (P = .96) was found between infants who did (n = 14) or did not (n = 64) have doctor-diagnosed wheeze in the first 6 months of life.

Antibiotics were prescribed at least once during the intervention period for 12 (14.1%) of 85 infants in the vitamin D group compared with 22 (25.0%) of 88 infants in the placebo group (P = .11). Antibiotic use was not associated with UV exposure in the infants (P = .74).

Immune function outcomes

At 6 months of age, infants supplemented with vitamin D had lower production of the T-cell growth factor IL-2 in response to TLR2/6 ligand (P = .03) and TLR4 ligand (P = .02), with a similar trend for TLR7/8 ligand (P = .06), as shown in Fig 2. Differences in adaptive responses were also observed, with lower production of the proinflammatory cytokine IL-1β in response to OVA allergen in the vitamin D group (P < .01, Fig 2). Plasma 25(OH)D levels at 6 months of age were only inversely correlated with GM-CSF to OVA (rho = −0.350, P = .01, n = 50) and IL-4 to PHA (rho = −0.283, P = .03, n = 57). Table E1 in this article's Online Repository at www.jacionline.org reports all correlation results between 25(OH)D levels at 6 months of age and PBMC cytokine expression at 6 months of age. Plasma 25(OH)D levels at 3 months of age were inversely correlated with cytokine responses only to PHA (n = 46): IFN-γ (rho = −0.299, P = .04), IL-2 (rho = −0.388, P = .01), and monokine induced by IFN-γ (rho = −0.481, P < .01). Table E2 in this article's Online Repository at www.jacionline.org reports all correlation results between 25(OH)D levels at 3 months of age and PBMC cytokine expression at 6 months of age. Because of an insufficient number of infants with adequate blood volume collection for PBMC cytokine analysis, this was not undertaken at 3 months of age.

TABLE IV. Clinical allergic disease outcomes

	Placebo group, no. (%)	Vitamin D group, no. (%)	P value
Doctor-diagnosed eczema by 3 mo	6 (6.7)	9 (10.0)	.42
Doctor-diagnosed eczema by 6 mo	16 (19.3)	19 (21.8)	.68
Doctor-diagnosed wheeze by 3 mo	7 (8.0)	7 (8.0)	1.00
Doctor-diagnosed wheeze by 6 mo	14 (16.7)	16 (18.2)	.79

UV light exposure from 0 to 3 months of age was inversely correlated with IL-2 production to TLR2/6 (rho = −0.441, P = .02, n = 26), TLR4 (rho = −0.496, P < .01, n = 30), and TLR7/8 (rho = −0.502, P < .01, n = 26: Fig 3). Other correlations that emerged between UV exposure and cytokine production were lower levels of RANTES and eotaxin in response to TLR4 (rho = −0.402, P = .03, n = 30 and rho = −0.452, P = .01, n = 30, respectively; Fig 3). In addition, UV exposure was inversely correlated with production of GM-CSF to TLR4 and TLR7/8 (rho = −0.455, P = .01, n = 30, and rho = −0.630, P < .01, n = 26, respectively; Fig 3). Table V reports all correlation results between UV light exposure from 0 to 3 months of age and PBMC cytokine expression at 6 months of age.

DISCUSSION

This study is the first to demonstrate an association between greater direct infant UV light exposure in the first 3 months of life and both lower incidence of medically diagnosed infant eczema and lower levels of immune factors associated with allergic inflammation at 6 months of age. Greater UV light exposure was associated with lower production of IL-2, which plays a central role in differentiation of naive CD4 T cells into T_H2 cells, with potential implications for allergic disease development[17]; GM-CSF, which facilitates antigen-specific T_H2 responses and allergic inflammation[18]; and lower levels of

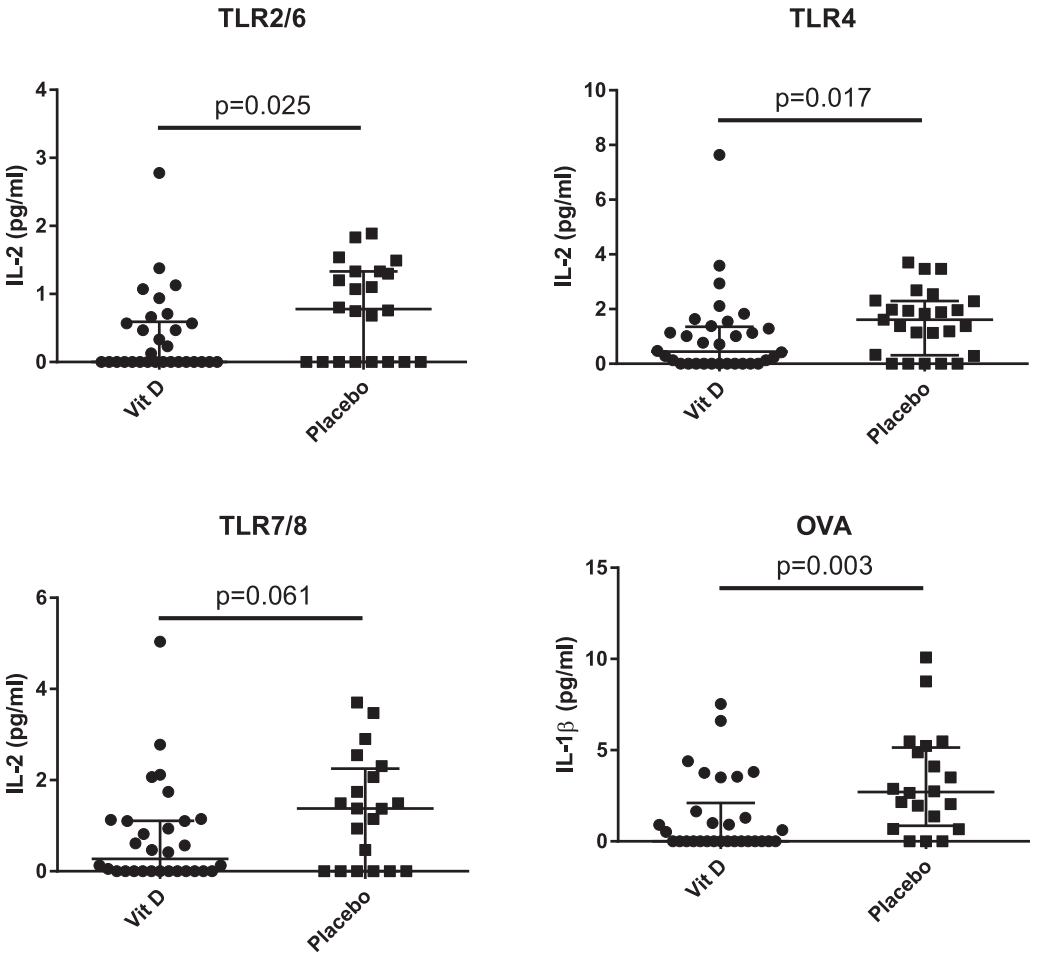

FIG 2. Scatter plots of cytokine (IL-2 and IL-1β production to TLR (TLR2/6, TLR4, and TLR7/8) ligands and OVA comparison between the intervention vitamin D–supplemented and placebo groups.

eotaxin, a chemokine associated with allergic inflammation.[19,20] Hence both early infancy clinical symptoms and immune markers of inflammation appear to be decreased by direct exposure of infants to UV light in the first few postnatal months. This is in accordance with the previous findings[2-5] of greater allergic disease incidence at higher latitudes, where there is less UV light exposure.

Our finding of no significant difference between the infant vitamin D–supplemented group and the placebo group for infant doctor-diagnosed eczema is consistent with vitamin D supplementation during pregnancy and offspring allergic disease outcome trials conducted over recent years.[21-24] In this study we also found no association between maternal late gestation vitamin D levels and infant doctor-diagnosed eczema by 6 months of age. This indicates that there might be local (skin) or systemic effects of UV radiation that are independent of the parallel effect that UV light exposure has on vitamin D status. It has been previously proposed that bioactive molecules, such as nitric oxide[25] and urocanic acid,[26,27] which are released from the skin into the systemic circulation after sunlight exposure, could mediate long-lasting, epigenetically imprinted effects on immune function.[28] Chimeric mouse studies have demonstrated altered functionality within cells of the myeloid lineage that is transferable from UV-irradiated donor mice to naive recipients.[28,29] For instance, UV-chimeric mice exhibit reduced responses to a contact hypersensitivity assay or skin treatment with an inflammatory stimulus.[28] The authors ascribe these findings to a reduced ability of myeloid-derived antigen-presenting cells to initiate responses to antigens and not to effects of UV radiation on T-cell proliferation or generation of IL-10.[28] The present study also finds UV exposure to be inversely correlated with antigen-presenting cell–dependent innate immune responses, suggesting that the observed association between UV and eczema might be subsequent to epigenetic changes in myeloid progenitors.

In addition, suberythemal doses of UV radiation can have localized vitamin D–dependent and independent effects that protect against development of allergic disease.[30] UV radiation can support skin barrier function through activation of the cutaneous vitamin D system, which in turns stimulates filaggrin, epidermal ceramide production, epidermal lipid synthetic enzymes, and involucrin.[31] Moreover, there is some evidence that direct suberythemal UV radiation can upregulate the synthesis of other significant stratum corneum lipids, including free fatty acids.[32] Ideally, future human studies need to more fully investigate the relationship between UV light exposure and these mechanistic and clinical outcomes in early life.

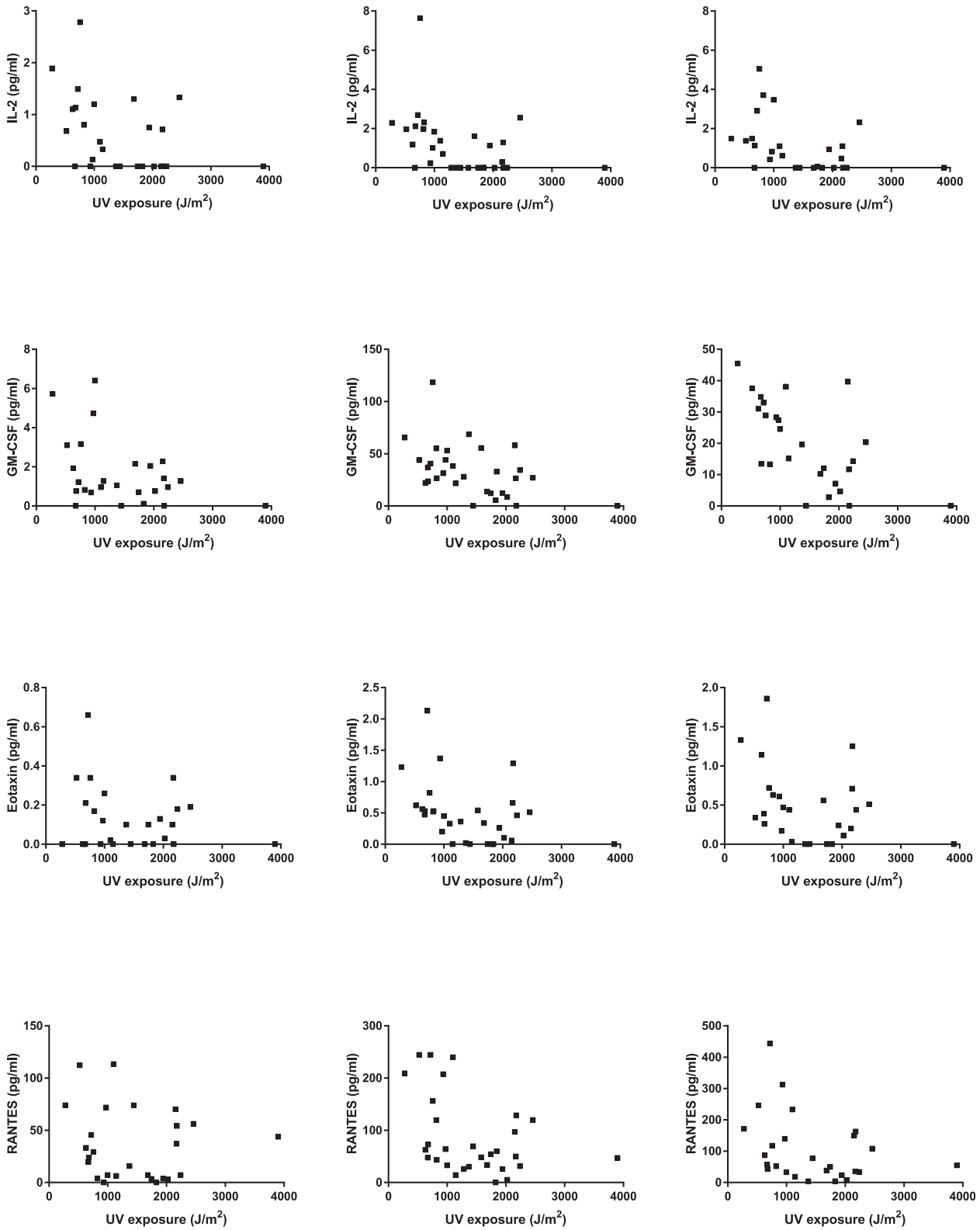

FIG 3. Scatter plots of cytokine (IL-2, GM-CSF, eotaxin, and RANTES) production to TLR (TLR2/6, TLR4 and TLR7/8) ligands in accordance with UV light exposure from 0 to 3 months of age.

In summary, our findings indicate that UV light exposure appears to be more beneficial than infant vitamin D supplementation as an allergy prevention strategy in early life. Future mechanistic studies need to investigate which bioactive molecules can be involved. However, further research is still required to determine how much sunlight exposure in infancy is ideal, and

TABLE V. Correlation coefficients between UV light exposure from 0 to 3 months of age and PBMC cytokine expression at 6 months of age

	TLR2/6 (n = 26)	TLR4 (n = 30)	TLR7/8 (n = 26)	HDM (n = 26)	OVA (n = 26)	PHA (n = 30)
IL-1β	−0.030, P = .88	−0.228, P = .23	−0.208, P = .31	−0.016, P = .94	−0.207, P = .31	−0.317, P = .09
IL-10	−0.112, P = .58	−0.147, P = .44	−0.201, P = .32	0.142, P = .49	−0.301, P = .13	0.132, P = .49
IL-13	−0.192, P = .35	−0.063, P = .74	−0.069, P = .74	−0.175, P = .39	−0.170, P = .41	−0.295, P = .11
IL-6	−0.238, P = .24	−0.279, P = .14	−0.275, P = .17	0.030, P = .88	−0.093, P = .65	−0.334, P = .07
IL-12	−0.049, P = .81	−0.205, P = .27	−0.181, P = .38	−0.117, P = .57	−0.087, P = .67	−0.112, P = .55
RANTES	−0.167, P = .42	**−0.402, P = .03**	−0.381, P = .05	−0.096, P = .64	−0.007, P = .97	**−0.383, P = .04**
Eotaxin	−0.082, P = .69	**−0.452, P = .01**	−0.339, P = .09	0.095, P = .64	0.024, P = .91	−0.190, P = .31
IL-17	0.057, P = .78	−0.083, P = .66	−0.055, P = .79	0.323, P = .11	0.072, P = .73	0.049, P = .79
MIP-1α (CCL3)	−0.169, P = .41	**−0.369, P = .04**	−0.319, P = .11	0.077, P = .71	0.005, P = .98	−0.166, P = .38
GM-CSF	−0.300, P = .14	**−0.455, P = .01**	**−0.630, P < .01**	0.176, P = .39	−0.138, P = .50	−0.166, P = .38
MIP-1β (CCL2)	−0.227, P = .26	−0.257, P = .17	−0.331, P = .09	−0.098, P = .63	−0.039, P = .85	−0.093, P = .62
MCP-1	−0.278, P = .17	−0.131, P = .49	−0.254, P = .21	−0.116, P = .57	−0.246, P = .22	−0.170, P = .37
IL-15	−0.364, P = .07	**−0.374, P = .04**	**−0.426, P = .03**	−0.314, P = .12	−0.366, P = .07	**−0.442, P = .01**
IL-5	−0.056, P = .79	0.060, P = .75	0.109, P = .59	0.142, P = .49	0.128, P = .53	−0.195, P = .30
IFN-γ	−0.307, P = .13	−0.183, P = .33	−0.259, P = .20	−0.039, P = .85	0.017, P = .93	−0.225, P = .23
IFN-α	−0.177, P = .39	−0.106, P = .58	−0.199, P = .33	−0.076, P = .71	−0.309, P = .12	−0.284, P = .13
IL-1RA	−0.187, P = .36	**−0.433, P = .02**	**−0.393, P = .04**	−0.153, P = .45	−0.171, P = .40	**−0.461, P = .01**
TNF-α	−0.125, P = .54	−0.244, P = .19	−0.151, P = .46	−0.116, P = .57	−0.056, P = .79	**−0.364, P = .04**
IL-2	**−0.441, P = .02**	**−0.496, P < .01**	**−0.502, P < .01**	0.086, P = .68	0.096, P = .64	−0.251, P = .18
IL-7	−0.322, P = .11	−0.348, P = .06	−0.298, P = .14	−0.182, P = .37	−0.056, P = .79	−0.171, P = .37
IP-10	−0.267, P = .19	−0.326, P = .08	−0.344, P = .08	−0.119, P = .56	0.063, P = .76	−0.307, P = .09
IL-2R	−0.028, P = .89	−0.133, P = .48	−0.031, P = .88	0.010, P = .96	−0.310, P = .12	0.019, P = .92
MIG	−0.164, P = .42	−0.178, P = .34	−0.186, P = .36	−0.041, P = .84	0.023, P = .91	−0.220, P = .24
IL-4	−0.214, P = .29	−0.229, P = .22	−0.260, P = .20	0.061, P = .77	−0.074, P = .72	−0.024, P = .90
IL-8	−0.268, P = .19	−0.266, P = .15	−0.281, P = .16	−0.241, P = .23	−0.254, P = .21	−0.140, P = .46

Boldface type indicates statistical significance.
HDM, House dust mite; *IL-1RA*, IL-1 receptor antagonist; *IL-2R*, IL-2 receptor; *IP-10*, interferon-inducible protein 10; *MCP*, monocyte chemoattractant protein; *MIG*, monokine induced by IFN-γ; *MIP*, macrophage inflammatory protein.

we need to weigh the benefits of potentially reduced allergic disease outcomes with potentially increased cancer risks also associated with sun exposure–related skin damage.

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Clinical implications: Direct UV light exposure in early infancy appears to be an important infant eczema prevention strategy.

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TABLE E1. Correlation coefficients between 25(OH)D levels at 6 months of age and PBMC cytokine expression at 6 months of age

	TLR2/6 (n = 52)	TLR4 (n = 57)	TLR7/8 (n = 50)	HDM (n = 52)	OVA (n = 50)	PHA (n = 57)
IL-1β	0.056, $P = .69$	−0.116, $P = .39$	−0.123, $P = .39$	0.074, $P = .61$	−0.211, $P = .14$	−0.013, $P = .93$
IL-10	−0.162, $P = .25$	−0.113, $P = .40$	0.005, $P = .97$	−0.132, $P = .35$	−0.297, $P = .05$	−0.180, $P = .18$
IL-13	−0.209, $P = .14$	−0.134, $P = .32$	−0.109, $P = .45$	0.003, $P = .98$	−0.057, $P = .70$	−0.133, $P = .32$
IL-6	0.153, $P = .28$	−0.097, $P = .47$	0.060, $P = .68$	0.156, $P = .27$	−0.040, $P = .78$	−0.116, $P = .39$
IL-12	0.113, $P = .42$	−0.005, $P = .97$	0.097, $P = .50$	0.150, $P = .29$	−0.090, $P = .53$	0.137, $P = .31$
IL-17	−0.046, $P = .75$	−0.028, $P = .84$	−0.060, $P = .68$	−0.033, $P = .82$	−0.069, $P = .63$	−0.086, $P = .53$
RANTES	0.000, $P = 1.00$	−0.134, $P = .32$	−0.127, $P = .38$	0.178, $P = .21$	−0.023, $P = .88$	0.069, $P = .61$
Eotaxin	−0.155, $P = .27$	−0.152, $P = .26$	−0.166, $P = .25$	−0.080, $P = .57$	−0.204, $P = .16$	−0.060, $P = .66$
GM-CSF	−0.120, $P = .40$	−0.187, $P = .16$	−0.267, $P = .06$	−0.260, $P = .06$	**−0.350, $P = .01$**	−0.030, $P = .83$
MIP-1α (CCL3)	0.098, $P = .49$	−0.170, $P = .21$	−0.024, $P = .87$	0.233, $P = .10$	−0.018, $P = .90$	0.161, $P = .23$
MIP-1β (CCL4)	0.013, $P = .93$	−0.060, $P = .66$	−0.006, $P = .97$	0.170, $P = .23$	0.127, $P = .38$	0.168, $P = .21$
MCP-1 (CCL2)	−0.100, $P = .48$	−0.087, $P = .52$	0.048, $P = .74$	0.133, $P = .35$	0.064, $P = .66$	0.039, $P = .77$
IL-15	−0.107, $P = .45$	−0.065, $P = .63$	−0.222, $P = .12$	−0.163, $P = .25$	−0.248, $P = .08$	0.014, $P = .92$
IL-5	−0.048, $P = .74$	−0.051, $P = .71$	−0.115, $P = .43$	0.047, $P = .74$	−0.169, $P = .24$	−0.175, $P = .19$
IFN-γ	−0.013, $P = .93$	0.191, $P = .15$	−0.037, $P = .80$	0.008, $P = .95$	−0.169, $P = .24$	0.202, $P = .13$
IFN-α	0.041, $P = .78$	−0.009, $P = .94$	0.150, $P = .30$	0.201, $P = .15$	0.071, $P = .63$	0.097, $P = .48$
IL-1RA	−0.087, $P = .54$	−0.176, $P = .19$	−0.170, $P = .24$	0.200, $P = .16$	0.094, $P = .52$	0.020, $P = .88$
TNF-α	−0.012, $P = .93$	0.002, $P = .99$	−0.064, $P = .66$	−0.141, $P = .32$	−0.037, $P = .80$	0.011, $P = .94$
IL-2	−0.135, $P = .34$	−0.170, $P = .21$	−0.250, $P = .08$	−0.198, $P = .16$	−0.182, $P = .21$	−0.134, $P = .32$
IL-7	0.122, $P = .39$	−0.208, $P = .12$	0.027, $P = .85$	0.120, $P = .40$	−0.014, $P = .93$	−0.083, $P = .54$
IP-10 (CXCL10)	−0.255, $P = .07$	−0.221, $P = .10$	−0.095, $P = .51$	−0.152, $P = .28$	−0.151, $P = .30$	0.016, $P = .91$
IL-2R	0.016, $P = .91$	−0.191, $P = .16$	−0.135, $P = .35$	0.112, $P = .43$	−0.034, $P = .81$	0.079, $P = .56$
MIG (CXCL9)	−0.123, $P = .39$	−0.092, $P = .50$	−0.001, $P = 1.00$	0.134, $P = .34$	0.159, $P = .27$	0.069, $P = .61$
IL-4	−0.159, $P = .26$	−0.097, $P = .47$	−0.044, $P = .76$	−0.118, $P = .41$	−0.267, $P = .06$	**−0.283, $P = .03$**
IL-8	−0.093, $P = .51$	−0.129, $P = .34$	−0.111, $P = .44$	0.055, $P = .70$	0.054, $P = .71$	−0.096, $P = .48$

Boldface type indicates statistical significance.

HDM, House dust mite; *IL-1RA*, IL-1 receptor antagonist; *IL-2R*, IL-2 receptor; *IP-10*, interferon-inducible protein 10; *MCP*, monocyte chemoattractant protein; *MIG*, monokine induced by IFN-γ; *MIP*, macrophage inflammatory protein.

TABLE E2. Correlation coefficients between 25(OH)D levels at 3 months of age and PBMC cytokine expression at 6 months of age

	TLR2/6 (n = 41)	TLR4 (n = 46)	TLR7/8 (n = 39)	HDM (n = 41)	OVA (n = 39)	PHA (n = 46)
IL-1β	0.048, $P = .77$	0.173, $P = .25$	0.013, $P = .94$	−0.196, $P = .22$	−0.293, $P = .07$	−0.081, $P = .59$
IL-10	0.083, $P = .61$	0.086, $P = .57$	−0.014, $P = .93$	−0.022, $P = .89$	0.051, $P = .76$	−0.133, $P = .38$
IL-13	−0.18, $P = .26$	0.065, $P = .67$	0.005, $P = .97$	−0.257, $P = .11$	−0.139, $P = .40$	−0.295, $P = .50$
IL-6	−0.01, $P = .95$	0.012, $P = .94$	−0.02, $P = .91$	−0.213, $P = .18$	−0.234, $P = .15$	−0.079, $P = .60$
IL-12	0.035, $P = .83$	−0.054, $P = .72$	−0.122, $P = .46$	−0.143, $P = .37$	−0.311, $P = .05$	0.079, $P = .60$
IL-17	−0.003, $P = .99$	0.044, $P = .77$	−0.063, $P = .70$	−0.166, $P = .30$	0.039, $P = .81$	−0.079, $P = .60$
RANTES	0.052, $P = .75$	0.015, $P = .92$	0.000, $P = 1.00$	−0.11, $P = .49$	−0.091, $P = .58$	−0.105, $P = .49$
Eotaxin	0.113, $P = .48$	0.189, $P = .21$	0.102, $P = .54$	0.209, $P = .19$	−0.071, $P = .67$	0.067, $P = .66$
GM-CSF	−0.06, $P = .71$	0.035, $P = .82$	0.039, $P = .81$	−0.156, $P = .33$	−0.195, $P = .23$	−0.234, $P = .12$
MIP-1α (CCL3)	−0.033, $P = .84$	0.003, $P = .98$	−0.015, $P = .93$	−0.229, $P = .15$	−0.256, $P = .12$	−0.186, $P = .22$
MIP-1β (CCL4)	0.002, $P = .99$	0.013, $P = .93$	−0.037, $P = .82$	−0.278, $P = .08$	−0.176, $P = .28$	−0.108, $P = .47$
MCP-1 (CCL2)	0.107, $P = .50$	0.051, $P = .74$	−0.025, $P = .88$	−0.085, $P = .60$	−0.059, $P = .72$	0.000, $P = 1.00$
IL-15	−0.032, $P = .84$	0.043, $P = .78$	−0.11, $P = .50$	−0.197, $P = .22$	−0.069, $P = .67$	−0.221, $P = .14$
IL-5	−0.022, $P = .89$	−0.03, $P = .84$	−0.052, $P = .76$	−0.106, $P = .51$	−0.252, $P = .12$	−0.079, $P = .60$
IFN-γ	0.037, $P = .82$	−0.046, $P = .76$	−0.193, $P = .24$	−0.199, $P = .21$	0.002, $P = .99$	**−0.299, $P = .04$**
IFN-α	0.113, $P = .48$	0.029, $P = .85$	−0.087, $P = .60$	−0.125, $P = .44$	−0.171, $P = .30$	−0.044, $P = .77$
IL-1RA	−0.016, $P = .92$	−0.011, $P = .94$	−0.156, $P = .34$	−0.131, $P = .41$	0.023, $P = .89$	−0.189, $P = .21$
TNF-α	0.153, $P = .34$	0.121, $P = .43$	0.136, $P = .41$	−0.134, $P = .41$	0.126, $P = .44$	−0.067, $P = .66$
IL-2	−0.040, $P = .80$	−0.076, $P = .62$	−0.043, $P = .80$	−0.303, $P = .05$	−0.162, $P = .32$	**−0.388, $P = .01$**
IL-7	0.047, $P = .77$	0.097, $P = .52$	0.140, $P = .40$	0.098, $P = .54$	0.191, $P = .24$	0.118, $P = .44$
IP-10 (CXCL10)	0.040, $P = .80$	−0.065, $P = .67$	−0.066, $P = .69$	−0.095, $P = .56$	−0.037, $P = .82$	−0.278, $P = .06$
IL-2R	0.064, $P = .69$	0.181, $P = .23$	0.198, $P = .23$	−0.257, $P = .11$	−0.187, $P = .25$	−0.290, $P = .05$
MIG (CXCL9)	0.177, $P = .27$	0.115, $P = .45$	0.051, $P = .76$	−0.131, $P = .41$	−0.173, $P = .29$	**−0.481, $P < .01$**
IL-4	0.156, $P = .33$	0.142, $P = .35$	0.105, $P = .53$	−0.09, $P = .57$	0.010, $P = .95$	0.026, $P = .86$
IL-8	−0.001, $P = 1.00$	0.012, $P = .94$	0.107, $P = .52$	−0.191, $P = .23$	−0.162, $P = .32$	−0.194, $P = .20$

Boldface type indicates statistical significance.

HDM, House dust mite; *IL-1RA*, IL-1 receptor antagonist; *IL-2R*, IL-2 receptor; *IP-10*, interferon-inducible protein 10; *MCP*, monocyte chemoattractant protein; *MIG*, monokine induced by IFN-γ; *MIP*, macrophage inflammatory protein.