Antibiotic Use and Vaccine Antibody Levels

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abstract

BACKGROUND: The majority of children are prescribed antibiotics in the first 2 years of life while vaccine-induced immunity develops. Researchers have suggested a negative association of antibiotic use with vaccine-induced immunity in adults, but data are lacking in children.

METHODS: From 2006 to 2016, children aged 6 to 24 months were observed in a cohort study. A retrospective, unplanned secondary analysis of the medical record regarding antibiotic prescriptions and vaccine antibody measurements was undertaken concurrently. Antibody measurements relative to diphtheria-tetanus-acellular pertussis (DTaP), inactivated polio (IPV), *Haemophilus influenzae* type b (Hib), and pneumococcal conjugate (PCV) vaccines were made.

RESULTS: In total, 560 children were compared (342 with and 218 without antibiotic prescriptions). Vaccine-induced antibody levels to several DTaP and PCV antigens were lower ($P < .05$) in children given antibiotics. A higher frequency of vaccine-induced antibodies below protective levels in children given antibiotics occurred at 9 and 12 months of age ($P < .05$). Antibiotic courses over time was negatively associated with vaccine-induced antibody levels. For each antibiotic course the child received, prebooster antibody levels to DTaP antigens were reduced by 5.8%, Hib by 6.8%, IPV by 11.3%, and PCV by 10.4% (all $P \leq .05$), and postbooster antibody levels to DTaP antigens were reduced by 18.1%, Hib by 21.3%, IPV by 18.9%, and PCV by 12.2% (all $P < .05$).

CONCLUSIONS: Antibiotic use in children <2 years of age is associated with lower vaccine-induced antibody levels to several vaccines.

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Dr Chapman conceived the study, analyzed data, and drafted the manuscript; Drs Pham and Bajorski performed the statistical analysis and contributed to manuscript writing; Dr Pichichero conceived the study, enrolled children in the study, oversaw data collection from child subjects, oversaw antibody measurements, oversaw statistical analysis, prepared the final version of the manuscript, and secured funding for the project; and all authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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WHAT'S KNOWN ON THIS SUBJECT: In mice and limited studies in adults, antibiotic exposure has been shown to result in reduced antibody responses to vaccination.

WHAT THIS STUDY ADDS: This study is the first in children to show an association of antibiotic use and reduction in vaccine-induced antibody levels.

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Vaccination has revolutionized human health and longevity, inducing immune protection from life-threatening infectious diseases.[1] Young children are especially susceptible to a variety of pathogens that are currently targeted by vaccines, making early-life vaccination a priority.[2] As a result, diphtheria-tetanus-acellular pertussis (DTaP) vaccine, *Haemophilus influenzae* type b (Hib) vaccine, inactivated polio vaccine (IPV), and pneumococcal conjugate vaccine (PCV) are given in a primary series of 3 immunizations before 6 months of age, with a booster immunization after 12 months of age. Although this approach to vaccination is robust for most, the immune response to vaccines has interindividual variability that is not easily attributable to immune deficiency or genetic factors.[3–6] Geography and environmental factors seem to contribute,[7–11] but the reasons for variability in vaccine responses are incompletely understood.

Within the past decade, human studies researchers have suggested that alterations in the composition, diversity, and abundance of the gut microbiome are associated with health outcomes, providing support for the hypothesis that human health requires a healthy microbiome.[12] Given the appreciated connection between microbiome and immunity, antibiotic usage may affect the immune response to vaccines.[13] In previous work in mouse models, researchers suggested that antibiotic-induced reduction in the abundance and diversity of gut microbiota negatively affects the immune response to vaccination in both the generation and the maintenance of vaccine-induced immunity.[14–16] Hagan et al[17] were the first to perform a human trial of antibiotics given or not to adults before seasonal influenza vaccination, which supported animal model findings. On the basis of their findings, the authors could clearly propose a mechanism of how microbiota-derived signals may potentiate acute vaccine responses by demonstrating that antibiotics kill important commensal bacteria in the gut that favorably modulate immune responses to vaccination. However, there are no published data in young children that support these findings, which is critical given the labile nature of microbiota during early life when vaccines are given.[18] In addition, mouse studies have revealed that the detrimental effect of antibiotics on vaccine responses may be more pronounced during early life than in adulthood.[14]

To explore whether an association exists between antibiotic use and vaccine-induced antibody levels in children, we performed a retrospective analysis in an age-matched pediatric cohort study of children with a history of antibiotic prescriptions and those without. Antibiotic use was hypothesized to be negatively associated with vaccine-induced antibody levels in the first 2 years of life.

METHODS

Subjects and Study Design

The study population was derived from a prospectively enrolled cohort study of 6- to 24-month-old children (2006–2016) that focused on respiratory infections in the primary care setting, especially acute otitis media. In the primary study, blood was collected during regularly scheduled well-child visits at 6, 9, 12, 15, 18, and 24 months of age and at the onset of acute otitis media infection, although not all samples were collected because of technical or time challenges or parental refusal, resulting in some missing data. We did not observe any patterns in the missing data beyond the reduced sample size, but the missing data prevented a full longitudinal approach to our analysis. Children with immunocompromise were not enrolled in the study population. No child received chemotherapy or long-term corticosteroids.

In the course of the study, nurses reviewed the medical record and questioned parents about illnesses and antibiotic prescriptions. All children who completed study visits to 24 months of age and from whom we procured at least 1 blood sample from which vaccine antibody levels could be measured were included in the current analysis. Children were from a suburban, mostly non-Hispanic White community in Rochester, New York. Virtually all medical care was provided at a single private clinical practice site of the senior author (M.E.P.). Medical encounters of cohort children at any other regional site (eg, urgent care, emergency department) were shared electronically with the primary study site, and data on antibiotic prescriptions given at those facilities were captured. All children in the cohort followed the recommended vaccination schedule. In reference to this study, DTaP, Hib, and IPV were given at 2, 4, 6, and 18 months of age. PCV was given at 2, 4, 6, and 15 months of age. Other vaccine responses were not studied because the laboratory of the senior author only has validated assays for the antigens tested. Demographics collected included sex, race (parent self-report), siblings, breastfeeding, day care attendance, tobacco smoke exposure, and atopy (diagnosed by a board-certified allergist). The Rochester General Hospital institutional review board approved the study, and written informed consent was obtained from parents before enrollment. The medical record was the source of antibiotic

prescription data for analysis of both recent and cumulative antibiotic use.

Vaccine Antibody Measurement

For analysis of vaccine-induced antibody levels after recent antibiotic use, antibody measurements were used from blood samples provided at these visits only. The control group included all vaccine-induced antibody measurements from children who met the study inclusion criteria and had no antibiotic prescriptions in the medical record. Serum antibody levels to vaccine components of DTaP (diphtheria toxoid [DT], pertussis toxoid [PT], tetanus toxoid [TT], pertactin [PRN], and filamentous hemagglutinin [FHA]), Hib conjugate (polyribosylribitol phosphate [PRP]), IPV (polio 2), and PCV (serotypes 6B, 14, and 23F) were measured in available samples.[3] Protective serum antibody levels used for purposes of this study were based on established criteria[19,20] (DT and TT, 0.1 IU/mL; PRP, 0.15 μg/mL; polio 2 antigen, reciprocal 1/8 dilution; PCV serotypes, 0.35 μg/mL). For acellular pertussis antigens, correlates of protection are not established; therefore, a titer of 8 EU/mL (for PT) and 8 IU/mL (for PRN and FHA) was used as the correlate of protection for this study, as previously suggested.[21,22] All antibody measurements were performed in a Good Laboratory Practices setting with established standard operating procedures at Rochester General Hospital, as previously described.[23–26]

Statistical Analyses

Mann-Whitney test was used to compare raw antibody levels between groups. Fisher's exact test was used to compare the proportion of subprotective antibody levels between subgroups of children. One-way analysis of variance was used to determine the association of child age with the normalized vaccine-induced antibody level at each age time point. Univariate risk factors associated with antibiotic status were compared using χ^2 tests. The relationship between a child's antibody level and accumulated number of antibiotics was determined using linear models. Missing blood draws prevented us from utilizing longitudinal statistical models. The dependent variables were natural log of normalized antibody levels at 12 to 15 months (Supplemental Fig 3, Supplemental Table 2) or 18 to 24 months (Supplemental Fig 4, Supplemental Table 3) of age, and independent variables were the accumulated number of antibiotic courses up to 12 or 15 months of age, respectively. All 4 vaccines were treated the same for modeling purposes. To adjust for the influence of covariates, we used multivariable linear regression because of heterogeneous risk factor distributions. The risk factors were separately included in the regression model as categorical variables if statistically significant. In Fig 2 A and B, we show the normalized antibody levels without the log transformation, and consequently, they become exponential functions of the number of antibiotic courses. For analysis of the overall antibiotic effect in Supplemental Figs 4 and 5, we used the Wei-Lachin multivariate test.

RESULTS

A total of 560 children (65.3%) met the inclusion criteria for the antibiotic association with vaccine response analysis. From these children, 11 888 antibody levels to vaccine antigens were measured. A total of 342 children with 1678 antibiotic courses prescribed were compared with 218 children with no antibiotic prescriptions. The predominant antibiotics prescribed were amoxicillin, cefdinir, amoxicillin/clavulanate, and ceftriaxone. Fifty-seven antibiotic courses (3.4%) were for recommended doses of trimethoprim/sulfamethoxazole or cephalexin for 10 days for skin/soft tissue infections or urinary tract infections, and those prescriptions were included in all other data presented. Cohort demographics are presented in Table 1. We found that day care attendance was associated with antibiotic prescriptions (42.7% in antibiotic group vs 20.6% in the no antibiotics group; $P < .001$). Ethnicity was also different (Table 1).

Vaccine-induced antibody levels were determined for 10 antigens included in 4 vaccines: DTaP, Hib, IPV, and PCV. Vaccine-induced antibody levels significantly changed with respect to child age, with higher levels observed after the primary vaccine series and after booster vaccination, along with waning antibody levels between primary and booster immunizations (Supplemental Fig 3).

Of possible high clinical relevance, from 9 to 24 months of age, children with antibiotic use had a higher frequency of vaccine-induced antibody levels below protection compared with children with no antibiotic use, placing them at risk to contract a vaccine-preventable infection for DTaP antigens DT, TT, and PT and for PCV serotype 14 (Fig 1A). Comparing all vaccine-induced antibody levels at each age, children with antibiotic use had a higher frequency of antibody levels below the protective threshold at 9 and 12 months of age (Fig 1B); other time points were not statistically different between groups. For time points where antibody levels were determined within 30 days of completion of a course of antibiotics (recent antibiotic use), individual antibiotics

TABLE 1 Demographics of Study Cohort

	All Children (n = 560), n (%)		No Antibiotics History (n = 218), n (%)		Antibiotics History (n = 342), n (%)		
Variable	Yes	Not Recorded	Yes	Not Recorded	Yes	Not Recorded	P
Daycare	191 (34.1)	0 (0)	45 (20.6)	0 (0)	146 (42.7)	0 (0)	<.001
Siblings	348 (62.1)	18 (3.2)	132 (60.6)	1 (0.4)	216 (63.2)	17 (4.9)	.210
Feeding	—	92 (16.4)	—	5 (2.3)	—	87 (25.4)	.230
Breastfed	115 (20.5)	—	56 (25.7)	—	59 (17.3)	—	
Formula	270 (48.2)	—	114 (52.3)	—	156 (45.6)	—	
Both	83 (14.8)	—	43 (19.7)	—	40 (11.7)	—	
Smoking	61 (10.9)	0 (0)	28 (12.8)	0 (0)	33 (9.6)	0 (0)	.300
Atopy	161 (28.8)	0 (0)	54 (24.8)	0 (0)	107 (31.3)	0 (0)	.120
Sex	—	0 (0)		0 (0)	—	0 (0)	.260
Male	285 (50.9)	—	104 (47.7)	—	181 (52.9)	—	
Female	275 (49.1)	—	114 (52.3)	—	161 (47.1)	—	
Race	—	1 (0.2)	—	0 (0)	—	1 (0.3)	.005
Black	38 (6.8)	—	22 (10.1)	—	16 (4.7)	—	
Multiracial	63 (11.2)	—	32 (14.7)	—	31 (9.1)	—	
Other	18 (3.2)	—	9 (4.1)	—	9 (2.6)	—	
White	440 (78.6)	—	155 (71.1)	—	285 (83.3)	—	

—, not applicable.

were analyzed for impact on antibody levels below protective levels. Across all vaccine antigens measured, amoxicillin was not significantly associated with antibody measurements below protective levels compared with no antibiotics (35 [16.4%] of 213 vs 448 [13.5%] of 3323; P = .22; odds ratio [OR], 1.26), but ceftriaxone (65 [17.8%] of 365; P = .03; OR, 1.39), amoxicillin/clavulanate (147 [19.8%] of 744; P < .001; OR, 1.58), and cefdinir (59 [19.7%] of 299; P = .004; OR, 1.58) were (Fig 1C). For 47% (349 of 744) of amoxicillin/clavulanate prescriptions, the medical record specified whether a 5- or 10-day course was given. Therefore, it was possible to also compare the effect of shorter versus longer courses of amoxicillin/clavulanate. Amoxicillin/clavulanate in a 5-day course was not associated with subprotective antibody levels (26 [13.9%] of 187; P = .827; OR, 1.04), whereas 10-day

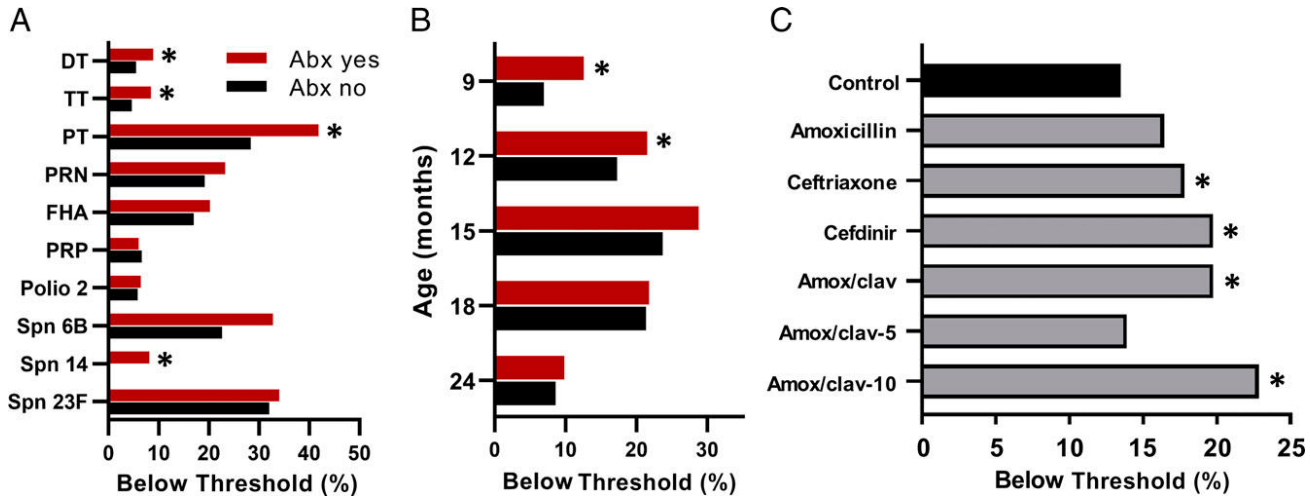

FIGURE 1

Frequency of subprotective vaccine antibody levels from 9 to 24 months of age. A, The frequency of subprotective vaccine levels were determined for children with antibiotic prescriptions and those with no antibiotic prescriptions for each vaccine antigen from 9 to 24 months of age combined. *Streptococcus pneumoniae* (Spn) and the specific serotype in the PCV tested are shown as numbered. B, Data for all vaccine antigens compared by child age. C, Time points where a single antibiotic prescription was completed within 30 days of measurement of vaccine-induced antibody levels were compared with those of children with no antibiotic prescriptions (control). Dosing: amoxicillin, 80 to 100 mg/kg/day divided twice daily for 10 days; cefdinir, 14 mg/kg/day once daily for 10 days; amoxicillin/clavulanate, 80 to 100 mg/kg/day on the basis of the amoxicillin component divided twice daily for 5 or 10 days; and ceftriaxone, 50 mg/kg intramuscular injection per day for 3 sequential days or every other day. Amox/clav-5 and Amox/clav-10 groups represent children who received 5- and 10-day courses of amoxicillin/clavulanate, respectively. *P < .05 by Fisher's exact test. Abx, antibiotics.

amoxicillin/clavulanate (37 [22.8%] of 162; $P = .002$; OR, 1.90) was (Fig 1C).

We next asked whether accumulation of antibiotic courses in the first year of life had an association with subsequent vaccine-induced antibody levels. Children were grouped according to the number of antibiotic courses prescribed up to 12 months of age and compared with children with no antibiotic history. All children with antibody levels measured between 12 and 15 months of age were included in the analysis. In a regression model of accumulated antibiotic courses up to 1 year of age and antibody levels at 12 to 15 months of age, antibody levels had a negative association with increasing antibiotic courses for DTaP, Hib, IPV, and PCV (Fig 2A, Supplemental Table 2). Each antibiotic prescription was associated with a reduction in the median antibody level for DTaP by 5.8% ($P = .01$), Hib by 6.8% ($P = .05$), IPV by 11.3% ($P = .04$), and PCV by 10.4% ($P = .01$). The actual change in the antibody level varied. Supplemental Table 5 provides the cumulative percent reduction in vaccine-induced antibody level for multiple antibiotic prescriptions.

To determine if booster vaccination influenced this association, a second analysis was performed using antibiotic prescriptions up to 15 months of age. Antibody levels at 18 to 24 months of age were used in this analysis, as booster PCV (15 months of age) and DTaP, Hib, and IPV (18 months of age) were given previously. Each antibiotic prescription was associated with a reduction in vaccine-induced antibody level for DTaP by 181% ($P = .001$), Hib by 21.3% ($P = .02$), IPV by 18.9% ($P = .02$), and PCV by 12.2% ($P = .03$) (Fig 2B, Supplemental Table 3). Supplemental Table 6 provides the cumulative percent reduction in vaccine-induced antibody level for multiple antibiotic prescriptions. In a multivariable regression analysis, race, atopy status, and siblings in the home were included as covariates, if significant, and results are shown in Fig 2 A and B and Supplemental Table 4. All P values as presented were adjusted for covariates.

In additional analysis, antibody levels from 9 to 24 months of age were compared between groups. The median antibody level across all ages and vaccine antigens was significantly lower ($P < .05$) in the antibiotic group for 10 of 50 datasets (Supplemental Fig 4). In the majority of the remaining datasets (28 of 40), the antibiotic effect was also negative but not statistically significant when considered separately. To measure the joint antibiotic effect over all datasets, we used a multivariate test, which showed a highly significant negative antibiotic effect ($P < .0001$). Several

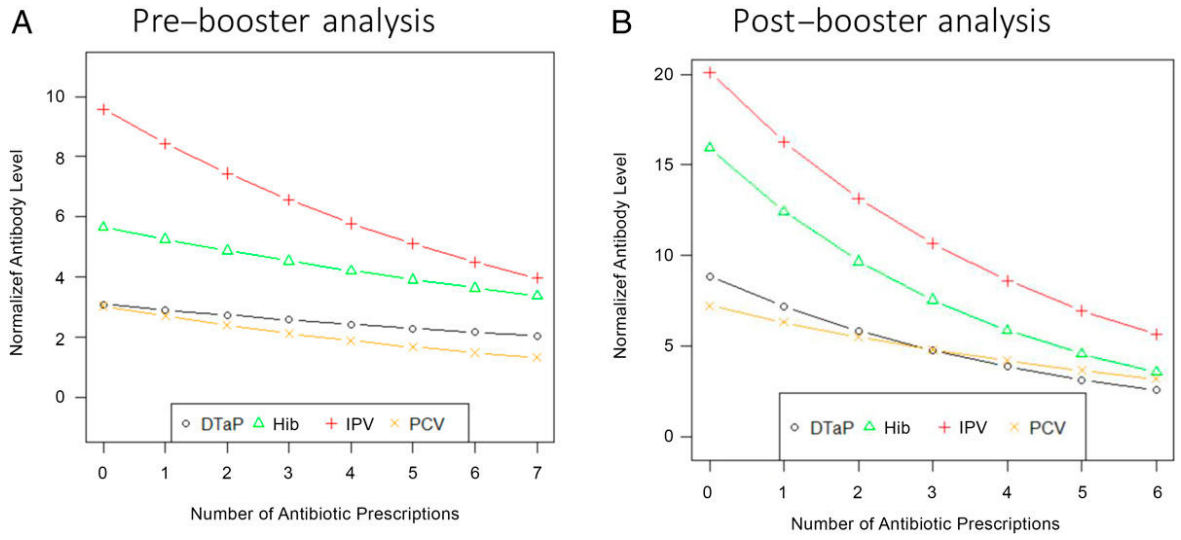

FIGURE 2

Association of antibiotic courses with pre- and postbooster vaccine antibody levels. A and B, Regression analyses of log-transformed vaccine-induced antibody levels across groups were performed for DTaP, Hib, IPV, and PCV as linear functions of the number of antibiotic prescriptions from 6 to 12 months (A) or from 6 to 15 months (B) of age. To show the impact on the nontransformed antibody level, the exponential relationship is shown. Prebooster antibody levels to DTaP antigens were reduced by 5.8% ($P = .01$), Hib by 6.8% ($P = .05$), IPV by 11.3% ($P = .04$), and PCV by 10.4% ($P = .01$) for each antibiotic course the child received. Postbooster antibody levels to DTaP antigens were reduced by 18.1% ($P = .001$), Hib by 21.3% ($P = .02$), IPV by 18.9% ($P = .02$), and PCV by 12.2% ($P = .03$) for each antibiotic course the child received. The associated intercepts, slopes, and errors in vaccine-induced antibody per antibiotic course for each vaccine are shown in Supplemental Table 2 for panel A (prebooster) and Supplemental Table 3 for panel B (postbooster). The effect of antibiotic days can be inferred because each of 1678 antibiotic courses was 10 days, except the subset of 187 (11%) children who received only 5 days of amoxicillin/clavulanate. Given the half-life of ceftriaxone, the 3 injections would constitute ~10 days of antibiotics.

DTaP- and PCV-associated antigens were significantly lower in children with antibiotic use at individual time points (Supplemental Fig 4). DT antibody levels at 18 months of age was the only time point where higher levels were measured in the no antibiotics group. For DTaP and Hib antigens, there were sufficient data to compare vaccine-induced antibody levels between control and recent antibiotic use groups for children who completed an antibiotic course within 30 days of a blood sample in which serum antibody levels were determined. Individual time points for DT, TT, PT, PRN, and FHA antigens were all lower in the recent antibiotic use group, with 8 of 30 comparisons significant ($P < .05$) (Supplemental Fig 5). Here, we also used a multivariable test, which again revealed a highly significant negative antibiotic effect ($P < .0001$).

We performed an analysis of changes in antibody levels from baseline in individual children for whom data were available (Supplemental Table 5) The percent reduction in antibody levels in the antibiotic use group was always larger than that in the no antibiotic group for all 6 antigens (DT, TT, PT, PRN, PRP, and FHA). When tested individually, PT ($P = .004$), PRN ($P = .02$), and PRP ($P = .02$) were statistically significant. Multivariate testing revealed a highly significant negative antibiotic effect ($P < .001$).

We compared the group of children included in this study versus those who were excluded (Supplemental Table 6). Significant differences between the 2 groups were in day care attendance, having siblings, and race. These differences should not have a significant impact on the major results of our study. We also listed the counts of the blood samples used for antibody testing in children at various ages, as shown in Supplemental Table 7.

DISCUSSION

In this study, we examined the association of antibiotic prescription frequency, type of antibiotic, and duration of antibiotic prescription with vaccine-induced antibody levels in young children. The results reveal that antibiotic use is associated with lower antibody levels to several vaccine antigens and a frequency-dependent effect of antibiotic courses over time in the first 2 years of life. Thus, clinicians are provided with more evidence that antibiotic prescriptions should be made judiciously, with narrow-spectrum drugs and for the shortest duration possible to avoid an adverse impact on vaccine-induced immunity. Our findings are consistent with those of reports in mouse models,[14,15] as well as with an adult clinical trial of the association of reduced seasonal influenza vaccine response with antibiotic exposure.[17] However, our study is the first in young children during the early age window where vaccine-induced immunity is established. Antibody levels fell below established protective thresholds for some antigens significantly more frequently in antibiotic-exposed young children. Specifically, antibiotic use was associated with increased frequency of subprotective antibody levels for DTaP and PCV antigens in children up to 2 years of age. This outcome would potentially influence herd immunity and leave children vulnerable to vaccine-preventable diseases. Outbreaks of vaccine-preventable diseases, such as pertussis, may be a consequence of multiple courses of antibiotics suppressing vaccine-induced immunity.

Amoxicillin was not associated with antibody measurements below protective levels, whereas amoxicillin/clavulanate, ceftriaxone, and cefdinir were. Published studies have shown that antibiotic exposure in early life can disrupt gut microbiota diversity for an extended time after administration.[27–29] Therefore, it is possible that broad-spectrum antibiotics disrupt gut homeostasis in early life and subsequently alter metabolic signals required for optimal plasma B-cell function.[30,31] Additional work is needed to determine a mechanistic link between our observations and the impact on the gut microbiome.

Amoxicillin/clavulanate was the only antibiotic with sufficient data to compare 5- and 10-day courses. A 10-day course was negatively associated with protective antibody levels within 30 days of completion of the antibiotic course, but a 5-day course was not. The issue of antibiotic overuse is well documented worldwide[32–34] and contributes to development of antibiotic resistance and occurrence of adverse drug effects. Shorter courses of antibiotics may be as effective as longer courses to treat most respiratory infections,[35] and we suggest an added benefit of limiting duration of antibiotics, especially broad-spectrum agents, when possible.

A goal of this study was to explore potential acute and long-term effects of antibiotic exposure on vaccine-induced antibody levels. Modeling of accumulated antibiotic courses up to booster immunization was associated with decreased vaccine antibody levels both before and after booster, suggesting that booster immunization was not sufficient to change the negative association with antibiotic exposure. The results were similar for all vaccines tested, suggesting that the specific vaccine formulation was not a factor.

Although we have focused on an association of antibiotic exposures adversely affecting vaccine-induced antibody responses in the first 2 years of life, the observations should be considered in the broader context of immune responsiveness. In our earlier studies with this primary cohort, we observed that a high percentage of children prone to recurrent acute otitis media, all of whom received multiple courses of antibiotics, had below-protective levels of antibody to multiple childhood vaccines.[36] In other studies, we showed that otitis media–prone children have immunity problems in their first 2 years of life that include poor responses of B cells, T cells, antigen-presenting cells, and generation of immune memory.[37] Some of these immunity deficits were also shown to occur in low responders to primary vaccinations.[3] Moreover, we found that young children who respond poorly to vaccinations and are prone to acute otitis media are significantly more prone to other respiratory infections, such as influenza, sinusitis, and pneumonia,[38] and these children have dysbiosis in their nasopharyngeal microbiome[39,40] and experience asthma significantly more often.[40] Taken together, it seems that a mechanistic link (or endotype) may clinically manifest as low vaccine responsiveness, high infection susceptibility, and a propensity to develop asthma that may be influenced by excessive early-life antibiotic use. However, the immune deficits in otitis media–prone[37] and low vaccine responders[3] occurred in 10% to 15% of the children our primary cohort, whereas reduced antibody levels to vaccinations associated with antibiotic exposure in the current report involved predominantly children who would be classified as normal vaccine responders, with normal B-cell, T-cell, and antigen-presenting cell function.[3] Therefore, we attribute the effect we report here to the impact of antibiotic exposure, consistent with the mechanism of killing protective commensal bacteria in the gut that favorably modulate vaccine-induced immunity.[17] Previous studies have revealed that mild illness does not adversely affect vaccine responses (see Supplemental Information).

Our study has several limitations. The antibiotic prescription data and measurements of vaccine-induced antibody levels were recorded and measured prospectively; however, our analysis was done retrospectively. The study cohort was derived from a single community in upstate New York, and approximately three-fourths of the children were reported as non-Hispanic White. Some children had to be excluded from the study because of the lack of antibody testing results needed for our analyses; the number of vaccine antibody measurements was limited by serum availability at some sampling time points in some children; and sometimes, the serum samples were collected far apart, which weakened our ability to perform longitudinal analyses. The available cohort samples did not include stool samples for the study of the potential impact of antibiotic courses on the gut microbiome.

In conclusion, our results reveal possible negative associations of antibiotic prescriptions with vaccine-induced immunity among young children. We provide new evidence to suggest caution about overprescribing antibiotics because an adverse effect seems to extend to reduction in vaccine responses. Our data reveal that the judicious choice of narrow-spectrum antibiotics for a shorter duration, where appropriate, may help to preserve vaccine-induced immunity in young children. Additional studies should be undertaken where children are monitored more regularly and with more frequent antibody testing. Other studies should also be designed to identify mechanistic interactions between the microbiome and immunity in young children receiving antibiotics.

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ABBREVIATIONS

DTaP: diphtheria-tetanus-acellular pertussis
FHA: filamentous hemagglutinin
Hib: *Haemophilus influenzae* type b
IPV: inactivated polio vaccine
OR: odds ratio
PCV: pneumococcal conjugate vaccine
PRN: pertactin
PRP: polyribosylribitol phosphate
PT: pertussis toxoid
TT: tetanus toxoid

REFERENCES

1. Rappuoli R, Pizza M, Del Giudice G, De Gregorio E. Vaccines, new opportunities for a new society. *Proc Natl Acad Sci U S A*. 2014;111(34):12288–12293
2. Wang H, Dwyer-Lindgren L, Lofgren KT, et al. Age-specific and sex-specific mortality in 187 countries, 1970-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012;380(9859):2071–2094
3. Pichichero ME, Casey JR, Almudevar A, et al. Functional immune cell differences associated with low vaccine

responses in infants. *J Infect Dis.* 2016;213(12):2014–2019

4. Querec TD, Akondy RS, Lee EK, et al. Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans. *Nat Immunol.* 2009;10(1):116–125
5. Payton T, Girgenti D, Frenck RW, et al. Immunogenicity, safety, and tolerability of 3 lots of 13-valent pneumococcal conjugate vaccine given with routine pediatric vaccinations in the United States. *Pediatr Infect Dis J.* 2013;32(8):871–880
6. Nakaya HI, Hagan T, Duraisingham SS, et al. Systems analysis of immunity to influenza vaccination across multiple years and in diverse populations reveals shared molecular signatures. *Immunity.* 2015;43(6):1186–1198
7. Valdez Y, Brown EM, Finlay BB. Influence of the microbiota on vaccine effectiveness. *Trends Immunol.* 2014;35(11):526–537
8. Grassly NC, Jafari H, Bahl S, et al. Mucosal immunity after vaccination with monovalent and trivalent oral poliovirus vaccine in India. *J Infect Dis.* 2009;200(5):794–801
9. Levine MM. Immunogenicity and efficacy of oral vaccines in developing countries: lessons from a live cholera vaccine. *BMC Biol.* 2010;8(1):129
10. Velasquez DE, Parashar U, Jiang B. Decreased performance of live attenuated, oral rotavirus vaccines in low-income settings: causes and contributing factors. *Expert Rev Vaccines.* 2018:17(2):145–161
11. Smolen KK, Ruck CE, Fortuno ES III, et al. Pattern recognition receptor-mediated cytokine response in infants across 4 continents. *J Allergy Clin Immunol.* 2014;133(3):818–26.e4
12. Belkaid Y, Harrison OJ. Homeostatic immunity and the microbiota. *Immunity.* 2017;46(4):562–576
13. Lynn DJ, Pulendran B. The potential of the microbiota to influence vaccine responses. *J Leukoc Biol.* 2018;103(2):225–231
14. Lynn MA, Tumes DJ, Choo JM, et al. Early-life antibiotic-driven dysbiosis leads to dysregulated vaccine immune responses in mice. *Cell Host Microbe.* 2018;23(5):653–660.e5
15. Oh JZ, Ravindran R, Chassaing B, et al. TLR5-mediated sensing of gut microbiota is necessary for antibody responses to seasonal influenza vaccination. *Immunity.* 2014;41(3):478–492
16. Hall JA, Bouladoux N, Sun CM, et al. Commensal DNA limits regulatory T cell conversion and is a natural adjuvant of intestinal immune responses. *Immunity.* 2008;29(4):637–649
17. Hagan T, Cortese M, Rouphael N, et al. Antibiotics-driven gut microbiome perturbation alters immunity to vaccines in humans. *Cell.* 2019;178(6):1313–1328.e13
18. Yatsunenko T, Rey FE, Manary MJ, et al. Human gut microbiome viewed across age and geography. *Nature.* 2012;486(7402):222–227
19. Plotkin SA. Correlates of protection induced by vaccination. *Clin Vaccine Immunol.* 2010;17(7):1055–1065
20. Plotkin SA. Updates on immunologic correlates of vaccine-induced protection. *Vaccine.* 2020;38(9):2250–2257
21. Cherry JD, Gornbein J, Heininger U, Stehr K. A search for serologic correlates of immunity to Bordetella pertussis cough illnesses. *Vaccine.* 1998;16(20):1901–1906
22. Storsaeter J, Hallander HO, Gustafsson L, Olin P. Levels of anti-pertussis antibodies related to protection after household exposure to Bordetella pertussis. *Vaccine.* 1998;16(20):1907–1916
23. Pichichero ME, Passador S. Administration of combined diphtheria and tetanus toxoids and pertussis vaccine, hepatitis B vaccine, and *Haemophilus influenzae* type b (Hib) vaccine to infants and response to a booster dose of Hib conjugate vaccine. *Clin Infect Dis.* 1997;25(6):1378–1384
24. D'Angio CT, Maniscalco WM, Pichichero ME. Immunologic response of extremely premature infants to tetanus, *Haemophilus influenzae,* and polio immunizations. *Pediatrics.* 1995;96(1 pt 1):18–22
25. Englund JA, Decker MD, Edwards KM, Pichichero ME, Steinhoff MC, Anderson EL. Acellular and whole-cell pertussis vaccines as booster doses: a multicenter study. *Pediatrics.* 1994;93(1):37–43
26. Pichichero ME, Deloria MA, Rennels MB, et al. A safety and immunogenicity comparison of 12 acellular pertussis vaccines and one whole-cell pertussis vaccine given as a fourth dose in 15- to 20-month-old children. *Pediatrics.* 1997;100(5):772–788
27. Azad MB, Konya T, Persaud RR, et al; CHILD Study Investigators. Impact of maternal intrapartum antibiotics, method of birth and breastfeeding on gut microbiota during the first year of life: a prospective cohort study. *BJOG.* 2016;123(6):983–993
28. Doan T, Arzika AM, Ray KJ, et al. Gut microbial diversity in antibiotic-naive children after systemic antibiotic exposure: a randomized controlled trial. *Clin Infect Dis.* 2017;64(9):1147–1153
29. Korpela K, Salonen A, Virta LJ, et al. Intestinal microbiome is related to lifetime antibiotic use in Finnish pre-school children. *Nat Commun.* 2016;7(1):10410
30. Kim M, Qie Y, Park J, Kim CH. Gut microbial metabolites fuel host antibody responses. *Cell Host Microbe.* 2016;20(2):202–214
31. Lam WY, Becker AM, Kennerly KM, et al. Mitochondrial pyruvate import promotes long-term survival of antibody-secreting plasma cells. *Immunity.* 2016;45(1):60–73
32. Wang CN, Huttner BD, Magrini N, et al; Collaborative Working Group of the Pediatric Subgroup of the China Society of Infectious Diseases. Pediatric antibiotic prescribing in China according to the 2019 World Health Organization Access, Watch, and Reserve (AWaRe) antibiotic categories. *J Pediatr.* 2020;220:125–131.e5
33. Størdal K, Wyder C, Trobisch A, Grossman Z, Hadjipanayis A. Overtesting and overtreatment—statement from the European Academy of Paediatrics (EAP). *Eur J Pediatr.* 2019;178(12):1923–1927
34. Korenstein D, Falk R, Howell EA, Bishop T, Keyhani S. Overuse of health care services in the United States: an understudied problem. *Arch Intern Med.* 2012;172(2):171–178
35. Pichichero ME. Short course antibiotic therapy for respiratory infections: a review of the evidence. *Pediatr Infect Dis J.* 2000;19(9):929–937

36. Pichichero ME, Casey JR, Almudevar A. Nonprotective responses to pediatric vaccines occur in children who are otitis prone. *Pediatr Infect Dis J*. 2013;32(11):1163–1168

37. Pichichero ME. Immunologic dysfunction contributes to the otitis prone condition. *J Infect*. 2020;80(6):614–622

38. Pichichero ME, Chapman TJ, Bajorski P. Pneumonia, sinusitis, influenza and other respiratory illnesses in acute otitis media-prone children. *Pediatr Infect Dis J*. 2021;40(11):975–980

39. Xu L, Earl J, Bajorski P, Gonzalez E, Pichichero ME. Nasopharyngeal microbiome analyses in otitis-prone and otitis-free children. *Int J Pediatr Otorhinolaryngol*. 2021;143:110629

40. Chapman TJ, Morris MC, Xu L, Pichichero ME. Nasopharyngeal colonization with pathobionts is associated with susceptibility to respiratory illnesses in young children. *PLoS One*. 2020;15(12):e0243942