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Dental Sealants and Composite Restorations and Longitudinal Changes in Immune Function Markers in Children

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SUMMARY

Objective—Resins used in dental composites, derived from bisphenol-A (BPA), have been shown to alter immune cells. The objective of this study was to explore children's immune function changes in relation to resin-composites treatment.

Design—We conducted secondary data analysis of the New England Children's Amalgam Trial immune function substudy (N=59). Immune function was measured pre-treatment and up to 5 times post-treatment through 5 year follow-up. Multivariable generalized linear regression models were used to estimate the association between three classes of resin-composites (bisphenol-A-diglycidyl-dimethacrylate [BisGMA]-based flowables used for preventive sealants; urethane dimethacrylate [UDMA]-based compomer restorations; bisGMA-based restorations) and changes in immune function markers measured annually.

Results—Total white blood cell counts and responsiveness of T-cells or neutrophils were not appreciably altered by composite treatment levels. Changes in B-cell responsiveness were greater throughout follow-up among children with more bisGMA-based composite restorations, which opposed findings for amalgam treatment levels. Monocyte responsiveness changes were decreased at 6-months with greater treatment, but not over longer follow-up.

Conclusions—Results of this analysis showed no overt immune function alterations associated with resin-composites. Additional research regarding lymphocyte activation may be warranted given the consistency of results within these analyses and with a prior study showing increased B-cell activation.

INTRODUCTION

Dental resin composite materials have become an integral part of comprehensive dental care and have proven versatile in areas of restorative and preventive dentistry. It is estimated that more than 10 million composite restorations are placed in children each year in the U.S. alone, and sealant prevalence on permanent teeth is on the rise.[1,2] Resin composite has been available for over fifty years, when a resin made from the monomer bisphenol-A-diglycidyl-dimethacrylate (BisGMA) was first introduced. More popular than the acrylic-based resins before it, bisGMA resins offered lower polymerization shrinkage and greater strength.[3] BisGMA is predominant in most composites current manufactured worldwide, although

monomers such as urethane dimethacrylate (UDMA) and triethylene glycol dimethacrylate (TEGDMA) are often used in place of or in addition to BisGMA depending upon the desired strength and viscosity of the material.[4,5] The ability to modify the properties of the resin material has allowed composites to be classified by their composition and viscosity. Packable (or "non-flowable") composites are commonly used for restoration of caries, while flowable composites are used for preventive restorations and fissure sealants.

Despite the wide use of composite resins in dentistry, concerns regarding their biological safety remain.[6,7] Numerous laboratory studies have shown genotoxic, cytotoxic, and mutogenic effects of resin-composite materials.[6–11] An unresolved question is whether bisphenol A (BPA), a synthetic resin shown to leach from bisGMA-based composites,[12–14] has relevance to possible adverse effects in humans. BPA is used in the synthesis of bisGMA, but not of UDMA or TEGDMA. BPA has been shown to have a variety of adverse effects in laboratory studies, including capacity for endocrine disruption, neuroendocrine signaling, altered metabolism and immune function.[15] In vivo studies have shown both the estrogenic and anti-androgenic effects of BPA on hormonal signaling and regulation, including effects on estrogen receptor gene expression and sex-hormone binding globulin levels.[16–18] In the general population of men and women, urinary BPA concentrations were associated with coronary artery disease risk in both US and UK studies[19–21], and with obesity and insulin resistance in children and adults.[22–25]. Immune function alterations in association with BPA have been observed in laboratory experiments.[26–29] BPA has been found to increase the activity of B cells,[26,30] which results in overproduction of antibodies and overstimulation of the immune system. Murine models have also found prenatal BPA exposure inhibited the induction of antigen-specific T-cell tolerance and resulted in a concomitant increase in antigen specific antibodies.[31]

BisGMA has also been implicated in immune function deregulation. BisGMA is capable of inducing double-strand DNA breaks in human lymphocytes and gingival fibroblasts.[32,33] A study of Swedish adolescents assessing the genotoxicity of composite and amalgam fillings found that with increasing numbers of composite fillings, B-cell lymphocyte micronuclei were increased; of note, the opposite association was found for amalgam fillings, and there were no differences in T4 or T8 lymphocyte micronuclei.[34]

The New England Children's Amalgam Trial (NECAT) was a randomized clinical trial that found no adverse neuropsychological effects of dental amalgam compared to resin-composite restorations.[35] NECAT included a substudy to examine effects on the immune system. Primary analyses using the randomized treatment group assignment showed no overt immune deficits in children assigned to amalgam through 5-year follow-up.[36] However, the effect of increasing treatment levels of resin-composites on measurable effects on immune function parameters was unexamined. The possible relevance of composite treatment levels was identified in secondary NECAT analyses, showing that treatment with bisGMA-based composites was associated with slightly poorer behavioral measures,[37] though not with neuropsychological tests or physical development.[38,39] No prior studies have examined dental resin-composites and immune function changes in children over time.

In this secondary analysis of the NECAT immune function substudy, we examined treatment levels of resin-composite materials, examining both flowable (e.g. sealants) and non-flowable (e.g. restorative fillings) types, and changes in immune cells of children through 5-years follow-up.

MATERIALS AND METHODS

Study Population

Participants were a sub-sample of NECAT, a two-group randomized safety trial of amalgam and composite restorations conducted from 1997–2005 at five clinics in the urban Boston area and one clinic in rural Farmington Maine. NECAT and the immune function substudy were approved by the institutional review boards of all participating sites. Details of both study protocols have been published.[35,36,40]

NECAT eligibility criteria were: aged 6–10 y; English fluency; no amalgam restorations; ≥2 posterior teeth with caries requiring restoration on occlusal surfaces; and, by parent-report, no physician-diagnosed immunosuppressive, psychological, behavioral, neurological, or renal disease. Of 5,110 children screened for eligibility, 598 were eligible, and 534 had written parental informed consent and child assent. For immune substudy recruitment, children were sampled equally from each randomly-assigned treatment group. Of 257 children invited to participate in the substudy, 66 (26%) provided consent/assent (35 amalgam group, 31 composite group). The primary reason for refusal into the substudy was fear of blood draws (mentioned by 75%), followed by time commitment (40%).

Immune function data at both baseline and 5-year follow-up, which were required for inclusion in this analysis, were available for 59 children (29 amalgam, 30 composite). A profile of substudy participants and comparisons to all NECAT participants were published in the previous report of treatment group effects.[36] Substudy participants were more likely to be from the rural study site (57.6% vs. 45.5%) and non-Hispanic white (89.8% vs. 62.1%), but similar to those in the parent study regarding the presence of allergy or asthma and the extent of dental treatment needs.

Interventions

Participants received comprehensive dental care semi-annually during their 5-year participation. Standard dental care included exams, cleaning, fluoride application, sealant placement, and restorative treatment. Dental procedures and materials were standardized across study sites, following manufacturer’s indications for use. Table 1 lists the sealant and restorative composite materials used.

Immune Function Measurement

Blood samples were collected at baseline and at 5 time-points after the initial dental treatment: 5–7 days, 6 months, 12 months, 18 months, and 5 years. Heparinized blood samples were shipped overnight in insulated containers with gel packs prewarmed to 30°C to a central laboratory (University of Pennsylvania, Philadelphia), where all assays were

performed. Laboratory technicians were blinded regarding dental treatments and checked assays for reproducibility as described previously.[36]

Four categories of immune parameters were assessed: (i) white blood cell (WBC) enumeration; (ii) T-cell responsiveness; (iii) B-cell responsiveness; and (iv) neutrophil and monocyte responsiveness. Total WBC enumeration was performed using Wright's stain and hemocytometer. Distribution of neutrophils, monocytes, T-cells, B-cells, natural killer cells, and cluster of differentiation (CD) subtypes CD4 and CD8 were determined by flow cytometry using the IMK+ Simultest kit (BD Biosciences, San Jose, Calif.). Functional analysis of T-cells after mitogenic activation involved two approaches: analysis of activation markers and cell cycle distribution. T-cells were incubated with phytohemagglutinin (PHA) (5 μg/mL) for 24 hr to assess expression of activation markers; CD69 and CD25 expression were determined by immunofluorescence using flow cytometry as described previously.[41] Cell cycle distribution was assessed after 72 hours' incubation in the presence of PHA.[42] B-cell activation was monitored by analyzing expression of CD69 and increased expression of CD23 after stimulation with pokeweed mitogen (PWM) (10 μg/mL).

Functional status of neutrophils and monocytes was determined by monitoring the oxidative burst in response to stimulation with phorbol myristate acetate (PMA) (0.5 μg/mL). The fluorescent probes dihydroethidium and dihydrorhodamine were used to assess superoxide ($O_2^{-\bullet}$) and hydrogen peroxide (H_2O_2) generation, respectively; fluorescence was determined by flow cytometry 30 minutes after cell activation.[43]

Statistical Methods

With 59 children, the substudy has 80% power to detect a correlation coefficient of 0.35 between exposure levels and changes in immune function markers. Interpretation of these results is based on consistency, rather than statistical significance. Therefore, we first visually examined graphical plots of immune changes over time, overall and by resin-composite levels. Where standard laboratory reference values were available, we descriptively compared the percentage of children who had cell counts outside standard ranges by dental treatment exposure group (using references values from the Mayo Clinic Medical Laboratories http://www.mayomedicallaboratories.com/test-info/pediatric/refvalues/reference.php).

We separately examined three classes of resin-composite materials: (i) flowable composites used as preventive sealants and preventive resin restorations (PRR), (ii) non-flowable standard bisGMA-based composite used for restoration of permanent tooth caries, and (iii) non-flowable polyacid-modified UDMA-based composite (compomer) used for restoration of primary (deciduous) tooth caries (Table 1). Although there were two different bisGMA-based flowable products used for PRRs and sealants, the specific flowable product used was not consistently recorded on NECAT data collection forms; thus, we combined them in analysis for an overall measure of bisGMA-based flowable resin-composites. Exposure levels to the dental materials were examined as the current number of treated surfaces present in the mouth at the time of the immune function measurement. For resin-composites used on permanent teeth, we also analyzed cumulative exposure levels, using a metric of

surface-years (each treated tooth surface weighted by the number of years present in the mouth).

To estimate the association between exposure to resin composites and immune function changes from baseline values, we used multivariable linear mixed effects models with repeated measures of immune function at 6 months, 12 months, 18 months, and 5 years follow-up. Data on immune function measured 5–7 days post-treatment were not analyzed because of uncertainty regarding the current number of treated surfaces in the mouth on the day of the blood draw (initial treatment needs often required repeat dental visits over multiple days for completion). For the repeated measures models, because plots showed a non-linear relationship between time and immune function, models included a quadratic term for time. In addition, we created separate models for each follow-up visit to examine the effect of time. All multivariable models were adjusted for baseline age, blood lead level, asthma, allergy, and study site. We also considered sex, birth weight, and urinary mercury concentrations, but did not include them as covariates, because they were statistically insignificant at P=0.20 and did not change estimates for resin-composites. There were no detectable interactions between the presence of asthma/allergy at baseline and resin-composites exposure in immune function changes (not shown). In sensitivity analyses (not shown), we excluded 5 children who had resin-composites present prior to the baseline blood draw; results were similar to those presented. Lastly, to evaluate the possibility of residual confounding in the analyses of restorative composites, we replicated the analyses using amalgam exposure levels. Because amalgam/composite groups were randomly assigned, results consistent between amalgam treatment levels and composite treatment levels in posterior teeth could indicate that findings may be the result of confounding by factors associated with dental disease, rather than dental materials.

RESULTS

Most children received some form of resin-based dental treatment at the initial dental treatment visit (n=48, 89%), and by the end of their 5-year follow-up, all substudy participants had received resin-composites (Table 2). For restorations, compomer was more commonly used at the start of the study given the prevalence of primary teeth caries, while composite was used with increasing frequency during follow-up, as new decay on permanent teeth occurred. Of note, most compomer-filled primary teeth had exfoliated by the end of follow-up; only 4 children had compomer present at Year 5. At year 5, 58% of children had composites present on permanent teeth, many of which were newly placed after baseline (surfaces newly placed, mean=1.9, SD=2.8). Exposure accumulated most for sealants during the study (surface-years, mean=28.1, SD=19.6), as these were routinely placed where clinically appropriate.

Asthma (n=11) or allergy (n=9) was reported by 29% participants at baseline. Overall, baseline counts of lymphocytes and granulocytes were within the normal reference values. Although 15% and 20%, respectively, fell outside this range, there were no consistent changes in these percentages over follow-up, and no differences by dental composite/amalgam treatment group (data not shown). Monocyte counts were commonly higher than

expected (44%) at baseline, and post-treatment 5–7 days and 6-months, but generally declined to normal values with longer follow-up.

Table 3 provides the multivariable model association between the number of resin-composite treated surfaces present at a blood draw and the corresponding change in immune function from the pre-treatment measurement. No consistent associations were seen for WBC counts or lymphocyte function across material types. A positive association between current number of non-flowable bisGMA-based composites and changes in B-cell activation, indicating increased activation, was present at both 6-months and 1-year, but not at the 5-year visit. As the number of bisGMA-based flowable (sealant/PRR) or non-flowable composites increased, monocyte and neutrophil function were decreased at both 6-months and 1-year follow-up, but not at year 5.

Using repeated measures of immune function over the entire 5-year follow-up in a multivariable model, associations between the present number of resin-composite surfaces and immune function changes were generally of negligible magnitude (Table 4). However, the present number of bisGMA-based standard composite restorations was associated with increased B-cell activation markers over follow-up (B-cell %CD69+PWM, β=1.7, 95% CI: 0.6,2.7; %CD23+PWM, β=2.2, 95% CI:0.5,4.0). In contrast, for amalgam, both B-cell markers indicated decreases in responsiveness since baseline (B-cell %CD69+PWM, β= −2.1, 95% CI:−3.8,−0.4; %CD23+PWM, β=−3.8, 95% CI:−6.2,−1.4). Positive associations between cumulative treatment levels of composite over follow-up and B-cell responsiveness changes persisted in secondary analyses (not shown); furthermore, estimates were similar for flowable and non-flowable bisGMA-based resin-composites (B-cell %CD69+PWM, flowable resin-composites 10-SY β=1.0, 95% CI:−0.1,2.7; non-flowable resin-composites 10-SY β=1.2, 95% CI:−2.1,4.5).

DISCUSSION

This analysis of longitudinal data from a randomized clinical trial showed that dental composite treatment levels were not consistently associated with WBC counts and responsiveness of T-cells or neutrophils. Monocyte responsiveness decreased during the first 6 months of follow-up for all participants, with greater bisGMA-based composite restorations showing the sharpest decline, but the trend did not persist with longer follow-up. Changes in B-cell responsiveness were greater throughout follow-up among children with more bisGMA-based composite restorations, which opposed findings for amalgam treatment levels.

A strength of this study is that the data were obtained as part of a randomized clinical trial, with rigorous data collection methods and outcome ascertainment. Although NECAT was not designed to examine type of dental composite material (e.g., compomer vs. composite), the available data proved useful to identify associations between specific composite materials and health outcome measures in prior analyses.[37] Furthermore, the unique availability of data on children randomized to amalgam in this study was important, both here and in previous analyses, to identify[38] or rule out[37,39] confounding by factors related to extent of tooth decay. While it remains unclear whether BPA exposure from dental

composites leads to systemic health effects, findings from NECAT suggest that further investigation is warranted. Greater exposure to bis-GMA based dental composites was found to be associated with impaired psychosocial function when assessed by two validated instruments.[37] Though no positive associations were found between composite material and anthropometric measurements, exploratory analyses among girls found that those randomly assigned to composites were less likely to have reached menarche during 5 years of follow up compared to girls assigned to amalgam for posterior restorations.[38]

The current analysis goes beyond the previous to also use data on flowable resin-composites. Flowable composites have higher resin content and in general, inferior mechanical properties which contribute to greater degradation and leaching, compared to standard hybrid composites.[44] However, standard non-flowable composites that are placed in bulk and undergo bulk polymerization could lead to more incomplete curing,[45] hence more exposure to resin monomers. Indeed, the few associations observed in our study occurred with standard (non-flowable) bulk composite. In addition, inferences from our results on flowable composites warrant greater caution, because two differently manufactured bisGMA-based flowable materials (preventive resin restorations and sealants), which may have different mechanical properties, were combined for an overall measure of exposure to flowable resins.

There exists little prior data with which to compare our results. Basic epidemiological data is lacking on the natural course of changes in immune function over time during development in healthy children, and there are no standard reference values for the activation markers. This makes it difficult to determine, e.g., whether the overall decrease in monocyte responsiveness that was observed in NECAT reflects normal changes over years of growth. For these reasons, we also examined associations within age strata in sensitivity analyses, and our primary analyses focused on relative differences in changes from baseline. Although prior longitudinal data were not available, cross-sectional studies of healthy children suggest that our observed overall trends may be similar in other populations; lymphocyte and monocyte counts have been shown to be higher among younger schoolchildren and linearly decrease with age,[46] and the irrelevance of sex or race/ethnicity has been noted.[46,47] The seemingly high percentage of asthma or allergy among NECAT participants is actually in line with the prevalence published by local public health officials,[48] although the national prevalence of childhood asthma is lower.[49] Our preliminary analysis examined effect modification by asthma or allergy status, and results were not different, suggesting that these findings could apply to the larger population beyond New England rural and urban children.

To our knowledge, only one prior study has presented data on dental composite treatment and changes in immune function in a patient population.[34] Interestingly, our findings regarding T-cell and B-cell responsiveness are somewhat consistent with this prior study. The researchers examined a Scandinavian adolescent population and reported no association for T-cell function markers (measured as the number of micronuclei present in either T4 or T8 lymphocytes). Lymphocyte B -cell micronuclei were increased among the Scandinavian adolescents having greater numbers of composite fillings, whereas the opposite was observed with greater numbers of amalgam fillings. Similarly, NECAT data showed increased B-cell responsiveness with more composite fillings, and decreased responsiveness

with more amalgam fillings. Of note, these findings were particular to permanent tooth restorations. The reason for this is unclear, but it may be biologically plausible given that standard bisGMA-based resin-composite was used for permanent tooth caries, whereas polyacid-modified UDMA-based material was used for primary teeth. Furthermore, for both amalgam and composite, few NECAT children had primary tooth restorations remaining in the mouth at the end of follow-up, making it difficult to analyze in light of the background decrease in B-cell activation occurring among all participants over time. Although the clinical significance of the magnitude of these associations for B-cell responsiveness is uncertain, they may be considered biologically relevant. Studies in a larger sample size are warranted to attempt a more definitive replication of results.

Overall, results of this exploratory investigation showed no overt immune function alterations associated with resin-composites. Additional research regarding lymphocyte activation is warranted given the consistency of the results within these analyses and with a prior study regarding increased B-cell activation. Such research is necessary for definitive clinical implications due to the exploratory nature of this study.

Why this paper is important to dentists:

- This study alerts dentists to the issue that the long-term safety of chemicals used in certain dental resin composites, particularly those derived from bisphenol-A (BPA), is uncertain.
- Patients might inquire about dental materials and express concern regarding safety of composites that might release BPA. This study provides evidence that dental composite resins used for restorations and sealants do not overtly alter immune function in children, thereby providing data for reassurance to patients and dentists.

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Table 1

Resin-Based Composite Dental Restorative Materials used in the New England Children's Amalgam Trial, 1997–2005[a]

Treatment type	Standardized Material	Manufacturer	Main Resin Monomers
Sealant[b]	Ultraseal XT	Ultradent (South Jordan, Utah)	bisGMA, diurethane dimethacrylate
Preventive resin restoration[c]	Revolution	Kerr (Orange, Calif.)	bisGMA
Restorations on primary teeth	Dyract AP Compomer	Dentsply Caulk (Milford, Del.)	UDMA, trimethacrylate resins
Restorations on permanent teeth	Z100 Composite	3M ESPE (St. Paul, Minn.)	bisGMA, TEGDMA

Abbreviations: bisGMA= bisphenol A diglycidyl dimethacrylate; TEGDMA=triethylene glycol dimethacrylate; UDMA= urethane dimethacrylate.

[a] As part of the randomized trial, children randomized to the amalgam group received amalgam (Dispersalloy, by Dentsply Caulk) for all posterior tooth restorations; composites were used for anterior tooth restorations for all study subjects, per standard clinical practice guidelines. Sealants and preventive resin restorations were placed as needed regardless of assigned treatment group, and a bonding agent (Optibond, by Kerr) composed of bisGMA and hydroxyethyl methacrylate (HEMA) was applied before restorations.

[b] Flowable composite used for prophylactic sealing of sound pits and fissures of posterior teeth.

[c] Flowable composite used for preventive resin restorations, which treated shallow, incipient caries that did not extend into the dentin.

Table 2

Treatment Exposure Levels of Preventive and Restorative Composite Materials at the Initial Dental Treatment Visit (Visit 1) and during 5-Year Follow-up among 59 Children in the NECAT Immune Function Substudy, 1997–2005.

	Sealant	PRR	Sealant and/or PRR	Compomer	Composite	Total
Number of treated surfaces						
Visit 1 (treated at initial visits)	3.1 (3.2)	1.0 (1.7)	4.2 (3.3)	3.5 (4.7)	1.2 (2.4)	8.9 (6.1)
Year 5 Final Visit (present or treated at final visit)	7.3 (5.8)	2.2 (2.3)	10.1 (6.4)	0.8 (2.7)	2.9 (4.1)	14.2 (7.3)
All Visits (ever treated)	9.9 (7.2)	2.7 (2.8)	12.6 (7.7)	4.8 (6.1)	3.0 (4.2)	20.9 (10.3)
Surface-years of exposure	28.1 (19.6)	6.9 (8.9)	36.9 (20.0)	11.2 (17.7)	9.6 (15.1)	60.6 (28.9)

Columns present mean (SD) of the specified treatment material and are not mutually exclusive. Total column presents sum of flowable (sealant or PRR) and non-flowable (compomer or composite) materials. Children were aged 6–10 years at baseline (mean 8.1 y, SD=1.4) and most had mixed primary and permanent dentition during the study.

PRR= preventive resin restoration.

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Table 3

Association between composite-resins present at follow-up and changes from baseline in immune function (β estimates)[a]

	Flowable Composites[b] (Preventive)			Non-Flowable Compomer[b] (Restorative)			Non-Flowable Composite[b] (Restorative)		
Follow-up visit:	**6 mo.**	**1 year**	**5 years**	**6 mo.**	**1 year**	**5 years**	**6 mo.**	**1 year**	**5 years**
No. participants	53	38	43	53	38	43	53	38	43
No. with any treated surfaces:	39	32	41	31	22	4	19	15	25
No. surfaces, mean±sd[c]	5.2±2.9	5.9±3.2	10.6±6.1	7.2±5.2	6.3±5.1	6.5±7.1	4.5±3.4	3.9±3.1	5.3±3.0
Total White Blood Cell Count (× 10^6)	−0.1	0.1	0.2	0.0	−0.1	0.4	−0.1	0.1	0.1
Lymphocytes	0.0	0.0	0.1	0.0	0.0	0.2	0.0	0.0	0.0
Granulocytes	−0.2	0.1	0.1	−0.1	−0.2	0.4	0.1	0.2	0.1
Monocytes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lymphocyte Function									
T-cell function									
%CD69 + PHA	1.1*	0.7	0.1	−0.6	−0.2	−1.4*	0.8	0.6	−0.3
%CD25 + PHA	0.4	1.2	0.4	−0.6	−0.1	−0.6	1.2	1.4	−0.4
B-cell function									
%CD69 + PWM	−0.8	−0.4	0.6	−0.2	−0.2	−0.4	1.6*	1.7	0.3
%CD23 + PWM	−2.5	−0.1	0.5	0.2	−0.8	0.5	2.1	2.8	0.4
Monocyte Function									
%DHE + PMA	−0.9	−1.3	0.9	0.9	1.5	−0.3	−3.1*	−0.8	0.5
%Rho + PMA	−0.2	−0.4	0.8	1.2	0.6	−0.2	−2.4	−0.4	0.1
Neutrophil Function									
%DHE + PMA	−0.3	−0.3	−0.1	0.2	0.2	0.3	−0.6	−0.3	0.1
%Rho + PMA	−0.3	0.1	0.2	0.3	0.0	0.4	−0.1	0.7	0.2

[a] From multivariable models adjusting for each type of material (number of surfaces currently treated with sealant/preventive resin restoration, compomer, or composite), age, time from baseline visit, study site, baseline blood lead level, and parent-reported asthma or allergy.

[b] Flowable composite was used for sealants on sound surfaces and for preventive resin restorations on shallow decay. Non-flowable UDMA-based compomer (polyacid-modified composite) was used for primary tooth restorations. Non-flowable standard bisGMA-based minifill composite was used for permanent tooth restorations. Materials were not used exclusively in each child; most children received treatment with more than one type of resin-composite.

[c] Among children with that type of resin-composite material present in the mouth at that time point, the mean ± standard deviation number of composite-treated surfaces present.

* $P<0.05$.

Abbreviations: PHA=phytohemagglutinin; PWM=pokeweed mitogen; PMA=phorbol myristate acetate.

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Table 4

Association [β estimates (95% confidence interval)] between present number of tooth surfaces with resin-composites or amalgam and immune function changes from baseline, from repeated measures models over 5-year follow-up[a]

	Flowable Composites (Preventive)	Non-Flowable Compomer (Restorative, Primary Teeth)	Non-Flowable Composite (Restorative, Permanent Teeth)	Amalgam (Restorative, Primary Teeth)	Amalgam (Restorative, Permanent Teeth)
Total White Blood Cell Count (× 10^6)	0.2 (0.03, 0.3)*	0.0	0.0	−0.0	0.0
Lymphocyte Cell Count	0.0	0.0	0.0	−0.0	0.0
Granulocyte Cell Count	0.0	0.0	0.1	−0.0	−0.1
Monocyte Cell Count	0.0	0.0	0.0	−0.0	0.0
Lymphocyte Function					
T-cell					
%CD69 + PHA	−0.0	−0.1	0.5	0.0	−0.5
%CD25 + PHA	−0.1	−0.0	0.7	−0.3	−0.2
B-cell					
%CD69 + PWM	−0.0	−0.2	1.7 (0.6, 2.7)***	0.1	−2.1 (−3.8, −0.4)**
%CD23 + PWM	−0.2	−0.3	2.2 (0.5, 4.0)**	0.3	−3.8 (−6.2, −1.4)***
Monocyte Function					
%DHE + PMA	−0.6	0.7	−0.6	−0.7	−0.0
%Rho + PMA	−0.3	1.1 (0.1, 2.0)*	0.3	−1.1	−0.6
Neutrophil Function					
%DHE + PMA	−0.2 (−0.4, −0.04)*	0.2	−0.0	0.1	0.2
%Rho + PMA	−0.2	0.2	0.4	−0.1	−0.2

[a]Multivariable models for resin-composite materials were adjusted for each type of resin-composite material (number of surfaces currently treated with sealant/preventive resin restoration, compomer, or composite), age, time from baseline visit, study site, baseline blood lead level, and parent-reported asthma or allergy. Models for amalgam were created to parallel models for composites, and therefore mutually adjusted for amalgam on primary and permanent teeth, as well as sealant/preventive resin restorations, age, time from baseline visit, study site, baseline blood lead level, and parent-reported asthma or allergy. Models included immune function changes from pretreatment at 6-months, 1-year, 1.5-year, and 5-year follow-up visits.

Abbreviations: PHA=phytohemagglutinin; PWM=pokeweed mitogen; PMA=phorbol myristate acetate.

*P=0.02

**P=0.01

***$P=0.002$