Published in final edited form as:

Psychoneuroendocrinology. 2015 September ; 59: 81–90. doi:10.1016/j.psyneuen.2015.04.021.

Secretory IgA Reactivity to Social Threat in Youth: Relations with HPA, ANS, and Behavior

Heidemarie K. Laurent^{*,a,b}, Laura R. Stroud^{c,d}, Bridget Brush^{c,d}, Christina D'Angelo^{c,d}, and Douglas A. Granger^{b,e}

^aUniversity of Oregon Psychology Department, Eugene, OR 97403

^bInstitute for Interdisciplinary Salivary Bioscience Research, Arizona State University, Tempe, AZ 85281

^cDepartment of Psychiatry and Human Behavior, Warren Alpert Medical School, Brown University, Providence, RI 02912

^dCenters for Behavioral and Preventive Medicine, The Miriam Hospital, Providence, RI, 02912

^eJohns Hopkins University School of Nursing; Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD 21218

Abstract

Although the role of immune marker secretory immunoglobulin A (SIgA) in stress-related health outcomes is gaining recognition, SIgA responsiveness to acute stress has rarely been assessed in adults, and not at all in children. This study was designed to clarify developmental origins of differential immune function-related health risks by investigating youth SIgA responses to psychosocial stressors, including both normative responses and variability related to behavioral problems. Children and adolescents from a larger study ($n = 82$) gave 6 saliva samples during a laboratory session in which they were exposed to a series of performance or interpersonal stressors. Samples were assayed for SIgA, as well as cortisol (representing hypothalamic-pituitary-adrenal axis activity) and alpha-amylase (sAA; representing autonomic nervous system activity). Behavioral problems were assessed with parent-report measures of youth internalizing and externalizing. Youth SIgA trajectories followed a normative pattern of reactivity and recovery around the stressors; however, these responses were blunted in youth with higher externalizing scores. SIgA showed differential associations with cortisol and sAA, and with positive and negative affect; whereas overall levels of SIgA related to cortisol output and positive affect,

* Address correspondence to HKL, 1227 University of Oregon, Eugene, OR 97403; 541-346-7051; hlaurent@uoregon.edu.

Conflict of Interest: In the interest of full disclosure, DAG is founder and chief Scientific and Strategy Advisor at Salimetrics LLC and Salivabio LLC, and these relationships are managed by the policies of the committees on conflict of interest at Johns Hopkins University School of Medicine, and the Office of Research Adherence and Integrity at Arizona State University. HKL, LRS, BB, and CD have no conflicts of interest to report.

Author Contributions: LRS conceived and designed the study, HKL analyzed the data, and all authors contributed to interpretation of the data. HKL drafted the article, and all authors helped to revise the manuscript and approved the final submitted version.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

changes in SIgA over time synchronized with changes in sAA and negative affect. In contrast to SIgA, neither cortisol nor sAA related significantly to behavioral problems. Implications for the role of SIgA during psychosocial stress in the development of immune function-related health risks are discussed.

Keywords

SIgA; Immunity; Stress; Children; Adolescents; Externalizing; Cortisol; sAA

1. Secretory IgA Stress Response in Youth: Relations with Other Stress Systems and Behavior

Childhood behavioral problems are associated with increased vulnerability to infection and disease (e.g., Jokela et al., 2009a; Odgers, et al., 2007). A possible link in this chain involves stress-related change in secretory immunoglobulin A (SIgA)—the main immunoglobulin in mucous, SIgA plays a key role in protection from pathogens. Surprisingly little is known about effects of acute psychosocial stress on children's SIgA, let alone links with other stress-responsive systems and individual differences associated with behavioral problems. This paper addresses a gap in the developmental health literature by examining the SIgA stress response in youth, elucidating associations with hypothalamic-pituitary-adrenal (HPA) and autonomic nervous system (ANS) responses, momentary affect, and internalizing/externalizing behaviors. Given that most infectious agents enter the body via mucosal surfaces, SIgA serves as a critical first line of defense against infection (Mazanec et al., 1993). Indeed, SIgA has been shown to guard against respiratory, intestinal, and urinogenital infections, as well as periodontal disease and caries (Evans et al., 1995). SIgA can be detected in saliva in the first days of life, with normative increases evident across infant/toddler development (Cole et al., 1999; Mellander et al., 1986; Seidel et al., 2001). Thus, SIgA offers a minimally invasive measure of children's resilience against infectious disease (Cieslak et al., 2003).

Individual differences in immune responses to stressful situations may be especially important in defining infectious health risks, given links between stress, adversity, and health. A growing body of research suggests that whereas acute stressors evoke transient increases in SIgA, longer-term/chronic stress exposure diminishes SIgA (see review by Segerstrom & Miller, 2004). Only links between longer-term stress and SIgA have been explored in childhood. One study showed that toddlers exposed to lower-quality (less sensitive) caregiving had lower daily SIgA (Vermeer et al., 2012). Childhood adversity may exert persistent effects on immunity; adolescents who were institutionalized or suffered physical abuse as children failed to limit viral reactivation, which in turn related to SIgA levels (Shirtcliff et al., 2009). These findings converge with studies demonstrating inverse relationships between life stress and SIgA in adults (e.g., Phillips et al., 2006) to suggest that long-term stressors influence SIgA. To our knowledge, there are no published studies of SIgA response to acute stress in children or adolescents. Investigating normative youth SIgA responses to psychosocial stress and variations related to both other stress-responsive

systems and behavioral problems would advance understanding of developing immune-health relationships.

Research addressing SIgA responses alongside other stress systems—in particular, the HPA axis (tapped by cortisol) and the ANS—has yielded inconclusive findings regarding cross-system linkages. There is evidence in adults that acute stress leads to parallel increases in SIgA and cortisol (Viena, 2012), yet other research suggests an inverse association between cortisol and SIgA following psychosocial stress (Campisi et al., 2012). Mouse research has shed further light on mechanistic associations across systems, demonstrating ANS involvement in SIgA secretion via cholinergic/adrenergic innervation, and either excitatory or inhibitory effects of corticosteroids on SIgA depending on dosage (Jarillo-Luna et al., 2015). Another important aspect of the stress response is subjective emotion. Studies with adults typically show lower immune response to acute stress in those expressing higher negative affect (e.g., Mills et al., 1996), though it may be important to distinguish between-person differences in affect and SIgA from within-person processes (Evans et al., 1993). Research directly addressing SIgA relations with HPA, ANS, and affect during acute stress in humans is needed to describe this immune marker's role within a larger stress response matrix. Further understanding of SIgA's significance in stress adaptation will come from observing associations with behavioral problems.

Adult research suggests a modest association between behavioral adjustment and SIgA levels (see review by Valdimarsdottir & Stone, 1997), and an acute depression induction was shown to reduce SIgA levels (Tsuboi et al., 2008). In children, there is scattered evidence that SIgA levels relate to behavioral problems, with children undergoing a stress management intervention showing simultaneous decreases in anxiety and depression and increases in positive mood and SIgA (Hewson-Bower & Drummond, 2001). The direction of effects, however, is not consistent across studies and may depend on individual difference moderators such as sex. One study showed higher SIgA related to higher impulsivity and delinquency among girls, but marginally lower impulsivity and distress among boys (Keller et al., 2010). Clearly, more study is needed to discern when and how SIgA relates to youth behaviors.

Another question is whether behavioral problem-SIgA associations parallel or diverge from known associations with other stress systems. Previous research in the current study sample demonstrated an association between internalizing behaviors and the interaction of cortisol and salivary alpha-amylase (sAA, an ANS marker), but no main effects of internalizing or externalizing on cortisol or sAA (Allwood et al., 2011). Comparing behavioral correlates of SIgA against those of cortisol and sAA would further help situate the former as part of an adaptive stress response.

The present study addresses gaps in knowledge about normative development of SIgA stress responsivity, its place within a multisystem stress response, and variations related to behavioral problems. This study diverges from previous work in this sample by including SIgA, and by examining all stress markers using growth curve modeling. We addressed the following unanswered questions in a sample of normally developing children and adolescents: Do youth show dynamic changes in SIgA during exposure to acute

performance or interpersonal stress? We hypothesized that youth, as a group, would show changing SIgA levels in response to acute psychosocial stressors, but that there would be significant between-child variability in responses.

Do SIgA responses relate to HPA, ANS, and/or affective responses? We expected SIgA would show associations with cortisol, sAA, and negative affect at the level of overall output (between-person effect) and/or synchrony over time (within-person effect).

How do youth behavioral problems (internalizing/externalizing) relate to SIgA responses? We hypothesized that youth with more problems would typically show attenuated SIgA responses, but this could vary by sex. Secondly, we wished to establish whether behavioral effects were similar to or different from those for cortisol and sAA.

2. Method

2.1 Participants

Participants were drawn from a larger study of the influence of development and stressor type on neuroendocrine and cardiovascular stress response over the adolescent transition (Stroud et al., 2009). The larger sample included 82 healthy children and adolescents (49% male; age range 7-17, $M = 12.5$, $SD = 2.5$) recruited through community and online postings. Potential participants were screened for exclusion criteria, which included use of any prescription medications (including oral contraceptives, thyroid medications, steroids, and psychotropic medications) or other substances known to influence cortisol, as well as diagnosed mental or physical illness (including asthma and autoimmune diseases) that could interfere with study participation (see Stroud et al., 2009 for further information about the study sample). The majority of participants were Caucasian (73%), with married parents (83%). Parents had typically completed at least some college (78% of mothers, 68% of fathers) and median household income was between \$60,000 and \$80,000.

The current study involved the participants for whom complete behavioral problem data ($n = 63$) were available. A comparison of this group with those not included in the analytic sample revealed no significant differences in any available variables, including youth and parent demographics and random assignment to stressor type. Previous published research within this sample had focused on developmental influences across HPA and ANS responses (Stroud et al., 2009) or HPA and ANS response related to child internalizing/externalizing behaviors (Allwood et al., 2011), but neither of these addressed immune markers.

2.2 Procedure

Protocols and procedures were reviewed and approved by Lifespan Hospitals Institutional Review Board. Informed consent was obtained from mothers and assent from children and adolescents. The study included two sessions, each lasting approximately 2 hours, conducted on separate days (median time between sessions = 15 days). Participants were accompanied by their mothers to the laboratory for both sessions. In the first “rest” session, participants watched child-appropriate movies and television shows and completed questionnaires. The primary purpose of the rest session was to allow participants to habituate to the laboratory

and physiological monitoring prior to the stress induction session. With the influence of laboratory novelty attenuated, differences in reactivity could be attributed to the stress induction. The second (stress) session involved random assignment to the performance (62%) or interpersonal (38%) stressors. Fewer participants received the latter condition due to scheduling constraints for the peer rejection actors. Both stress sessions included a 20-25 minute baseline period where participants watched child-appropriate movies and television shows (some also read easy early primary school-level books); three stressors, lasting 10, 5, and 5 minutes respectively; and a one-hour recovery period in which participants completed questionnaires and again watched child-appropriate movies and television shows.

The performance stress session included speech (5 minutes preparation, 5-minute speech), mental arithmetic (5 minutes) and mirror tracing (5 minutes) tasks; the interpersonal stress session involved three exclusion challenges (10, 5, and 5 minutes) with gender/age-matched confederates. All mothers were required to observe the stressor portion of the session from an observation room. Six saliva samples were taken over the baseline, stressor, and recovery periods. All sessions began between 14:00 and 17:00 to control for diurnal variation in biomarkers. Participants were asked to refrain from food and drink (besides water) for two hours prior to the stress session, from exercise for 24 hours prior to the session, and from caffeine beginning the evening before the stress session. Following the stress sessions, participants were extensively debriefed, and participants and mothers were compensated for their time.

2.3 Stressors

2.3.1 Performance Challenges—Performance-oriented tasks were based on an adaptation of the Trier Social Stress Test for Children (TSST-C; Buske-Kirschbaum et al., 1997; see Stroud et al., 2009, for further details). The first segment was a public speaking task in which participants were given 5 minutes to prepare, then were asked to speak on academic topics (e.g., English, Science, History) for 5 minutes. Specifically, participants were asked to give a book report on a book of their choice, a science lesson (e.g., the planets), and a history lesson (e.g., describe their favorite president and why). Participants then completed a mental arithmetic task involving serial subtraction under time pressure for 5 minutes. The mirror tracing task also lasted 5 minutes and was adapted from Allen and Matthews (1997). This task involved tracing the figure of a six-sided star while viewing only its mirror image using a mirror star tracing apparatus (Layfayette Instruments, 1987). Mistakes were counted and marked by a sound and a white light. All tasks were performed before a two-member audience who remained stern during the procedures and pretended to take notes.

2.3.2 Interpersonal (Peer Rejection) Challenges—These challenges involve three peer rejection interactions based on an adaptation for children and adolescents of the Yale Interpersonal Stressor (YIPS; Stroud et al., 2000; see Stroud et al., 2009 for further details). The Child-YIPS (YIPS-C) involved interactions with two trained, same-sex, similar-age confederates who subtly excluded the participant by bonding with each other, leaving the participant out of their conversations, and having different interests and activities than the participant. Youth confederates were extensively trained child actors or trained college

students. Exclusion interactions focused around three topics: weekend activities, family, and friends.

2.4 Saliva Sampling

Seven to nine whole saliva samples were collected from each participant by passive drool over the course of each stress session. Participants were asked to fill saliva collection vials to a designated line using a straw or by salivating directly into the tube. Following collection, samples were frozen at -80° Celsius until shipment overnight delivery on dry ice to Salimetrics Laboratories (State College PA).

Figure 1 offers an overview of timing for the six saliva samples used in this study (see Allwood et al., 2011 and Stroud et al., 2009 for further description of the sampling rationale and procedure).

2.5 Measures

2.5.1 SIgA—SIgA was assayed using a commercially available indirect competitive immunoassay without modification to the manufacturers recommended protocol (Salimetrics, State College, PA). Samples were run in duplicate and mean values were calculated for each sample. On average, inter and intra-assay coefficients of variation were less than 15 and 10% respectively. The minimal concentration of SIgA that can be distinguished from 0 is 2.5 $\mu\text{g/mL}$. All of the salivary SIgA samples were within this assay detection limit. Figure 2 shows mean SIgA levels across sampling time points.

2.5.2 Cortisol—Salivary cortisol was analyzed in duplicate using a commercially available enzyme immunoassay without modification to the manufacturer's protocol (Salimetrics, State College, PA), range of sensitivity .007-3.0 $\mu\text{g/dL}$ and intra- and inter-assay coefficients of variation < 5 and 10%, respectively.

2.5.3 sAA—Alpha-amylase is an enzyme produced in the oral cavity that serves as a surrogate marker of autonomic activation (see Granger et al., 2007). sAA was measured using a kinetic reaction assay that employs a chromogenic substrate, 2-chloro-p-nitrophenol, linked to maltotriose (Salimetrics, State College, PA). Intra- and inter-assay coefficients of variation were < 7.5 and 6%, respectively.

2.5.4 Momentary affect—Self-reported affect was assessed at six points during each stress session: once at baseline, three times following the stress tasks (participants reported how they felt *during* each task), and twice during the recovery period. Affect measures comprised mood adjectives adapted from the State-Trait Anxiety Inventory for Children (STAI-C; Spielberger et al., 1973) including “upset,” “nervous,” “sad,” “happy,” “relaxed,” and “scared” rated along 3-point Likert scales. Affect measures included emotion faces at the extremes of each scale to assist participants in anchoring high and low levels of each emotion. Based on the results of a principal components analysis, we created two overall affect scales (negative affect: “upset,” “nervous,” “sad,” “scared” and positive affect: “happy,” “relaxed”).

2.5.5 Youth behavioral problems – Child Behavior Checklist 6-18 (CBCL)—The CBCL is a widely used parent-report measure assessing adjustment for both children and adolescents (Achenbach & Rescorla, 2001). Parents rated 113 behaviors along a 3-point Likert scale (0 = “not at all true,” to 2 = “very true”) to yield two broadband problem scales—Internalizing and Externalizing—and eight problem behavior subscales. Anxious/Depressed, Withdrawn/Depressed, and Somatic Complaints subscales contributed to the Internalizing composite, whereas Rule Breaking and Aggressive Behavior subscales contributed to the Externalizing composite. Test-retest reliability and internal consistency of both broadband scales and subscales have been shown to be excellent (Achenbach & Rescorla, 2001). Broadband problem scale *T*-scores ranged from 24 to 75, with relatively few participants showing clinically elevated Internalizing (6%) or Externalizing (3%) scores ($T > 65$). Due to restricted range of the *T*-score distributions and the non-clinical nature of this sample, raw CBCL scale scores (log-transformed to correct for positive skew) were utilized in all analyses.

2.6 Analytic Strategy

The data under investigation are dependent (i.e., SIgA scores over time clustered within an individual). Therefore, hierarchical linear modeling (HLM; Raudenbush & Bryk, 2002) was selected to model youth SIgA trajectories throughout the sampling period. Level 1 modeled within-person variation in SIgA over time with growth parameters that were allowed to vary across participants. At Level 2, between-person variability in these growth parameters could be explained by youth behavioral problems. All behavioral problem effects were continuous, based on CBCL scale scores.

In addition to the SIgA response trajectories themselves, we were interested in their relation to other stress markers over the course of the session. Thus, a second set of models included each of the other physiological or subjective stress markers—i.e., cortisol, sAA, or momentary affect (both positive and negative)—simultaneously as group mean-centered time-varying covariates predicting SIgA at Level 1, and grand mean-centered average scores for those markers across the session as Level 2 predictors of SIgA intercepts. These models allowed simultaneous examination of (a) between-person differences in overall stress system activation related to SIgA, and (b) within-person synchrony of changes in these stress systems and SIgA over time. First, baseline models with no explanatory predictors were fit, providing a description of average SIgA response trajectories to answer the first study question. Next, models including cortisol, sAA, or momentary affect scores as predictors of SIgA at Level 1 and Level 2 were fit as described above to address the second study question. Finally, participant CBCL scores were entered as predictors of variability in trajectory components for SIgA, as well as for cortisol and sAA, to address the third study question. Growth curve models were centered at the onset of the second stress task and included an intercept (representing SIgA levels at the beginning of the second stressor), slope (the instantaneous rate of change of the curve at the beginning of the second stressor, represented by time), quadratic term (the acceleration/deceleration describing the rate of change in slope, represented by time²), and—if merited according to model fit change—a cubic term (the overall curvature of the trajectory's changing acceleration/deceleration rate, represented by time³). Whereas intercepts reflected levels of SIgA during the stress tasks,

the other terms offered insight into the dynamics of reactivity and recovery across the entire session.

For illustration, the 2-level equations assessing relations between youth SIgA response trajectories and (a) cortisol responses, and (b) behavioral problems are given below:

a. *Level 1:* $\text{SIgA} = \beta_0 + \beta_1(\text{cortisol at same sample}) + \text{error}$

Level 2: $\beta_0 = \gamma_{00} + \gamma_{01}(\text{mean cortisol}) + \text{error}$

$\beta_1 = \gamma_{10} + \text{error}$

b. *Level 1:* $\text{SIgA} = \beta_0 + \beta_1(\text{time}) + \beta_2(\text{time}^2) + \beta_3(\text{time}^3) + \text{error}$

Level 2: $\beta_0 = \gamma_{00} + \gamma_{01}(\text{CBCL Internalizing}) + \gamma_{02}(\text{CBCL Externalizing}) + \text{error}$

(similar equations used to predict β_1 - β_3)

3. Results

3.1 Control Variables

A number of variables that could impact stress physiology were examined. These included participant age (in years) and sex, pubertal status (youth-reported and physician-reported Tanner stage), menstrual status (females' self-report of whether they had begun menstruating and menstrual phase, based on date of expected next period), socioeconomic status (highest grade completed by parents and total household income), body mass index, and sleep (hours of sleep the night before). None of these variables was found to significantly influence SIgA, and controlling for all of these covariates failed to alter the effects noted below (all coefficients within 98% confidence interval of original estimate). Stressor type (i.e., peer rejection vs. performance) also was not significantly related to SIgA responses, and neither stressor type nor youth sex moderated behavioral problem-SIgA associations. Thus, the more parsimonious models including only hypothesized predictors are reported below.

3.2 Question 1: Do youth show changes in SIgA during psychosocial stress exposure?

First, a repeated-measures ANOVA was conducted to determine whether SIgA levels changed over time in the sample as a whole. This test was significant, $F(5, 395) = 16.99, p < .001$. Difference contrasts revealed a significant rise in SIgA from sample 1 (pre-stress) to sample 2 (post-stressor 1), $F(1, 79) = 27.18, p < .001$; and significant declines from sample 2 (post-stressor 1) to sample 3 (post-stressor 2), $F(1, 79) = 13.24, p < .001$, and from sample 3 (post-stressor 2) to sample 4 (post-stressor 3), $F(1, 79) = 15.38, p < .001$. Differences between samples 4 and 5, and between 5 and 6, were nonsignificant.

Next, baseline HLM models were fit to determine the shape of SIgA trajectories in the sample as a whole. A cubic function provided the best fit to the SIgA data, based on comparisons of quadratic and cubic models to the simpler linear one (change in deviance from linear to cubic model $\chi^2[9] = 87.14, p < .001$; from quadratic to cubic model $\chi^2[5] = 82.96, p < .001$). This model reflected the fact that SIgA levels rose early in the session (during the initial stress task) to peak during the second stress task and fall during the final task, only to start rising again at the end of the session. Because the model was centered at

the onset of the second stress task, a significant quadratic term ($b = -291.83, p < .001$) confirmed a reactivity/recovery dynamic during the heart of stress exposure, and a significant cubic term ($b = 298.74, p < .001$) confirmed an overall rising, falling, rising dynamic across the session. At the same time, significant variability in each of the trajectory components, $\chi^2(62) = 125.95-831.81$, all $p < .001$, suggested individual differences in the level and dynamics of SIgA response that could be explained by youth behavioral problem predictors.

3.3 Question 2: How do both momentary changes in and overall levels of cortisol, sAA, and momentary affect relate to SIgA responses?

To determine how youth SIgA related to known stress-responsive systems, cortisol and sAA scores were used to explain variation in SIgA at both Level 1 and Level 2. Whereas mean cortisol across the session predicted higher mean SIgA levels (between-person effect), relative increases in sAA predicted increases in concurrent SIgA across samples (within-person effect). That is, youth with higher levels of cortisol overall displayed higher SIgA levels, and sample-to-sample increases in sAA were accompanied by increases in SIgA. The cortisol model explained 9.5% of the variance in SIgA intercepts, and the sAA model explained 12.2% of the residual within-person variability in SIgA. See Table 2, top panel, for these effects.

Next, momentary positive and negative affect were examined as predictors of SIgA at both Levels 1 and 2. Whereas mean positive (but not negative) affect predicted higher SIgA levels (between-person effect), relative increases in negative (but not positive) affect predicted increases in concurrent SIgA across samples (within-person effect). That is, youth with higher levels of positive affect overall displayed higher SIgA levels, and momentary increases in negative affect were typically accompanied by increases in SIgA. This model explained 6.3% of the variance in SIgA intercepts and 25.4% of the residual within-person variability in SIgA. See Table 2, lower panel, for these effects.

3.4 Question 3: How do youth behavioral problems relate to SIgA stress responses? (And how does this compare to relations with cortisol and sAA responses?)

To determine if youth SIgA responses varied by behavioral problems, a series of models was tested. First, broadband scale scores—i.e., Internalizing and Externalizing—were examined. If a significant effect was found for the broadband scale, individual subscales were then investigated to better understand the source of the effect/s. Externalizing, but not Internalizing, significantly predicted SIgA levels (intercepts) and dynamics (quadratic and cubic terms). Specifically, youth with higher externalizing scores exhibited lower, less rapidly changing SIgA responses during the stressor. Figure 3 shows predicted trajectories for youth with high (75th percentile) or low (25th percentile) Externalizing scores. This model explained 17.2% of the variance in SIgA intercepts, 23.2% of the variance in quadratic terms, and 24.5% of the variance in cubic terms. When specific behavior subscales were probed, only Aggressive Behavior predicted these patterns (Rule-Breaking effects were nonsignificant). See Table 3 for CBCL broadband scale effects on SIgA.

In contrast to SIgA, but consistent with prior work in this sample using a different analytic approach, cortisol and sAA showed no significant main effect associations with CBCL scores.

3.5 Summary

Taken together, the above models demonstrate that youth spanning a range from childhood through adolescence displayed SIgA reactivity to and recovery from acute stress. Relations with cortisol and sAA responses, and with positive and negative affect, reflected different forms of cross-system linkage. Finally, youth showing more externalizing behaviors were characterized by attenuated, less dynamic SIgA responses (but no differences in cortisol or sAA responses).

4. Discussion

This study represents a step toward identifying individual differences in stress-related health disparities based on impaired immune function during childhood and adolescence. In particular, we showed that typically developing youth respond to acute psychosocial stress with increased SIgA. We further showed that SIgA during acute stress relates in distinct ways to HPA and ANS response components, as well as to positive and negative affect. Finally, we found differences in SIgA responses related to youth externalizing behaviors. Below, we consider implications of these findings for understanding child psychosomatic risk and possible points of intervention.

First, we aimed to establish whether youth show an acute SIgA stress response. We found that children and adolescents typically responded to developmentally relevant laboratory stressors with SIgA reactivity, followed by recovery. Finding a normative SIgA response in children highlights intriguing possibilities for future research to investigate developmental immune-health relations in the context of psychosocial stress. Compared to previous findings in adults, the current results reveal points of both convergence and divergence. Whereas a study of men undergoing the TSST also demonstrated a significant task-related increase in SIgA from similar starting levels, the magnitude of peak SIgA in that study was roughly twice that of the current sample (Romero-Martinez et al., 2014). This suggests an immune component to the prototypical acute stress response that strengthens with age (though not necessarily across the age range in this sample), which could help to buffer individuals from external threat during challenging situations.

In order to understand how SIgA fits within the larger human stress response, it is necessary to define links with known stress-responsive systems. We found that SIgA related positively to both HPA and ANS response components, though at different levels of analysis. Whereas youth with higher SIgA levels during the stress session also showed higher cortisol levels—a between-person difference—increases and decreases in SIgA across the session tended to synchronize with changes in sAA—a within-person effect. These findings help to shed light on possible cross-system linkages in humans; as suggested by rodent research, moderate acute stress elevations in HPA activity are generally paired with SIgA increases (even as more extreme or chronic HPA activation has a suppressive effect), and ANS activation appears to exert a more direct stimulatory effect on SIgA (Jarillo-Luna et al., 2015). We

were also interested in how youth positive and negative affect relate to SIgA responsiveness at within- and between-person levels of analysis. Mean positive affect predicted higher SIgA activation, as did momentary increases in negative affect across the session. This finding aligns with previous research in adults highlighting links between SIgA and both transient increases in subjective stress (within-person association with negative mood) and lower levels of overall life stress (between-person association with desirable events) (Evans et al., 1993). This pattern suggests a possible protective mechanism during acute stress; that is, when the demands of the situation are greater (eliciting more negative affect), oral-mucosal defenses appear to be strengthened. Further research will be needed to discern how generalizeable these links are across stress situations, and whether variations in the degree of cross-system association have implications for mental/physical health. Our final question was whether youth behavioral problems related to differences in SIgA responses. As expected, more problems—specifically, higher externalizing behaviors—related to blunted SIgA responses to the stress tasks. This finding could help to explain increased immune function-related health vulnerabilities in children showing behavioral problems, especially if they are exposed to repeated or chronic stress. While it is too early to conclude that internalizing behaviors are unrelated to SIgA responses, it is interesting that previous adult work showing differences in stress-induced SIgA focused on aggressive (interpersonal violence) behaviors (Romero-Martinez et al., 2014). This provides a contrast to previous findings in this sample, which related cortisol x sAA responses to internalizing (but not externalizing) behaviors (Allwood et al., 2011), and may highlight the importance of different stress response branches for understanding different mental health-related risks. Research on health outcomes associated with externalizing and internalizing has tended to highlight health-risking behaviors and injury as reasons for poor health in the former, and an overall degradation of perceived health and fitness in the latter (Laukkanen et al., 2002; Jokela et al., 2009b). The current results support impaired immune defense against infection as another potential reason for the elevated mortality risk associated with childhood externalizing behaviors (Jokela et al., 2009a). Of course, implications for long-term health outcomes must be approached with caution given the non-clinical nature of this sample and the cross-sectional design; longitudinal research in clinical samples represents an important future direction for this area of study.

Unlike the prior study involving children's diurnal SIgA (Keller et al., 2010), we did not detect gender differences in effects. Besides the difference in SIgA sampling context (basal vs. acute stress), differences in age (2nd-3rd grade vs. middle childhood through late adolescence) could have played a role. Replication in larger samples representing a greater range of ages, sampling protocols, and internalizing/externalizing behaviors will help to clarify possible moderators of behavior-SIgA associations. In contrast to SIgA, neither cortisol nor sAA responses showed main effect relations with behavioral problems in this sample. As conceptualizations of the stress response expand to consider multiple interlocking systems (e.g., Laurent et al., 2013), the time is ripe to include SIgA function as a unique protective facet of the stress response. It is possible that SIgA activity reflects an underlying stress-responsive phenotype with adaptive significance. SIgA has broad importance for risk vs. resilience in the face of threat, given its position as a first line of defense against infections, as well as strong links between oral and systemic health (e.g.,

Cullinan et al., 2009; Garcia et al., 2000; Otomo-Corgel et al., 2012). As the role of the immune system in stress-responsive physiology becomes clearer, it makes sense that children who are able to devote more biological resources to immune function are better able to adapt to a changing environment, which is manifested in both somatic resilience and fewer behavioral problems. Although an in-depth treatment of mechanisms is beyond the scope of the current study, externalizing behaviors are known to both result from and contribute to ongoing psychosocial stress. It is possible that these chronically stressed children, in turn, show a long-term suppressive effect of HPA activation and/or a weakening of the ANS response to acute stress that would result in diminished SIgA production. Beyond stress exposure itself, the coping strategies children use during stress may contribute to differences in immune function. Further probing of coping-SIgA links during acute and chronic stress will be needed to elucidate directional effects and adaptive implications in different contexts.

Limitations of the current study point toward next steps in this line of research. The present sample size was modest, and it was drawn from a sample of typically developing youth; as such, it did not offer extensive variability in behavioral problems. Future studies in larger samples that include clinical sub-groups may reveal additional effects involving internalizing behaviors and/or differences in behavioral problem-SIgA links by gender or stressor type. Similarly, a larger and more diverse sample might allow for the detection of age and/or pubertal effects. Other limitations that may have obscured effects of interest have to do with the lack of oral health screening and a sampling procedure that did not allow us to consider salivary flow rate in SIgA analyses. On the one hand, research has shown that flow rate can vary independently of SIgA concentrations (e.g., Rockenbach et al., 2006; Sag et al., 2007), and previous published research on child SIgA in relation to stressful caregiving conditions did not include flow rate (Vermeer et al., 2012); thus, there is no evidence of which we are aware that flow rate makes a difference in the ability to detect stress-related changes in SIgA or relations with behavior under normative sampling conditions with unstimulated whole saliva. On the other hand, it would be optimal to be able to examine the influence of flow rate on SIgA concentrations and, if influential, to eliminate noise in the data by controlling for this variable. Finally, based on the design of the original study, we collapsed across two stressor types to arrive at the present findings. Although effects of stressor type were not significant for SIgA in this sample, future studies with greater power to investigate different developmentally relevant stressors may yield additional information regarding the influence of the stress context on SIgA.

It is noteworthy that we were able to detect effects of youth externalizing behaviors on SIgA responses even with these limitations, and this study offers a strong starting point for further research on physiological and behavioral correlates of stress-induced SIgA. For now, this work confirms the existence of an acute SIgA stress response in normally developing youth that connects with other aspects of psychophysiological stress, while highlighting a potential risk factor—externalizing behaviors—that could interfere with this response, and thus with immune function-related health outcomes more broadly.

Acknowledgments

We thank Salimetrics for the donation of SIgA reagents, and Judy Quang for biotechnical support with the salivary assays. This research was supported in part by an NIMH Career Development Award (K23 MH65443), a National Alliance for Research on Schizophrenia and Depression Junior Investigator Award to the second author, and NCI grant P50 CA84719. We are indebted to the mothers and children who participated in this study.

Role of the Funding Source: The sources of funding for this research did not play a guiding role in the study design, data collection/analysis, or writing of this manuscript.

References

- Achenbach, TM.; Rescorla, LA. Manual for the ASEBA school-age forms and profiles. Burlington, VT: University of Vermont Research Center for Children, Youth, and Families; 2001.
- Allen MT, Matthews KA. Hemodynamic responses to laboratory stressors in children and adolescents: The influence of age, race, and gender. *Psychophysiol.* 1997; 34:329–339.
- Allwood MA, Handwerker K, Kivlighan KT, Granger DA, Stroud LR. Direct and moderating links of salivary alpha-amylase and cortisol stress-reactivity to youth behavioral and emotional adjustment. *Biol Psychol.* 2011; 88:57–64. [PubMed: 21771636]
- Buske-Kirschbaum A, Jobst S, Kirschbaum C, Rauh W, Hellhammer D. Attenuated free cortisol response to psychosocial stress in children with atopic dermatitis. *Psychosom Med.* 1997; 59:419–426. [PubMed: 9251162]
- Campisi J, Bravo Y, Cole J, Gobeil K. Acute psychosocial stress differentially influences salivary endocrine and immune measures in undergraduate students. *Physiol Behav.* 2012; 107:317–321. [PubMed: 22982515]
- Cieslak TJ, Frost G, Klentrou P. Effects of physical activity, body fat, and salivary cortisol on mucosal immunity in children. *J Appl Physiol.* 2003; 95:2315–2320. [PubMed: 12909608]
- Cole MF, Bryan S, Evans MK, Pearce CL, Sheridan MJ, Sura PA, Wientzen RL, Bowden GH. Humoral immunity to commensal oral bacteria in human infants: Salivary secretory immunoglobulin A antibodies reactive with *Streptococcus mitis* biovar 1, *Streptococcus oralis*, *Streptococcus mutans*, and *Enterococcus faecalis* during the first two years of life. *Infect Immun.* 1999; 67:1878–1886. [PubMed: 10085031]
- Cullinan MP, Ford PJ, Seymour GJ. Periodontal disease and systemic health: Current status. *Aust Dent J.* 2009; 54:S62–S69. [PubMed: 19737269]
- Evans P, Bristow M, Hucklebridge F, Clow A, Walters N. The relationship between secretory immunity, mood and life-events. *Br J Clin Psychol.* 1993; 32:227–236. [PubMed: 8318943]
- Evans, PD.; Hucklebridge, F.; Clow, A.; Doyle, A. Secretory immunoglobulin A as a convenient biomarker in health survey work. In: Rodriguez-Martin, J., editor. *Health psychology and quality of life research; Proceedings of the 8th Annual Conference of the European Health Psychology Society.* University of Alicante; Alicante. 1995. p. 541–549.
- Garcia RI, Henshaw MM, Krall EA. Relationship between periodontal disease and systemic health. *Periodontol.* 2000; 25:21–36.
- Granger DA, Kivlighan KT, El-Sheikh M, Gordis EB, Stroud LR. Salivary α -amylase in biobehavioral research. *Ann NY Acad Sci.* 2007; 1098:122–144. [PubMed: 17332070]
- Hewson-Bower B, Drummond PD. Psychological treatment for recurrent symptoms of colds and flu in children. *J Psychosom Res.* 2001; 51:369–377. [PubMed: 11448705]
- Jarillo-Luna RA, Rivera-Aguilar V, Pacheco-Yépez J, Godínez-Victoria M, Oros-Pantoja R, Miliar-García A, Campos-Rodríguez R. Nasal IgA secretion in a murine model of acute stress: The possible role of catecholamines. *J Neuroimmunol.* 2015; 278:223–231. [PubMed: 25468772]
- Jokela M, Ferrie JE, Kivimäki M. Childhood problem behaviors and death by midlife: The British National Child Development Study. *J Am Acad Child Adolesc Psychiatry.* 2009a; 48:19–24. [PubMed: 19218894]
- Jokela M, Powers C, Kivimäki M. Childhood problem behaviors and injury risk over the life course. *J Child Psychol Psychiatry.* 2009b; 50:1541–1549. [PubMed: 19573032]

- Keller PS, El-Sheikh M, Vaughn B, Granger DA. Relations between mucosal immunity and children's mental health: The role of child sex. *Physiol Behav.* 2010; 101:705–712. [PubMed: 20732336]
- Laukkanen E, Shemeikka S, Notkola I, Koivumaa-Honkanen H, Nissinen A. Externalizing and internalizing problems at school as signs of health-damaging behavior and incipient marginalization. *Health Promot Int.* 2002; 17:139–146. [PubMed: 11986295]
- Laurent HK, Powers SI, Granger DA. Refining the multisystem view of the stress response: Coordination among cortisol, alpha-amylase, and subjective stress in response to relationship conflict. *Physiol Behav.* 2013; 119:52–60. [PubMed: 23684904]
- Mazanec MB, Nedrud JG, Kaetzel CS, Lamm ME. A three-tiered view of the role of IgA in mucosal defense. *Immunol Today.* 1993; 14:430–435. [PubMed: 8216720]
- Mellander L, Carlsson B, Hanson LA. Secretory IgA and IgM antibodies to E. coli O and poliovirus type I antigens occur in amniotic fluid, meconium and saliva from newborns. A neonatal immune response without antigenic exposure: A result of anti-idiotypic induction? *Clin Exp Immunol.* 1986; 63:555–561. [PubMed: 3011325]
- Mills PJ, Dimsdale JE, Nelesen RA, Dillon E. Psychologic characteristics associated with acute stressor-induced leukocyte subset redistribution. *J Psychosom Res.* 1996; 40:417–423. [PubMed: 8736422]
- Odgers CL, Caspi A, Broadbent JM, Dickson N, Hancox RJ, Harrington H, Poulton R, Sears MR, Thomson WM, Moffitt TE. Prediction of differential adult health burden by conduct problem subtypes in males. *Arch Gen Psychiatry.* 2007; 64:476–484. [PubMed: 17404124]
- Otomo-Corgel J, Pucher JJ, Rethman MP, Reynolds MA. State of the science: Chronic periodontitis and systemic health. *J Evid Based Dent Pract.* 2012; 12:20–28. [PubMed: 23040337]
- Phillips AC, Carroll D, Evans P, Bosch JA, Clow A, Hucklebridge F, Der G. Stressful life events are associated with low secretion rates of immunoglobulin A in saliva in the middle aged and elderly. *Brain Behav Immun.* 2006; 20:191–197. [PubMed: 16055305]
- Raudenbush, SW.; Bryk, AS. Hierarchical linear models: Applications and data analysis methods. 2nd. Sage; Thousand Oaks, CA: 2002.
- Rockenbach MI, Marinho SA, Veeck EB, Lindemann L, Shinkai RS. Salivary flow rate, pH, and concentrations of calcium, phosphate, and sIgA in Brazilian pregnant and non-pregnant women. *Head Face Med.* 2006; 2:44. [PubMed: 17132167]
- Romero-Martinez A, Lila M, Conchell R, Gonzalez-Bono E, Moya-Albiol L. Immunoglobulin A response to acute stress in intimate partner violence perpetrators: The role of anger expression-out and testosterone. *Biol Psychol.* 2014; 96:66–71. [PubMed: 24315951]
- Sag C, Ozden FO, Acikgoz G, Anlar FY. The effects of combination treatment with a long-acting beta2-agonist and a corticosteroid on salivary flow rate, secretory immunoglobulin A, and oral health in children and adolescents with moderate asthma: A 1-month, single-blind clinical study. *Clin Ther.* 2007; 29:2236–2242. [PubMed: 18042480]
- Segerstrom SC, Miller GE. Psychological stress and the human immune system: A meta-analytic study of 30 years of inquiry. *Psychol Bull.* 2004; 130:601–630. [PubMed: 15250815]
- Seidel BM, Schubert S, Schulze B, Borte M. Secretory IgA, free secretory component and IgD in saliva of newborn infants. *Early Hum Dev.* 2001; 62:159–164. [PubMed: 11282225]
- Shirtcliff EA, Coe CL, Pollak SD. Early childhood stress is associated with elevated antibody levels to herpes simplex virus type I. *Proc Natl Aca Sci USA.* 2009; 106:2963–2967.
- Spielberger, CD.; Edwards, CD.; Lushene, RE.; Montuori, J.; Platzek, D. Preliminary manual for the State-Trait Anxiety Inventory for Children. Consulting Psychologists Press; Palo Alto, CA: 1973.
- Stroud LR, Foster E, Papandonatos GD, Handwerger K, Granger DA, Kivlighan KT, Niaura R. Stress response and the adolescent transition: Performance versus peer rejection stressors. *Dev Psychopathol.* 2009; 21:47–68. [PubMed: 19144222]
- Stroud LR, Tanofsky-Kraff M, Wilfley DE, Salovey P. The Yale Interpersonal Stressor (YIPS): Affective, physiological, and behavioral responses to a novel interpersonal rejection paradigm. *Ann Behav Med.* 2000; 22:204–213. [PubMed: 11126465]
- Tsuboi H, Hamer M, Tanaka G, Takagi K, Kinane N, Steptoe A. Response of ultra-weak chemiluminescence and secretory IgA in saliva to the induction of angry and depressive moods. *Brain Behav Immun.* 2008; 22:209–214. [PubMed: 17845839]

- Valdimarsdottir HB, Stone AA. Psychosocial factors and secretory immunoglobulin A. *Crit Rev Oral Biol Med.* 1997; 8:461–474. [PubMed: 9391755]
- Vermeer HJ, van Ijzendoorn MH, Groeneveld MG, Granger DA. Downregulation of the immune system in low-quality child care: The case of secretory immunoglobulin A (SIgA) in toddlers. *Physiol Behav.* 2012; 105:161–167. [PubMed: 21878345]
- Viena TD, Banks JB, Barbu IM, Schulman AH, Tartar JL. Differential effects of mild chronic stress on cortisol and S-IgA responses to an acute stressor. *Biol Psychol.* 2012; 91:307–311. [PubMed: 22910371]

Highlights

We tested youth secretory immunoglobulin A (SIgA) responses to psychosocial stress.

Children and adolescents showed normative SIgA stress reactivity and recovery.

Different aspects of SIgA response were associated with cortisol and alpha-amylase.

SIgA responses were blunted in youth with externalizing problems.

Figure 1.

Session and saliva sample timing.

Note. Stress tasks 1 (10 min), 2 (5 min), and 3 (5 min) shown in numbered boxes. There was some variability in timing among participants—sample times were within a 5-minute window of times given above.

Figure 2.

Observed SIgA levels across samples (gray area represents stress tasks).

Note. Error bars represent the standard error of the mean at each sample.

Figure 3.

Youth externalizing behaviors predict SIgA responses to acute psychosocial stress.

Note. Continuous effect illustrated by predicted trajectories at ± 1 SD from the mean of CBCL Externalizing scores.

Table 1
Study Measure Descriptives

Measure	<i>M, SD</i>
SIgA (µg/mL)	155.03, 112.48
Cortisol (µg/dL)	.14, .11
sAA (µg/mL)	125.59, 107.76
CBCL Internalizing	5.36, 5.18
CBCL Externalizing	2.04, .78

Note. Raw scores shown. Measures found to be skewed were transformed prior to analysis.

Table 2
Youth SIgA Trajectories Related to Other Aspects of the Stress Response

Predictor	Coeff., SE	p
Stress Physiology		
SIgA Intercept		
Mean Cortisol	.29, .10	.007
Mean sAA	.01, .13	.937
Cortisol-SIgA Association	.11, .11	.305
sAA-SIgA Association	.36, .10	.001
Momentary Affect		
SIgA Intercept	137.60, 10.53	< .001
Mean Positive Affect	47.17, 22.34	.039
Mean Negative Affect	31.03, 37.58	.412
Positive Affect-SIgA Association	.46, 9.49	.962
Negative Affect-SIgA Association	41.57, 14.13	.005

Note. All coefficients are standardized. Significant effects ($p < .05$) highlighted in bold.

Table 3
Youth SIGA Trajectories Related to Behavioral Problems

Predictor	Coeff, SE	p
Intercept (level at 2 nd stressor)		
Internalizing	.12, .13	.354
Externalizing	-.53, .20	.010
Slope(recovery at 2 nd stressor)		
Internalizing	-.04, .20	.840
Externalizing	.51, .31	.102
Quadratic (reactivity/recovery curve around 2 nd stressor)		
Internalizing	.02, .47	.973
Externalizing	1.65, .54	.004
Cubic (overall response curvature)		
Internalizing	-.11, .53	.830
Externalizing	-1.89, .65	.006

Note. All coefficients are standardized. Significant effects ($p < .05$) highlighted in bold.