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Depressive symptoms and immune response to meningococcal conjugate vaccine in early adolescence

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Abstract

Research findings in psychoneuroimmunology document reliable, bidirectional linkages among psychological processes, the nervous system, and the immune system. However, available data are based almost entirely on animal and adult human studies; the application to children and adolescents is uncertain. We capitalized on the experimental leverage provided by a routine vaccination to examine the link between mood symptoms and the immune response to a vaccine challenge in early adolescence. One hundred twenty-six 11-year-olds for whom vaccine response data were available were assessed at prevaccination and 4 weeks, 3 months, and 6 months following vaccination; self-report ratings of depression and anxiety as well as measures of psychosocial and somatic risk were assessed prior to vaccine response. Analyses indicated that children's internalizing mood symptoms were associated with elevated and persistently higher antibody responses, with evidence extending to two of the four serogroups. The associations remained after controlling for multiple possible confounders (social class, body mass index, sleep, psychosocial risk, and pubertal status). The observed enhanced vaccine response associated with depressive and anxious symptoms in early adolescence may reflect an important developmental difference in immune system–brain interplay between adults and children, and it underscores the need for further developmental studies of psychoneuroimmunology.

Numerous studies demonstrate dynamic, variegated, and bidirectional associations between the immune system and the brain, a principle underlying the concept of psychoneuroimmunology (PNI; Ader, Cohen, & Felten, 1995). The evidence base in animals is overwhelming (Ader & Cohen, 1975, 1993; Banks, Kastin, & Broadwell, 1995; Kelley, Arkins, & Li, 1992; Moynihan, Ader, Grota, Schachtman, & Cohen, 1990; Sheridan, Stark, Avitsur, & Padgett, 2000). The now prolific adult human literature on PNI dates back several decades and illustrates many different kinds of connections across conditions and research paradigms (Blackmore et al., 2011; Kiecolt-Glaser et al., 1984, 2005; Miller, Kemeny, Taylor, Cole, & Visscher, 1997; Wright, Strike, Brydon, & Steptoe, 2005). Applications to models of psychopathology have grown out of this work and are beginning to attract significant attention (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008) and suggest avenues for intervention (Creswell et al., 2012; Raison et al., 2013).

A key limitation of this work is that it is based largely on animal and adult human studies; extension to pediatric samples is uncommon, and so there are only the beginnings of a developmental model of the interplay among psychological processes, the nervous system, and the immune system (Ader, 1983; Coe, 1996; O'Connor, Moynihan, & Caserta, 2014). We leverage the power of a natural experiment of vaccine administration in 11-year-olds to test a PNI hypothesis that depressive and anxious symptoms would predict vaccine responses.

Several types of research designs have been employed to examine the nature of the linkages among psychological processes, the nervous system, and the immune system. In the current study, we use the vaccine challenge paradigm because (a) it is a well-established paradigm in the animal and adult work; (b) it provides rigorous experimental leverage that is not afforded by observational/correlational studies; (c) where vaccine responses are tracked over successive assessments, it may offer insight into immune response dynamics and possible mechanisms; and (d) understanding sources of variation in vaccine response may indicate avenues for promoting individual and public health. Specifically, we examined the response to a quadrivalent meningococcal polysaccharide-protein conjugate vaccine (Menactra, Sanofi Pasteur). This vaccine was licensed for use in individuals from 11 to 55

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years of age in January 2005 (Centers for Disease Control and Prevention [CDC], 2005); the CDC's Advisory Committee on Immunization Practices subsequently recommended adding meningococcal conjugate vaccine to the routine immunization schedule for all children at age 11–12 years (CDC, 2005). The vaccine contains purified capsular polysaccharide of Serogroups A, C, Y, and W-135 conjugated to diphtheria toxoid; elicits a T cell dependent antibody response; and induces immune memory (Borrow et al., 2003; Rennels, King, Ryall, Papa, & Froeschle, 2004). The vaccine has proven very effective, with 82%–97% of 11- to 18-year-olds achieving fourfold or greater rises in serum bactericidal antibody titers in a large trial (CDC, 2005).

A now familiar adult model of the vaccine challenge paradigm is that immune response is diminished in individuals exposed to chronic stress. For example, in their study of 52 older adults, Glaser, Sheridan, Malarkey, MacCallum, and Kiecolt-Glaser (2000) reported that current caregivers of spouses with dementia had initially comparable levels of antibody titers to pneumococcal polysaccharide vaccine compared with former caregivers and controls, but they showed a more rapid decline over the 6-month follow-up period. The findings are broadly consistent with other studies in adults (Gallagher, Phillips, Drayson, & Carroll, 2009; Kiecolt-Glaser, Glaser, Gravenstein, Malarkey, & Sheridan, 1996; Moynihan et al., 2004; Segerstrom, Hardy, Evans, & Greenberg, 2012; Snyder, Roghmann, & Sigal, 1993; Vedhara et al., 1999), although the specific nature of the effect varies somewhat across study (e.g., whether the effect is evident immediately after vaccination or only after a period of time). Particularly germane to the current study is one report that assessed response to meningococcal Serogroup C conjugate vaccine. In a sample of 60 college students, individuals who experienced high perceived stress or elevated anxiety and insomnia had lower antibody response, although the analysis was based on a single visit from 1 to 16 months following vaccination (Burns, Drayson, Ring, & Carroll, 2002). In addition, associations have been shown for different types of vaccine, such as T cell dependent (e.g., conjugate vaccines) and independent (e.g., nonconjugate pure polysaccharide vaccines) types.

Evidence that chronic stress may suppress vaccine response is only one example of how psychological states and exposures may alter vaccine responses, however. Reviews of human and animal research (Dhabhar, 2009; Powell, Allen, Hufnagle, Sheridan, & Bailey, 2011) note that vaccine responses can be both suppressed and enhanced by stress. For example, Edwards et al. (2008) demonstrated, in a sample of 31 healthy young adult men assigned to one of three conditions (physical stress, mental stress, or control), that those in either of the two stress conditions exhibited increases in serum immunoglobulin G (IgG) concentration to a polysaccharide meningococcal vaccine compared to those in the control group 4 weeks after vaccination; the effect was short lived (not evident at 20 weeks postvaccination), evident only for Serogroup A (not C) and not evident in women. Evidence of an enhanced influenza vaccine response was found for women in the same study as a function of prevaccination stress (Edwards et al., 2006); in that study, influenza and meningococcal vaccines were administered concurrently.

Opportunities to identify psychological or psychosocial predictors of vaccine response in children are numerous, given that vaccines against multiple infectious diseases are administered from birth to adolescence. Nonetheless, most studies of individual differences in vaccine responses consider basic questions about administration, such as whether or not booster doses are needed (Van Damme et al., 2010; Wu, Hwang, Goodman, & Beasley, 1999; Zanetti et al., 2012). Research examining a possible role for psychological factors or exposures in vaccine responses in children is rare (e.g., Meyer & Haggerty, 1962; O'Connor et al., 2013).

One starting point for research on psychological predictors of vaccine responses in children, which may inform a broader developmental model of PNI, is the applicability of established adult models. There are reasons for suspecting that the adult model may not apply in children. One reason is that the leading adult model focusing on chronic stress exposure may be questioned insofar as the notion of "chronic" stress in (young) children is ill defined and difficult to translate. That is, a major theme to emerge from adult work emphasizes the accumulation of (decades of) stress, the effects of which may only be observable in (older) adults with aged immune systems, a concept referred to as "immunosenescence" (Bauer, 2008; Franceschi et al., 2000). Another reason is that adult models of PNI may not extend to children because of the rapid and substantial developmental changes in the innate and adaptive components of the immune system from infancy to adulthood (Bauer, 2008; Kollmann, Levy, Montgomery, & Goriely, 2012; PrabhuDas et al., 2011).

Recent reviews (Mills, Scott, Wray, Cohen-Woods, & Baune, 2013; O'Connor et al., 2014) indicate that there is some evidence of "adultlike" patterns of associations between immune parameters and psychological symptoms or exposure in pediatric samples, but there are also strikingly contrary patterns. For example, Schleifer et al. (2002) found that depressed adolescents exhibited greater natural killer (NK) cell activity than did nondepressed adolescents. That observation comports with Caserta et al. (2008), who reported that an index of family stress predicted greater NK cell function in young children. However, these findings contradict the reliable finding in adults that stress and depression are associated with reduced NK cell cytotoxicity (e.g., Irwin et al., 1990). It is not easy to extrapolate from NK cell function, an index of innate immune response, to vaccine responses, a prototypic index of the adaptive immune system; however, these contrasting findings suggest that distinctly different patterns of the interplay among psychological processes, the nervous system, and the immune system may be found in adult and pediatric samples.

Given the rarity of research on the psychological correlates and predictors of immune responses in children, we regarded our current study on child mood predictors of vaccine responses as largely exploratory in nature. Nonetheless, several methodological safeguards were in place to assure that the

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robustness of any finding detected could be evaluated. We included as covariates those factors known to correlate with immune markers in adults that may confound a mood–immune response association in the current study, including somatic factors such as recent illness, body mass index (BMI), sleep quality (O'Connor et al., 2009), and sociodemographic risk. In addition, we assessed antibody responses at 4 weeks (when a peak antibody response would be evident) and 3 and 6 months postvaccination to track consistency in effect in a relatively wide window of response. In addition, although many PNI studies in children include highly selected and small samples, we assessed a comparatively large community sample. Finally, given the focus on internalizing symptoms in this Special Issue, we not only included child reports of depression and anxiety but also considered measures of stress and coping to examine the possible broader nature of the phenotype associated with altered immune response.

Methods

Sample and procedure

A group of 188 children 9–10 years of age and a primary caregiver (in 88.5% the biological mother) were recruited by an invitation letter from three pediatric practices serving diverse populations in a medium-sized city, with the intent of oversampling families at high psychosocial risk. Interested families were subsequently screened by phone for eligibility. Children were eligible if they were free of chronic illness affecting immune function and were able to complete the research measures in English. The aim of the study was to examine predictors of the immune response to the meningococcal conjugate vaccine, which is now routinely administered at approximately 11 years of age. Research visits were conducted with children and a primary caregiver 12 and 6 months prior to the vaccination and at the time of vaccination; for these visits, children and parents completed questionnaire assessments, and a physical and health assessment of the child was conducted by a research nurse. Measures of child internalizing symptoms as well as psychological and psychosocial covariates (see Measures, below) and sociodemographic and health covariates (see Measures, below) were taken from the study visit 6 months prior to vaccination. After vaccination, children returned for an abbreviated visit at 4 weeks, 3 months, and 6 months postvaccination, at which time a venous blood sample was obtained to assess the antibody response to four vaccine serogroups. All research visits took place in a hospital-based clinical research center. The study was approved by the local institutional review board; parents provided written informed consent and children provided written assent to participate.

Measures

Meningococcal serogroup specific antibody responses. Antibody responses were determined by enzyme-linked immunosorbent assay as described in the literature (Gheesling et al., 1994; Sikkema et al., 2000). Briefly, 96-well medium-binding EIA plates (COSTAR #3591, Fisher, Suwanee, GA) were coated with meningococcal polysaccharide Serogroups A, C, W-135, or Y with methylated human serum albumin (mHSA; National Institute for Biological Standards and Control, Hertfordshire, UK) in pyrogen-free phosphate buffered saline (PBS) at 100 μl per well. Final concentrations of the polysaccharide and mHSA were optimized for the plate lot based on box titrations. For Serogroup A, the final concentration was 2.5 μg/ml in 1.25 μg/ml mHSA; Serogroup C final concentration was 0.625 μg/ml in 0.625 μg/ml mHSA; Serogroup W-135 final concentration was 1.25 μg/ml in 2.5 μg/ml mHSA; and Serogroup Y final concentration was 1.25 μg/ml in 1.25 μg/ml mHSA. The plates were incubated overnight at 4 °C. Following three washes with pyrogen-free PBS with 0.05% Tween 20 (PBST), the plates were blocked for 1 hr with PBST containing 3% low-endotoxin vaccine grade Probumin (Millipore, Billerica, MA). Eleven serial dilutions of anti-meningococcal human reference serum CDC 1992 (National Institute for Biological Standards and Control, Hertfordshire, UK) starting at 0.36 μg/ml IgG antibodies for each serogroup were used to create a standard curve on each plate and four dilutions of subject serum sample starting at 1:50 or 1:100 were added to the plates as well as 2 wells of blanks containing PBST and incubated overnight at 4 °C. Plates were washed three times with PBST then incubated at room temperature for 2 hr with 100 μl per well goat antihuman IgG–alkaline phosphatase conjugate (Thermo Pierce–Fisher, Suwanee, GA). Following four washes with PBST, 100 μl per well of 1 mg/ml *p*-nitrophenyl phosphate substrate in 1X diethanolamine buffer (Thermo Pierce-Fisher, Suwanee, GA) was incubated for 40–60 min at room temperature until optical density at 405 nm of the first reference serum dilution reached 3.0, as read on a SeptraMAX 340 (Molecular Devices, Sunnyvale, CA). For each plate, reference optical density measurements at 490 nm were subtracted from the 405-nm values, and then a cutoff was calculated from 10 *SD* above the average of the blanks. Concentrations of IgG for each serogroup in each sample were extrapolated from the five-parameter regression of reference serum CDC 1992. An average of at least two dilutions of the sample was accepted as the final result. Any sample with a coefficient of variance above 15% was not used in the calculation. Competition experiments were performed to confirm the specificity of each assay as well as to determine the limit of detection of meningococcal IgG, which was 0.15 μg/ml for all serogroups. Antibody titer levels were assessed prevaccination and at 4 weeks, 3 months, and 6 months following the vaccination. This assessment schedule was based on our interest in assessing peak antibody response (which would be evident at 4 weeks) and decay in antibody response. All immunological assays were performed in collaboration with the Human Immunology Core Laboratory at the University of Rochester Medical Center.

Child internalizing symptoms. Child self-report of depressive symptoms was based on the short form of the Child Depression

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Inventory (CDI), a 10-item index that was derived from the longer well-validated CDI (Kovacs, 2001, 2003). The measure is widely used and has considerable evidence of reliability and validity (Allgaier et al., 2012), including as part of a previous PNI study from this group (Caserta, Wyman, Wang, Moynihan, & O'Connor, 2011). There are only limited data on the CDI short form for establishing cutoffs to establish clinical significance. In a pediatric patient sample, Allgaier et al. (2012) reported that a score of 3 or more had a sensitivity of 93% and a specificity of 71% in predicting disorder; in the current sample, approximately 17% of the children met that score, indicating a comparatively high degree of clinically significant symptoms. Child self-report of anxiety symptoms was based on the Revised Child Manifest Anxiety Scale (Reynolds & Richmond, 1985, 1997), a widely used index of anxious symptoms in children with considerable evidence of reliability and validity (e.g., Muris, Merckelbach, Ollendick, King, & Bogie, 2002). This 37-item questionnaire that uses a yes–no response format; there are three subscales (physiological, social, and worry) as well as the total summary scale. In the absence of a priori hypotheses about subscale, we used the total scale in analyses below.

Psychological and psychosocial covariates. In addition to ratings of internalizing symptoms, we also included a measure of coping based on children's reported perceived self-efficacy, a 14-item measure to assess children's perceived ability to achieve their goals in common situations and settings, including family, school, and community. The measure has documented evidence of reliability and validity (Cowen et al., 1991; Wyman et al., 1999), including data from a prior study of immune function (Caserta et al., 2011). Two measures of stress were also considered. Child stress exposure was based on the Stressful Life Events Conditions Checklist, an index of common negative life events that has been widely used in studies of risk and resilience (Wyman, Cowen, Work, & Kerley, 1993) At each study visit, respondents indicate which of 35 adverse events (e.g., violence exposure) and chronic processes (e.g., parent unemployment) are ongoing or have occurred during the prior 6-month interval. Another measure of child stress exposure was parent self-reports of their own psychological distress from the Brief Symptom Inventory, a 51-item symptom rating scale with several subscales indexing symptoms of depression, anxiety, and psychoticism (Derogatis, 1992). The measure has been shown to index clinical disturbance and has considerable evidence of reliability and validity (Derogatis, 1992; Derogatis & Melisaratos, 1983). The total score was used in analyses.

Sociodemographic and health covariates. Socioeconomic status was indexed by family income (an 8-point scale, from <$15,000/year to >$95,000/year) and education (categorized as less than high school degree, high school degree, less than 4-year college degree, college degree, or greater). A child-rated subjective measure of social class was obtained using the McArthur Scale of Subjective Social Status (Adler, 2000; Goodman et al., 2001); for this measure, children were asked to place themselves on the ladder following standard instructions, with 10 representing the highest level and zero the lowest in relation to "American society" and in relation to "your school." BMI was calculated according to the standard formula, weight (kilograms)/height (meters)^2. Information on sleep and sleep quality was derived from a diary assessment in which children reported on the time they went to sleep and woke up for a 14-day period. Children also reported the number of times they awoke each night and the quality of sleep on a Wong–Baker faces 6-point scale (*good* to *bad*). Reliability and validity of sleep diary data in early adolescent samples has been widely reported (e.g., Werner, Molinari, Guyer, & Jenni, 2008). Illness in the past 2 weeks was assessed from a brief medical history obtained at each visit (Caserta et al., 2008). Other potential covariates included child age and sex, pubertal status (derived from self-report Tanner stages), ethnicity (see categories in Table 1), and family size.

Data analyses

We present descriptive sample information, followed by attrition analyses that compare children on whom we obtained a prevaccination blood sample (1 year following initial study enrollment) with those children who dropped out of the study or did not agree to a blood collection. Prevaccination anti-

Table 1. *Descriptive data*

	Mean (*SD*)	*N*
Child Factors		
Age (years)	9.55 (0.50)	
Sex (female)		64 (51%)
Body mass index	20.05 (5.36)	
Race/ethnicity		
American Indian		3 (2%)
Asian		3 (2%)
Hispanic		4 (3%)
Black		65 (52%)
White		51 (41%)
Family Factors		
Education (mother)		
<High school		16 (13%)
High school degree		19 (15%)
>High school <college degree		44 (35%)
College degree or more		47 (37%)
Income[a]		
<$15,000/year		45 (38%)
$16,000–$25,000/year		17 (14%)
>$95,000/year		7 (6%)
Live-in partner		88 (72%)

Note: Descriptive data at the beginning of the study for those children for whom we obtained a prevaccination blood draw ($n = 126$).
[a]Not all income categories are displayed.

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Table 2. *Correlations between prevaccination mood symptoms and antibody concentrations through 6 months postvaccination*

	Depressive Symptoms	Anxiety Symptoms
Serogroup A		
Prevaccination	.21*	.33*
4 weeks	.09	−.10
3 months	.11	.04
6 months	.16	.07
Serogroup C		
Prevaccination	.14	.09
4 weeks	−.08	.01
3 months	.07	.07
6 months	.11	.11
Serogroup W135		
Prevaccination	.25*	.01
4 weeks	.23*	.06
3 months	.28*	.04
6 months	.31*	.07
Serogroup Y		
Prevaccination	−.09	.11
4 weeks	.00	.26*
3 months	.02	.22†
6 months	.03	.25*

Note: The 4-week antibody concentration is corrected for prevaccination levels; antibody concentration values within each assessment are adjusted for variation in time since vaccination; $n = 91$ at prevaccination; $n = 84$ at 6 months postvaccination.
†$p < .10$. *$p < .05$.

body levels to the four serogroups indicated evidence of prior exposure to *Neisseria meningitides* or cross-reacting commensal organisms via colonization, as would normally be expected (Table 2). Therefore, the prevaccination antibody level was subtracted from the 4-week antibody level to derive an initial vaccine response. Antibody measurement may be strongly associated with time since vaccination, and this is especially so for initial antibody responses (i.e., levels within the first month). Accordingly, time since dose was considered as a covariate; some cases for the initial sampling, targeted for 4 weeks, were deleted because of extreme or off-time blood draw compared with vaccination (see below). For the quadrivalent meningococcal polysaccharide–protein conjugate vaccine, we assessed A, C, W-135, and Y polysaccharide-specific antibodies; these are independent antibody responses. The hypothesis that mood symptoms would predict antibody titer was tested using correlation analyses to examine associations within each assessment; repeated measures multivariate analyses of variance were then used to examine if the prediction from mood symptoms prevaccination to the multiple postvaccination measures of each serogroup was constant over time. Between-subjects predictor variables were child internalizing symptoms (self-reported depression or anxiety); time (i.e., 4 weeks, 3 months, or 6 months) was the within-subjects factor. In supplementary analyses, we consider other predictors of vaccine response, namely, child stress, self-efficacy, and parental psychiatric symptoms, because these constructs have received attention in research on vaccine responses in adults or in more limited research on children. All analyses of antibody concentrations (other than for descriptive purposes) are based on ln-transformed data. The main analyses are based on continuous measure of antibody concentration, but we also analyzed data using a categorical cutoff to indicate seroprotection based on 2 μg/ml of IgG.

Results

Preliminary analyses

Of the 188 children originally recruited, 3 children were excluded because they were vaccinated off-schedule (e.g., before the scheduled prevaccination blood draw visit); in addition, because of manufacturer differences in the quadrivalent meningococcal conjugate vaccine, we restricted our analyses to those children who received the vaccine from Sanofi Pasteur and therefore excluded 4 children who received a vaccine manufactured by Novartis. One additional child was excluded because of an extensive series of medical problems and surgeries that would have confounded immune analyses. Of the remaining 180 children, we were able to collect a prevaccination blood sample 1 year after the initial study visit on 126 children (70%). Attrition analyses comparing the 126 children on whom we obtained a prevaccination blood sample 1 year after the start of the study to the 54 for whom we did not (but had complete data from the initial study visit) indicated few differences. There were no group differences at initial assessment (i.e., 1 year prior to vaccination) in child reports of mood or stress measures or health confounds; only two differences emerged: children for whom we obtained a prevaccination blood sample were more likely to have mothers with higher education, χ^2 (3) = 10.29, $p < .05$, and income, F (1, 167) = 4.08, $p < .05$. There was not significant evidence of attrition by race/ethnicity when all categories were considered (Table 1), but there was a significant group difference when race/ethnicity was defined as white versus minority (80% vs. 65%, respectively), χ^2 (1) = 4.61, $p < .05$.

Descriptive data at study entry for those children on whom we obtained a prevaccination blood draw (Table 1) indicate that the sample was diverse but overrepresented by families at elevated psychosocial risk, suggested by the finding that more than one-third (38%) of the families had annual incomes of $15,000 or less; there was also a relatively high proportion of minority families.

Although we obtained prevaccination blood samples on 126 children, antibody concentrations were available on slightly fewer children for each subsequent assessment: 121 at 4 weeks postvaccination, 119 children at 3 months postvaccination, and 116 children at 6 months postvaccination (these numbers are identical for concentrations of all serogroups). It is well established that timing of vaccination influences antibody concentration, with maximum response evident at approximately 28 days following vaccination. Antibody concentration obtained long before or after this 1-month target

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window would misestimate maximum response. Therefore, we limited analyses to those subjects on whom we were able to obtain blood between 18 and 38 days postvaccination (i.e., 10 days on either side of the 28-day window); 35 (28%) children were so excluded. This decision was made on an a priori basis and blind to titer results and all identifying information. Antibody concentration assessments at 3 and 6 months postvaccination would be less sensitive to time; therefore, no a priori inclusion criteria concerning timing of blood draw were set for these later assessments. The remaining analyses are therefore restricted to the 91 children with antibody concentration data for whom we obtained a first postvaccination blood sample within 18–38 days of vaccination.

Antibody concentration (nontransformed) for the four serogroups (Appendix A) indicated a marked increased in mean levels at 4 weeks, followed by a decline through 6 months. Preliminary analyses indicated that the variation in days since vaccination (i.e., within the 18- to 38-day window) was associated with antibody titer at the 4-week assessment for all four serogroups, although statistically significant only for Serogroup C, r (88) = –.26, $p < .05$; correlations ranged from –.10 to –.19 for other serogroups. Time (days) since vaccination was not significantly associated with antibody response for any serogroups at the 3-month time assessment (rs = .08 to –.14) or the 6-month assessment (rs = –.10 to –.19), although the effects were generally negative and of marginal interest. We partial out time since vaccination from antibody concentrations at each assessment for all subsequent analyses.

There was moderate to large overlap between children's self-reported anxiety and depression at the prevaccination visit ($r = .66, p < .001$), and between internalizing symptoms and other psychosocial stress measures considered as possible predictors. For example, for children's self-reports of depression, correlations were $r = .47, p < .001$ with the Stressful Life Events Conditions Checklist; $r = –.25, p < .05$ with self-efficacy; and $r = .23, p < .05$ with parent self-reports on the Brief Symptom Inventory. The correlations with children's self-reported anxiety symptoms were parallel, if somewhat weaker.

Predictors of antibody concentration through 6 months postvaccination

There was minimal evidence that sociodemographic and health covariates (see Measures Section) significantly predicted antibody responses to the four serogroups. For child sex, higher antibody concentration for Serogroup Y were found for girls at 3 months, F (1, 83) = 5.17, $p < .05$, and 6 months, F (1, 82) = 7.04, $p < .05$, but not at 4 weeks, or for any of the other serogroups. We retained child sex as a covariate for analyses of Serogroup Y, below. None of the other covariates showed a consistent pattern, operationalized as more than one occasion within a serogroup or for more than one serogroup. For example, although child BMI prior to vaccination was negatively correlated with 4-week antibody response to Serogroup C, r (86) = –.21, $p < .05$, the association was not significant at 3 months (r = –.07) or 6 months (r = .00) postvaccination, and there was not a significant association between child BMI and any of the other serogroups at any time point. We concluded that this did not constitute reliable evidence that BMI predicted antibody responses to vaccine. Therefore, only child sex was retained (for analyses of Serogroup Y) in analyses predicting vaccine responses from mood symptoms.

Table 2 includes the correlations between internalizing symptoms and antibody concentrations for the four serogroups at each time point, adjusted for time since vaccination for each postvaccination assessment. Results indicate that children's depressive symptoms were reliably associated with higher levels of antibody concentration for Serogroup W135, with significant correlations stable through 6 months postvaccination. In addition, child self-reports of anxiety were reliably associated with higher antibody concentration through 6 months for Serogroup Y. We thereof considered these associations further in a repeated measures analysis of variance.

A repeated measures analysis of variance was used to predict antibody concentration at 4 weeks through 6 months from internalizing symptoms assessed prevaccination. Based on results in Table 2, we considered depression as a predictor of Serogroup W135 and anxiety as a predictor of Serogroup Y vaccine response. For depression, repeated measures analyses indicated a significant between-subjects effect of depression, F (1, 77) = 8.12, $p < .05$; there was no evidence that the prediction from depression differed from 4 weeks through 6 month postvaccination, as indicated by a nonsignificant Depression × Time interaction, F (1, 77) = 0.09, *ns*. There was the expected significant linear time effect, F (1, 77) = 4.55, $p < .05$, indicating a decrease in antibody titer level from 4 weeks to 6 months postvaccination.

Parallel results were obtained for child self-reports of anxiety for the Serogroup Y in repeated measures analyses. Specifically, we obtained a significant between-subjects effect of anxiety, F (1, 68) = 6.36, $p < .05$, and no evidence of a significant change in the prediction from 4 weeks to 6 months postvaccination (Anxiety × Time interaction), F (1, 68) = 2.59, *ns*. As suggested by the preliminary analyses above, there was also a significant between-subjects effect for child sex (girls greater than boys): F (1, 68) = 5.14, $p < .05$. There was a significant within-subjects effect for the linear, F (1, 69) = 36.57, $p < .001$, and quadratic effect of time, F (1, 69) = 5.57, $p < .05$, for antibody concentration to Serogroup Y.

Supplementary analyses

Exploratory analyses indicated no reliable evidence that the prediction from mood symptoms to vaccine responses were moderated by child sex or other covariates listed above. Analyses using measures of seroprotection were not feasible for Serogroup A because the vast majority of children scored above the threshold of 2 μg/ml IgG cutoff (95% at 6 months postvaccination). Analyses using threshold concentration Serogroups

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C, W135, and Y were broadly consistent with analyses of the continuous measures of antibody concentration: children with concentrations above the threshold for Serogroup W135 exhibited higher levels of depression, and children with concentrations above the threshold for Serogroup Y exhibited consistently higher levels of anxiety (not tabled; contact first author for details).

We then examined additional measures of child stress and coping to determine if there was evidence that children's vaccine responses were predicted from a broader array of stress and coping measures. There was not. Specifically, there was not reliable evidence that children's self-efficacy or life event stress or parental psychiatric symptoms predicted children's antibody responses across time or serogroup (details available from the first author).

Discussion

The findings provide some of the only evidence to date that psychological factors predict vaccine responses in children. In a comparatively large sample of early adolescents in which we tracked antibody response from peak level (~4 weeks) to 6 months postvaccination, we found that children's self-reports of mood predicted greater antibody concentrations. The effects, where detected, were consistent across time and not confounded by sociodemographic or health covariates.

The question might be raised of how robust the associations are between mood symptoms and vaccine challenge responses. We found that children's self-reports of depression predicted greater antibody concentration from 4 weeks through 6 months for one of the four serogroups; children's self-reports of anxiety also predicted greater antibody responses from 4 weeks through 6 months postvaccination for one (different) serogroup. We suggest that these results constitute a reliable association for the following reasons. One is the consistency of positive findings across measure (depression and anxiety), persistence across time (4 weeks through 6 months), and across serogroups (W135 and Y). The rate of significant associations exceeds what would be expected by chance and, as noted, is congruent with other data suggesting stress/distress and increased immune activation in young people, as in the case of increased NK cell function as a function of depression (Schleifer et al., 2002) or family stress (Caserta et al., 2008).

However, the findings raise two questions for further study. One question concerns the nature of psychological processes or characteristics that predict vaccine responses. In the supplementary analyses, we found no consistent evidence that antibody responses were predicted from life events, perceived self-efficacy, or parental psychopathology; neither did socioeconomic stress predict vaccine responses. It is not clear if the stronger prediction from mood ratings reflects bone fide psychobiological differences among the potential predictor measures that may mediate immune system responses. For example, although there is evidence in the pediatric literature that stress measures and psychopathology may be associated with altered hypothalamic–pituitary–adrenal (HPA) axis function and glucocorticoid activity (Gunnar & Quevedo, 2007), it is not yet evident which constructs may be most closely connected with altered HPA axis function and be plausible candidates for understanding variation in immune activation. The second question for further study is why one but not another serogroup would be predicted from mood state, although studies using this or similar paradigm rarely observe effects across multiple serogroups, where results from multiple serogroups are reported (Edwards et al., 2008). It is noteworthy that the peak concentrations of antibody to Serogroups A and C measured at 4 weeks postvaccination were the highest found in the study, suggesting that psychological effects may only be measurable with less robust immune stimulation (i.e., Serogroups W135 and Y), which has also been suggested for the effect of exercise on antibody responses (Edwards et al., 2012).

By what mechanisms might depressed or anxious mood be associated with increased immune response to vaccination? One hint may be in the finding that depression and anxiety predicted prevaccination antibody concentrations for several serogroups. This may be because children with increased internalizing symptoms are more likely to have been exposed to either *Neisseria meningitides* or cross-reacting commensal organisms, although there is no reason to suspect differing exposures. An alternative explanation is that mood symptoms may have biological correlates that explain the immune outcomes. Several candidates are plausible mediators in this regard given that the body's stress systems (HPA axis and sympathetic nervous system) have close connections with the immune system (Ader et al., 1995; O'Connor et al., 2014). Associations between depression and elevated concentrations of interleukin 6 (IL-6) have been demonstrated in adults, suggesting that mood disturbances lead to a pro-inflammatory state (and the reverse may also be so). In addition, elevated levels of IL-6 have been associated with enhanced vaccine responses in young adults, suggesting a possible mechanism (Edwards et al., 2006), although studies of older adults have not consistently shown an association between antibody response to influenza vaccination and IL-6 concentrations (Segerstrom, et al., 2012). It seems unlikely for there to be a single biological mediator to account for these effects, given the inherent overlap in stress system responses.

A developmental perspective may point to further clues about mechanisms. Our observation that symptoms predicted increased immune response to vaccination is contrary to the findings in adults, showing that mood and stress are associated with reduced vaccine responses (e.g., Glaser et al., 2000). It is possible that this amplified immune response in pediatric samples but diminished response in (older) adult samples may not be so much a contrary pattern but rather different parts of a shared developmental response curve: stress or distress may initially amplify immune responses that, over time, show downregulation and diminished sensitivity. Although the mechanisms underlying this particular effect in humans are not known, evidence that prolonged stress expo-

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sure may alter stress sensitivity and immune function (leading to a qualitatively different effect over time) has been provided, such as in the particular case of the buildup of glucocorticoid resistance following prolonged stress exposure (Avitsur, Stark, & Sheridan, 2001; Miller et al., 2009). Furthermore, and perhaps in parallel, there are qualitative differences in vaccine responses in adults according to acute stress (increased) versus chronic stress (impaired), as described above.

There certainly remains too much variation among studies concerning the links between measures of stress or psychopathology and immune markers in children to derive firm conclusions about the reliability or direction of effects. Findings from the current study may have particular value because of the experimental leverage offered by a vaccine challenge paradigm. Nevertheless, patterns of results may well depend on how the immune system response is operationalized; effects found for one marker of innate immunity (e.g., NK cell function or IL-6 levels) may not extend to B or T cell immune measures (e.g., vaccine responses). The implication is that a developmental PNI model will require multiple kinds of research paradigms across alternative definitions of immune system response.

We had no a priori prediction about timing of effects. Repeated measures analyses indicated that the association between internalizing symptoms and antibody responses was not significantly different from the initial assessment at 4 weeks through to the assessment at 6 months. That is in contrast to Glaser et al. (2000), who found that the effects of chronic caregiving stress in the elderly predicted diminished immune response only after 6 months. Differences among studies may be explained by variation in the type of vaccine administered, which could account for variation in the magnitude and persistence of response. Follow-up longer than 6 months postvaccination is rare in studies that assess psychological predictors of response, but it is possible that a longer follow-up period would detect a differential rate of antibody decline long after vaccine administration.

The study does have limitations. First, we assessed response to only one type of vaccine. Whether or not the findings are particular to meningococcal conjugate vaccine is not clear; there is a need to consider if the same prediction effects hold for other vaccines. That work is needed to comprehend the clinical and public health significance of these results (given that the aim of this study was not to predict meningococcal conjugate vaccine response per se, but rather to use leverage of this vaccination to test hypotheses about the interplay among psychological processes, the nervous system, and the immune system). Second, we did not have cross-informant prediction of child mood symptoms. We did not have detailed measures of depression and anxiety ratings on the child from parents; however, a long history of research shows that parent reports on internalizing symptoms at this age are known to be discrepant with child reports, influenced by their own mental state, and generally less sensitive than child self-reports (e.g., Angold et al., 1987; Weissman et al., 1987). Furthermore, the usual concern about cross-informant consistency in studies of child health and development has to do with circumventing problems of shared method variance. That is obviously not a problem in the current study, in which we assess child self-reports to measures of cellular activity as assessed in detailed laboratory analyses. Third, analyses were available on the majority of children initially recruited, but there was attrition, and for other children we were unable to obtain a postvaccination blood sample within a relatively narrow time window for the 4-week antibody assessment. These results reflect the usual problems of attrition, and particularly nonrandom attrition common in longitudinal studies, as well as the added difficulty of obtaining repeated blood samples on a relatively high-risk sample within a narrow time frame. We did not have evidence that these factors likely altered the results; for example, none of the many sociodemographic or health measures considered was associated with antibody response. These limitations are offset by several strengths of the study, including comparatively large size, relatively high rate of compliance of blood collection on three occasions through 6 months, detailed measures of child symptoms and other plausible psychological predictors, and the consideration of multiple potential somatic and psychosocial confounds.

It may be premature to project clinical or public health applications of the findings. Replication is needed (in other samples and for other vaccines) before judging the practical implications of these results. In addition, it is important to note that the primary outcome in this study is variation in antibody concentrations primarily above the range of what would be considered a "protective" response. In any event, it would obviously not follow, at this stage of research, to suppose that treating symptoms would have unhelpful (or promoting internalizing symptoms would have helpful) influences on vaccine responses. Rather, what these findings suggest is the need for integrating clinical work with depressed and anxious children and adolescents in a broader (somatic) health context, and for considering what role immune factors may have in the developmental trajectories of neurodevelopment and affective symptoms. That is not now customary in ordinary child mental health practice. However, there is increasing evidence of associations between immune factors and psychiatric symptoms in children. One notable example is pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections, in which there is a sudden onset of obsessive–compulsive and tic disorders/Tourette syndrome symptoms, and perhaps other symptoms following streptococcal infection (Swedo, Garvey, Snider, Hamilton, & Leonard, 2001; Swedo & Grant, 2005). In addition, there is growing evidence for an association between markers of the immune system and autistic symptoms (e.g., Ashwood et al., 2011). In addition, although treatment applications remain uncertain, one recent study found that IL-6 was elevated in adolescent girls not treated with selective serotonin reuptake inhibitors for emotional disorders (Henje Blom et al., 2012). There may be sizable value in adopting methods and measures from a PNI model for understanding the childhood onset and origins of somatic disease and behavioral disorders.

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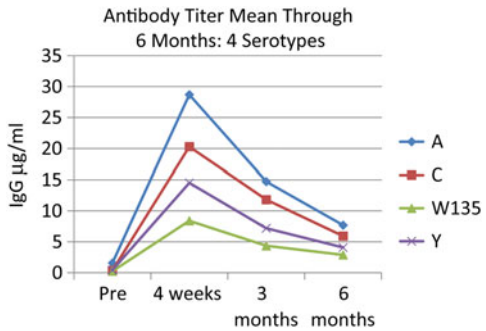
Appendix A



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