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Journal

Psychosomatic Medicine, 75(6)

ISSN

0033-3174

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Publication Date

2013-07-01

DOI

10.1097/psy.0b013e31829807fb

Peer reviewed

Published in final edited form as:
Psychosom Med. 2013 ; 75(6): . doi:10.1097/PSY.0b013e31829807fb.

Family Adversity and Autonomic Reactivity Association With Immune Changes in HIV-Affected School Children

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Abstract

Objective—To explore whether primary school entry is associated with changes in immune system parameters in HIV-affected children. HIV-affected children are vulnerable to psychosocial stressors, regardless of their own HIV serological status.

Methods—Data from 38 HIV+ and 29 HIV− children born to seropositive women were obtained before and after school entry. Measures included family adversity questionnaires, autonomic nervous system (ANS) reactivity (based on mean arterial responses to challenge tasks), and enumerative and functional changes in peripheral blood immune parameters.

Results—In comparison to children who were HIV−, children who were HIV+ at baseline had fewer CD4+ T lymphocytes (M = 916 vs. 1206 cells/mm^3 × 10^3; F = 7.8, p = .007), more CD8+ cells (M = 1046 vs. 720 cells/mm^3 ×10^3; F = 7.98, p = .006), and diminished NK cell cytotoxicity (M =−.29 vs. .41; F = 8.87, p = .004). School entry was associated with changes in immune parameters, but HIV status was not associated with the magnitude of changes. Changes in immune parameters following school entry were associated with family stress and pre school entry ANS reactivity. Highly ANS reactive children had either the greatest increase in CD8+ cells following school entry or the greatest decrease, depending upon reported levels of family adversity (B = 215.35; t = 3.74, p < .001). Changes in functional immune assays were significantly associated with the interactions between HIV status and ANS reactivity.

Conclusions—These results suggest that autonomic reactivity is associated with increased immunological sensitivity to adverse or challenging social contexts among children affected by HIV.

Keywords

HIV; children; stress; reactivity; immune

Introduction

Globally, there are more than 2 million HIV+ children, 16 million children who have lost either one or both parents to HIV-related illness, and even more who live with a parent with chronic HIV-related morbidity (1). HIV/AIDS remains the leading cause of mortality among women of reproductive age. In 2008, approximately 1.5 million HIV+ women gave birth (1). In resource-rich regions, the rate of mother to child transmission (MTCT) has been reduced

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to approximately two percent with highly active antiretroviral therapy (HAART) (2). As the rate of MTCT has slowed and life expectancy with HIV infection has increased, greater numbers of HIV-exposed but uninfected (HEU) infants have been born. As more children are now HEU, it is apparent that this group may differ systematically from unexposed counterparts and that maternal HIV illness confers a variety of biopsychosocial risks regardless of infant infection. Given this shared risk, we define all children born to HIV+ mothers (including both HIV+ and HEU children) as *HIV-affected*.

HEU children experience higher morbidity and mortality than their unexposed counterparts. Studies in Zimbabwe indicate that HEU children have 30% more sick clinic visits, 20% more hospitalizations (3), and at least twice the infant mortality compared to unexposed children (4). HEU children have decreased linear and ponderal growth, increased rates of other infections, poorer overall nutritional status, and a variety of neurologic abnormalities including motor delays relative to unexposed peers (5, 6). The causal mechanisms underlying these disparities are likely multi-factorial. Potential biologically based factors include lower specific antibody responses at birth (7), differences in cord blood lymphocytes and cytokines (8), and alterations in immunoglobulin levels (7), compared to unexposed children. HEU infants show increased risk of severe anemia following exposure to HAART in utero (9). Changes in breastfeeding practices as a result of maternal infection likely contribute to differences in nutrition and growth, especially in disadvantaged settings (10, 11).

Children with HIV+ mothers may also be vulnerable to a variety of psychosocial adversities, including instability in their primary caregiver's physical and mental health status and inconsistent parental custody (12). HIV-affected children may face stigmatization, and children whose parents have advanced disease may face the severe stressor of a parent's death. Although most HIV-affected children have increased exposure to psychosocial stressors, they vary in their response and susceptibility to these stressors. In healthy and HIV + adults, extensive literature documents how individual psychological and biological factors influence variability in disease progression (e.g. (13, 14)). In non-HIV affected adult and pediatric populations, individual differences in stress response have been associated not only with in vitro immune responses to stress, but also with variance in infectious illness rates (15, 16). Autonomic nervous system (ANS) reactivity has been shown to be a potential mediator between psychological factors such as social inhibition and the progression of HIV-illness (17) and a moderator of associations between adversity and clinical course among children with a chronic disease (18). ANS reactivity has been shown to differentially affect immunocellular responses among HIV+ and HIV− adults following an acute laboratory stressor (19).

Because prior research has identified primary school-entry as a normative developmental stressor capable of inducing immune changes in healthy children (20, 21), the school transition might be particularly evocative of shifts in immune competence and function among HIV-affected children. We hypothesized that HIV serostatus, exposures to environmental stressors, and individual differences in ANS reactivity would directly and/or interactively influence immune responses at primary school matriculation. To our knowledge, no previous research has examined stress reactivity and immune changes in response to a normative transition in a sample of HIV-affected children. Our exploratory hypotheses were that: 1) baseline and post-school entry differences in measures of immune competence and function would be found between groups of HEU and HIV+ children; 2) among HIV-affected children, significant changes in immune parameters would be identified following the school transition; and 3) family stress and children's HIV status would be associated with school entry-related changes in immune measures, and ANS reactivity would moderate those associations.

Methods

Participants

The present sample of 67 children was a subset of a larger study comprising HIV-affected and HIV-unaffected children entering kindergarten or first grade and recruited in four successive years, 1997–2000. The sub-sample was composed of two groups: HIV+ children of HIV+ mothers (N= 38) and HIV− children of HIV+ mothers (N= 29). Participants were identified through the patient registry of the Northern California Pediatric HIV Surveillance Study and were recruited from three local Pediatrics AIDS Clinics. Children with advanced HIV illness (CD4+ counts < 200 cells/mm^3) or other chronic medical conditions were excluded. The study was approved by the University of California, Berkeley and the University of California, San Francisco Institutional Review boards and informed consent was obtained from participant caregivers.

Procedure

Immune changes in response to starting school were assessed at two time points: approximately four weeks before school entry (Time 1) and another four weeks following (Time 2). At the first laboratory session, participants completed ANS reactivity testing and gave a peripheral blood sample to obtain baseline immune measures. Following school entry, participants returned for testing of school readiness, developmental status, and psychiatric morbidities and repeat blood sampling. The child's primary caretaker completed questionnaires assessing demographic and family context information.

Measures

Family adversity—To assess family adversity, the primary caretaker completed multiple, previously validated instruments, including the Major Life Events Questionnaire (derived from (22–24)), Perceived Stress Scale (25), Beck Depression Inventory (26), Chronic Health Conditions Questionnaire (created for this study), Sarason Social Support Questionnaire (27), Moos Family Relationship Index (28), Parental Attitudes toward Childrearing Scale (29), and Personal Lifestyle Questionnaire (30). To derive a composite measure of family adversity, all family context scales were entered into a principal components analytic (PCA) model. Four specific measures formed a single factor with eigenvalues greater than .60: the Major Life Events Questionnaire, Beck Depression Inventory, the maternal Chronic Health Conditions Questionnaire, and the conflict subscale of the Moos Family Relationship Index. Our decision to employ a PCA approach, reducing the number and complexity of the family adversities analyzed, has been used previously (31,32), was driven by our relatively small sample size, and reduced the likelihood of Type I error. In addition to demonstrating statistical coherence, these four measures were theoretically consistent in their focus on cumulative, rather than acute, family adversity. The latter may explain why the more chronic, enduring stressors of parental mood, chronic external stressors, and conflict loaded significantly into the PCA, while other measures such as perceived stress and parental attitudes did not. Using this principal components model, a family adversities factor score was computed as a composite measure comprising these four scales.

ANS reactivity—ANS reactivity was assessed at the first laboratory visit preceding school entry. The 30-minute, standardized laboratory protocol (see (20)) consisted of four ethologically valid challenges for five and six year old children: 1) a child interview from the Gesell school readiness screening test (33); 2) number recall from the Kaufman assessment battery for children (34); 3) lemon juice tasting; 4) emotion-evocative video clips. An automatic, oscillometric Dinamap monitor was used to assess mean arterial pressure (MAP), an integrative measure of sympathetic and parasympathetic activation. The protocol included seven measurements conducted during the stressors and four

measurements during resting. MAP reactivity was computed as a standardized residual score, calculated as the standardized difference between the mean of task measures and the mean predicted for an individual at the sample level by the regression equation y = a+b(x), where x is the mean of resting measures (20). To index the multidimensionality of the MAP responses to protocol (35), ANS reactivity was summarized using a PCA score that combined MAP variance, peak level, and standardized residual score derived from regressing the average of task measures on the average of baseline, control measures (eigenvalues = .80 – .93).

Enumerative and Functional Immune Parameters—Baseline and follow-up measures of immune parameters were conducted in the pediatric immunology research laboratory of co-author (DW) and consisted of two enumerative measures (counts of T lymphocyte CD4+ and CD8+ subsets) and two functional measures (NK cell cytotoxicity and lymphoproliferative responses to tetanus antigen). To obtain these measures, eight milliliters of venous blood were sampled by venipuncture, transferred to heparin tubes, and transported at room temperature to the immunology laboratory. Plasma was collected after slow centrifugation (300 g), and peripheral blood mononuclear cells (PBMCs) were isolated with Hypaque-Ficoll (Pharmacia,P iscataway,N J).

CD4+/CD8+ cell counts: For counts of T lymphocyte subsets, blood was stained within 3 hours of collection. Monoclonal antibodies (10μl)—comprising CD3 (T-cells), CD3/4 (T helper cells), CD3/8 (cytotoxic T cells), and CD56 (natural killer cells)—were added to the appropriate tubes with the subsequent addition of 100μl of whole, heparinized blood to each tube.

NK cytotoxicity: To assess NK cell lytic activity, effector cells were first prepared by isolating PBMCs from heparinized whole blood by Hypaque-Ficoll density gradient centrifugation. The K562 erythromyeloid cell line was used as target cells to detect NK cell-mediated lysis. Serial, two-fold dilutions of the effector cell preparation (5×10^6 cells/ml) were prepared to obtain effector:target cell ratios (E:T) of 50:1, 25:1, 12.5:1 and 6:1 with targets at 1×10^5 cells/ml. Each E:T ratio was replicated in triplicate in well v-bottom plates and the percent Chromium-51 release for each of the E:T ratios was calculated (36). As a means of summarizing NK cell cytotoxicity over all four E:T dilutions, principal components scores were computed for both the pre- and post-school entry values (eigenvalues = .93 – .99).

Lymphoproliferative assay: Lymphoproliferative assays were performed in flat-bottomed 96-well polystyrene microtiter plates (Nunc, Roskilde, Denmark) precoated overnight with tetanus antigen (50/L/well at 10/g/mL) in sterile carbonate/bicarbonate buffer (pH 9.6). Cryopreserved PBMCs were quick-thawed, washed in Hanks' buffered saline solution (HBSS), and re-suspended at 10^6/ mL in RPMI 1640 containing 5% autologous plasma, 10 mM HEPES (Life Technologies), and 50 μg/mL gentamicin (Life Technologies). Each plate contained wells coated with control and tetanus antigens to ensure equal culture conditions. Plates were incubated at 37°C in 5% CO_2 for 6 days before 1 μCi of tritiated thymidine (ICN Radiochemicals, Irvine, CA) was added to each well. Total cellular DNA was collected onto glass-fiber filters 24 h later using an automated harvester, and incorporated counts were measured by beta counter. Lymphoproliferative responses were expressed as counts per minute (cpm).

Statistical Analyses—All statistical procedures were performed using SPSS 17.0 for Windows or PASWStatistics 18 for Macintosh. Oneway analysis of variance was used to examine univariate differences in means between HIV+ and HEU groups. For all four

immune measures, both simple change scores and standardized residual scores (see (37)) were computed for the pre- to post-school entry observation period. In the case of each immune measure, Δ scores and residual scores were highly correlated (rs > .90), and the simpler Δ scores were therefore reported and used in subsequent analyses. For each of the immune change scores, multiple linear regression models were estimated, using HIV serostatus, family adversity, ANS reactivity, and all possible two-way interactions as predictor variables. Each independent variable was centered at its mean (38), and where significant interactions were found, moderator effects were probed using the approach of Aiken and West (39). The technique of Cohen and Cohen (40) of plotting interactions using 1 SD above and below the mean was used for each component variable.

Results

Table 1 shows the demographic characteristics of our 67-child sample, comprising 38 HIV+ and 29 HEU participants. The two groups of HIV-affected children were of similar age and race/ethnicity and had equivalent numbers of boys and girls. There was a statistical trend toward the HIV+ group having families with higher parental education and higher annual incomes (Fs = 3.19 and 3.31, respectively; ps = .08 and.07). These trends may be accounted for by the observation that significantly more HEU children (93%) lived with their biological mothers compared to HIV+ children (45%; $\chi^2 = 17.1$, $p < .001$), likely due to more advanced HIV-related disease among the mothers of HIV+ children. Although no significant relations were found between demographic variables and family adversity or ANS reactivity, subsequent analyses were run with and without adjustment for gender, parental education and residence with the biological mother to preclude confounding of the reported associations.

The full sample had significant school entry-associated change for CD8+ T lymphocytes (M = 921 cells/mm^3 at Time 1 to 1022 at Time 2; in repeated measures ANOVA, F = 11.89, p = .001), but not for CD4+ cells, NK cell cytotoxicity, or tetanus mitogen responses. Table 2 presents baseline and post-school entry immune parameters and immune changes from Time 1 to Time 2 for the two groups. Consistent with studies of healthy school age children (21), we found substantial individual variability in immune response to the normative, school entry stressor. For each immune measure, individuals showed broad ranges of post-school entry changes (e.g.,ΔCD8+ scores of −311 to +1414 and Δ%NK cytotoxicity scores of −5.12 to +2.18; see Table 2), including both down- and up-regulation and both minimal and large shifts in cell counts and immune function. As anticipated, HIV+ children at baseline had significantly fewer CD4+ T lymphocytes (M = 916 vs. 1206; F = 7.8, p = .007), more CD8+ cells (M = 1046 vs. 720; F = 7.98, p = .006), and diminished NK cell cytotoxicity (M =−.29 vs. .41; F = 8.87, p = .004), relative to their HEU counterparts. NK cell cytotoxicity was lower for HIV+ children at all levels of E:T dilution.

Hierarchical multiple regression models examining main and interactive effects of HIV serostatus, family adversity, and ANS reactivity on post-school entry immune changes are shown in Table 3. No consequential changes in the direction, magnitude or significance of the listed coefficients were found on re-computation of these models with controls for gender, parental education and residence with biological mother. For simplicity, we thus report the regression analyses without demographic covariates. HIV serostatus did not emerge as a main effect in any of the models. No significant main or interaction effects were observed for CD4+ cell change scores. In contrast, post-school entry changes in CD8+ cells were associated with a positive, main effect of family adversity (B = 164.53; t = 3.79, p < .001), and changes in tetanus mitogen responses were associated with a positive, main effect of ANS reactivity (B = 9007; t = 3.61, p < .001).

Plots of significant interaction terms, with their components assigned values one SD above and below the mean, are shown in Figures 1–3. Post-school entry changes in CD8+ cells were significantly predicted by the interaction of adversity and ANS reactivity (B = 215.35; t = 3.74, p < .001). As shown in Figure 1, children with low ANS reactivity showed almost no changes in CD8+ enumeration, irrespective of the level of family stress, while those with high ANS reactivity showed substantial differences, in both directions. Specifically, children with high ANS reactivity from low family stress environments had the greatest down-regulation of CD8+ cells, while those from high stress environments had the greatest up-regulation following school entry.

With regard to functional immune measures, HIV serostatus x ANS reactivity interactions were associated with changes in both NK cell cytotoxicity (Figure 2) and tetanus mitogenesis (Figure 3). Specifically, ANS reactivity most strongly differentiated NK cell responses among HEU children: the low reactivity subgroup showing down-regulation of NK cell cytotoxicity and the high reactivity children showing up-regulatory responses. In contrast, ANS reactivity differentiated changes in tetanus mitogen responses in *HIV+* children, with low reactivity individuals showing declines and high reactivity children showing increases in tetanus mitogenesis.

Discussion

This study produced three principal findings. First, the two groups of children, HIV+ and HEU, exhibited expected differences in baseline immune measures according to their HIV serostatus. Prior to school entry, HIV+ children had fewer CD4+ cells, more CD8+ cells, and less NK cell lytic activity than their HEU counterparts. These differences are consistent with current understanding of HIV pathophysiology (41, 42) and with a previous study comparing immune reactivity between HIV+ and HIV− adults (19). The present finding offers replication of previously observed immune differences between HIV+ and HIV− individuals and extends findings to the pediatric population.

Second, and also consistent with previous studies (15, 21), children sustained alterations in immune measures in response to the normative stressor of school entry. Specifically, both HIV+ and HEU children showed increases in CD8+ cell counts after starting school, despite differences in HIV serostatus and baseline immune parameters. The particular immune measure affected differs in cellular specificity from our previous work with HIV-unaffected children, which found increases in CD4+ and CD19+ cells, but not in CD8+ cells. However, the up-regulation in CD8+ cells is consistent with findings in adult samples following an acute laboratory stressor (43, 44). Further, each immune measure showed a wide range of reactivity to school entry, variability in immune response that has now been broadly documented (e.g., (14, 21, 43, 45, 46)). Such differences are likely multi-factorial in origin and may be affected by gene polymorphisms (47, 48), experiences of social subordination (49), and coping styles (50).

The third, most novel, and potentially heuristic finding was the array of interactions among pairs of independent variables. We found a significant interaction between ANS reactivity and family stress, such that children with high reactivity and high stress family environments sustained the greatest up-regulation in CD8+ cells, whereas children with equally high ANS reactivity and low stress family environments showed a down-regulation in CD8+ cells. By contrast, low reactivity children had approximately the same CD8+ cell count responses, irrespective of family adversity. This interaction effect was independent of HIV serostatus and is commensurate with a growing body of work on “biological sensitivity” or “differential susceptibility” to the social environment (see, e.g.: (51–54)). This evolutionary-developmental theory posits an early calibration of stress-responsive,

neurobiological systems, conditional upon properties of the rearing environment, which results in the emergence of a subgroup of children with exceptional sensitivities to both the deleterious and supportive dimensions of social context. These children, sometimes identified by their exaggerated reactivity to stress, show the most or least adaptive developmental and health outcomes, contingent on the psychosocial features of their families, schools or neighborhoods. Studies demonstrating this greater susceptibility of neurobiologically responsive children to both positive and negative aspects of their environments have included a wide variety of: a) stressors, including paternal depression (55), marital conflict (56, 57), parental psychopathology (58), and overall family adversity (59); b) positive environments, including parental warmth (60) and supportive interventions (61); and c) biological markers of susceptibility, including physiologic reactivity (e.g., (20, 62)), differences in brain circuitry (63), and gene polymorphisms (64, 65). Most importantly, highly susceptible children show bidirectional effects on outcomes in contrasting low and high stress settings—not simply an attenuation of negative effects in high stress circumstances. In the present study, independent of HIV serostatus, autonomically reactive, HIV-affected children exhibited the same propensity for extreme, socially contingent outcomes, within an immunological process.

Changes in both functional immune assays—NK cell cytotoxicity and tetanus mitogen responses—were also significantly predicted by interactions, in this case between HIV status and ANS reactivity. Children with high ANS reactivity had the greatest increases in NK cell lytic capacity and tetanus mitogen responses following school entry and those with low ANS reactivity the greatest declines. Such changes occurred primarily among HEU children in the case of NK cell function and among HIV+ children in the case of tetanus lymphoproliferative changes. The divergent configuration of these interactions cannot be accounted for with data available from the present study but may be attributable to HIV-associated differences in NK cell function (e.g., see: (66)) versus lymphoproliferative responses (e.g., see: (67)).

The observed up-regulation in CD8+ cells in both groups may be particularly notable. Since CD8+ cytotoxic cells are essential in the pathophysiology of HIV, one might expect CD8+ cells to behave differently in the two groups. Also, CD8+ cells in HIV+ individuals have shortened telomeres and less proliferative potential, perhaps as a result of replicative senescence (68, 69). The up-regulation of CD8+ cells following school entry is commensurate with the reality that all children with HIV+ mothers have increased vulnerability, whether HEU or HIV+. Biological influences that may be relevant to understanding risks incurred by HIV-affected children include in utero exposure to HAART (70, 71), the immunologic consequences of fetal HIV exposure (72, 73), and differences in breastfeeding and growth patterns (10, 11, 74). Potentially relevant psychosocial factors include stigmatization (75, 76), the adequacy of maternal access to healthcare (77), and maternal stress, substance use, and other psychiatric sequelae during the perinatal period (78, 79).

Although it is impossible to make definitive clinical interpretations of the interaction effects of Figures 2 and 3, there are emerging observations regarding the significance of CD8+ cell activation and NK cell lysis following a stressor. For example, one study comparing HIV+ and HIV− adults had strikingly similar findings: HIV+ adults with larger changes in plasma norepinephrine (NE) following an acute laboratory stressor had greater activation of CD8+ cells compared to those with less NE reactivity and compared to their HIV− counterparts(21). The same study also found that HIV+ individuals had diminished NK cell responses compared to HIV− counterparts and that HIV− individuals showed an association between plasma epinephrine activity and NK cell activity. The convergence of these findings within adult and pediatric populations is persuasive and likely indicates impaired

NK cell immunity in early HIV-related illness. Indeed, a recent paper showed increased CD8+ cell activation and decreased NK cell function as potential mediators between higher levels of psychological distress and greater disease severity in HIV+ adults (80).

The interpretation of these findings must be weighed within the context of several study limitations. Our report has been limited by the lack of an unexposed control group and our relatively small number of study participants. It is possible that a larger sample, offering greater statistical power, might have revealed even more significant associations between independent variables and the examined immune changes; nonetheless, the available sample was sufficient to detect several theoretically important relations that were unlikely to have been attributable to the operation of chance. Children with HIV+ mothers are still experiencing significant family stress (81, 82), but the quality of stress may be different in the era of HAART. The majority of HIV-affected children live outside of the United States, and our data may not be generalizable to that larger population. Finally, the clinical relevance of laboratory immune markers is often unclear. As discussed elsewhere, the specific timing and nature of stressors produce distinct and at times paradoxical immune responses (14, 83), and normative stressors in the genesis of pediatric immune responses are even less understood.

Although methodology and our understanding of the role(s) of immunocellular markers in disease pathogenesis have advanced since our data were collected, this study also has considerable strengths. Our observations extend previous work revealing stress reactivity as an index of biological sensitivity to context to HIV-affected children. Further, the study contributes to a growing literature on the role of biopsychosocial stressors in the increased morbidity and mortality of HEU children. Although this study had relatively small numbers of participants, the identified interactions are unlikely to be due to chance, given that field studies tend to underestimate and under-detect interaction effects (84).

Our findings suggest a broader public health approach to the vulnerable and growing population of HIV-affected children, including services that extend beyond the elimination of MTCT and routine clinical treatment. While extoling the significant reduction of MTCT of HIV globally, we should attend, as well, to the ongoing risks that HIV-affected children face. In efforts to interrupt the intergenerational transmission of adversity-related morbidity in HIV-affected children, new research on the biopsychosocial characteristics that promote resilience in such children should include measurement of relevant immune parameters and their trajectories of change during the developmentally critical first years of life.

Acknowledgments

Funding Acknowledgements: The research on which this manuscript was based was supported by grants to MT from the National Institute of Mental Health and to WTB from the National Institute of Mental Health, the John D. and Catherine T. MacArthur Foundation Research Network on Psychopathology and Development, and the Canadian Institute for Advanced Research.

Glossary

AIDS	acquired immune deficiency syndrome
ANS	autonomic nervous system
E:T	effector:target
HAART	highly active antiretroviral therapy
HBSS	Hanks' buffered saline solution

HEU	HIV-exposed but uninfected
HIV	human immunodeficiency virus
HIV−	HIV seronegative
HIV+	HIV seropositive
MAP	mean arterial pressure
MTCT	mother to child transmission
NE	norepinephrine
NK	natural killer
PBMC	peripheral blood mononuclear cell
PCA	principal components analysis
Δ	delta or change score

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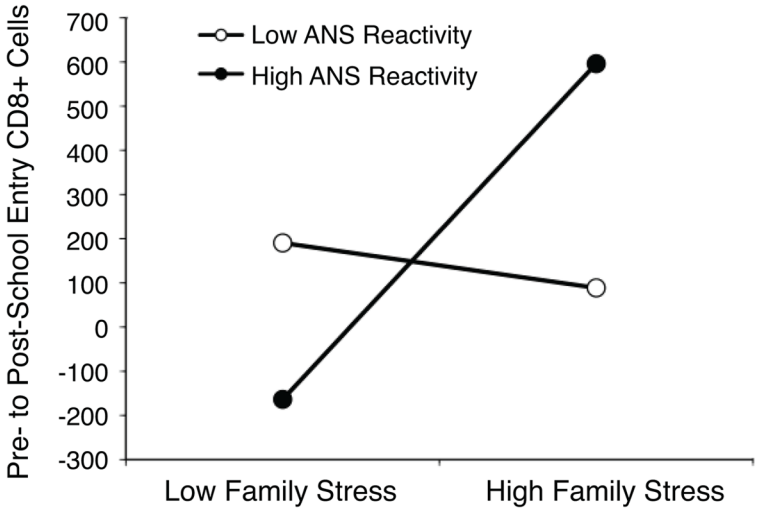

Figure 1.
CD8+ Cell Change by Family Stress and ANS Reactivity (SE of point estimates = ± 58)

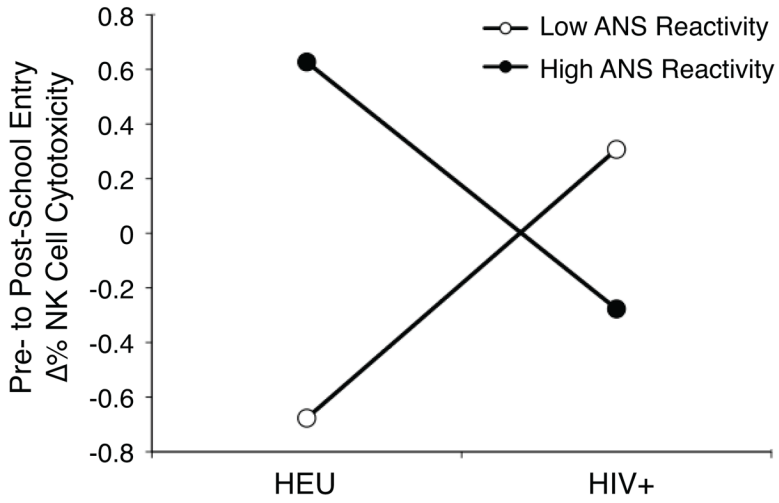

Figure 2.
Natural Killer Cell Lysis Change by Group and ANS Reactivity (SE of point estimates = ± 0.15)

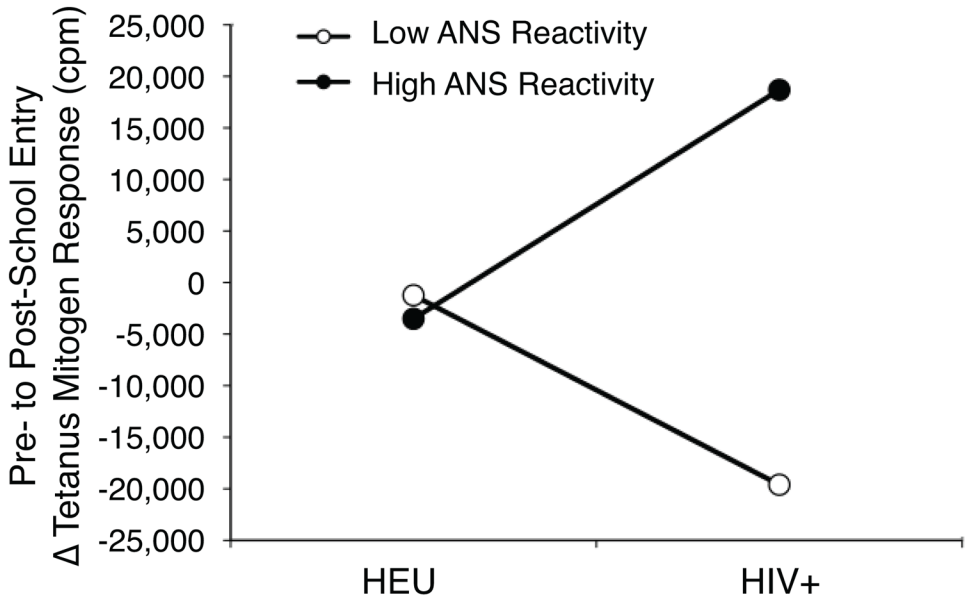

Figure 3.
Tetanus Mitogen Response Change by Group and ANS Reactivity (SE of point estimates = ± 2935)

Table 1

Demographic Characteristics [a]

Participant Characteristics	HIV+	HEU	X^2 [F] (p)
Sample Size	38	29	
Sex:			0.57 (p = .45)
Girls	53%	60%	
Boys	47%	40%	
Age (mean (SD) in years)	5.16 (.64)	5.21 (.56)	[0.11] (p = .74)
Grade			3.33 (p = .07)
K	76%	55%	
1st	24%	45%	
Parent education level (1=some grade school; 2=completed grade school; 3=some high school; 4=completed high school; 5=some college or 2-year college; 6=4-year college graduate; 7=some school beyond college; 8=professional or graduate degree)			[3.19] (p = .08)
Mean (SD)	4.9 (1.6)	4.2 (1.3)	
Range	1–8	1–8	
Annual household income (1=<$10,000; 2=10–14999; 3=15–19999; 4=20–29999; 5=30–39999; 6=40–49999; 7=50–59999; 8=60–69999; 9=70–79999; 10=80–89999; 11=90–99999; 12=>100000; 13=>160000)			[3.31] (p = .07)
Mean (SD)	4.2 (3.5)	2.9 (2.3)	
Range	1–13	1–10	
Race			2.08 (p = .35)
White	18%	10%	
Black	53%	45%	
Other	29%	45%	
Lives with Biological mom	45%	93%	17.07 (p < .001)

[a] Chi-square statistics and ANOVA have been used to test for differences between groups.

Table 2

Comparison of Baseline and Change Immune Measures Between Groups [a]

	HIV+		HEU		F(p)
	Mean (SD)	**Range**	**Mean (SD)**	**Range**	
CD4+Time 1 (cells/mm^3 ×10^3)	916(367)	378–1733	1206 (427)	468 – 2071	7.80 (p = .007)
CD4+Time 2	987 (401)	114–1945	1101 (494)	43 – 2208	1.00 (p = .32)
ΔCD4+Time 1 to 2	97 (278)	−434 – 688	−33 (479)	−1323–928	1.69 (p = .20)
CD8+Time 1 (cells/mm^3 × 10^3)	1046 (510)	475–3273	720 (266)	277 –1390	7.98 (p = .006)
CD8+Time 2	1157 (566)	323 – 3010	830 (470)	308–2116	5.64 (p = .02)
ΔCD8+Time 1 to 2	173 (319)	−311–1090	165 (450)	−422 –1414	0.01 (p = . 94)
% NK cytotoxicity Time 1 (factor score for 4 dilutions)	−.29 (.80)	−1.47–1.95	.41(1.12)	−1.24–4.39	8.87 (p = .004)
% NK cytotoxicity Time 2	−.22 (.89)	−1.39–2.04	.31(1.08)	−1.44–3.05	4.58 (p = .04)
Δ% NK cytotoxicity Time 1 to 2	.05 (.71)	−.78–2.12	−.07 (1.32)	−5.12–2.18	0.20 (p = .66)
Tetanus mitogen response Time 1 (cpm)	11034(15101)	51 – 60228	16267 (12668)	2710–46944	1.54 (p = .22)
Tetanus mitogen response Time 2 (cpm)	11307 (21234)	305 –105183	14985 (11938)	2496–40406	0.48 (p = .49)
ΔTetanus mitogen rspnse Time 1 to 2 (cpm)	344 (21711)	-55959 –83413	−1281 (5549)	−10783 –10064	0.11 (p = .75)

[a] One-way ANOVA tests have been used to analyze differences between HIV+ and HEU groups.

Table 3

Multivariate Regression Analyses Predicting ΔCD4+ Cells, ΔCD8+ Cells, ΔNK Cell Lysis, and Δ Tetanus Mitogen Response[a]

ΔCD4+ cells	**B(SE)**	**T(p)**	**R^2 (model ES)**	**F(P)**
Model Summary			.16(0.19)	1.60 (p = .17)
Main Effects				
HIVSerostatus	−39.60(50.98)	−0.78 (p = .44)		
Family Stress	−67.46(49.12)	−1.37 (p = . 18)		
ANS Reactivity	−75.33(53.37)	−1.41 (p = . 16)		
Interactions				
HIV Serostatus × Family Stress	−62.00(48.61)	−1.28 (p = .21)		
HIV Serostatus × ANS Reactivity	−19.35(65.49)	−0.30 (p = .77)		
Family Stress × ANS Reactivity	−48.39 (65.07)	−0.74 (p = .46)		
Δ CD8+ cells				
Model Summary			.59 (1.44)	4.45 (p = .001)
Main Effects				
HIV Serostatus	−63.73 (45.07)	−1.41 (p = . 16)		
Family Stress	164.53 (43.43)	3.79 (p<.001)		
ANS Reactivity	38.35(47.19)	0.81 (p = . 42)		
Interactions				
HIV Serostatus × Family Stress	−26.37 (42.98)	−0.61 (p = . 54)		
HIV Serostatus × ANS Reactivity	−99.63 (57.91)	−1.72 (p = .09)		
Family Stress × ANS Reactivity	215.35(57.53)	3.74 (p<.001)		
ΔNK cell cytotoxicity				
Model Summary			.45 (.82)	2.50 (p = .03)
Main Effects				
HIV Serostatus	−0.02 (0.13)	−0.16 (p = .88)		
Family Stress	0.05 (0.12)	0.37 (p = .71)		
ANS Reactivity	0.18(0.12)	1.47 (p = . 15)		
Interactions				
HIV Serostatus × Family Stress	−0.02 (0.12)	−0.13 (p = .90)		
HIV Serostatus × ANS Reactivity	0.47 (0.15)	3.09 (p = .003)		
Family Stress × ANS Reactivity	−0.26(0.16)	−1.69 (p = .10)		
ΔTetanus mitogen response				
Model Summary			.59 (1.44)	3.39 (p = .009)
Main Effects				
HIV Serostatus	−955 (2410)	−0.40 (p = .69)		
Family Stress	2346 (2413)	0.97 (p = .34)		
ANS Reactivity	9007 (2498)	3.61 (p = .001)		
Interactions				

ΔCD4+ cells	B(SE)	T(p)	R^2 (model ES)	F(P)
HIV Serostatus ×Family Stress	−4215 (2349)	−1.79 (p = .08)		
HIV Serostatus × ANS Reactivity	−10141 (2934)	−3.46 (p = .001)		
Family Stress × ANS Reactivity	3321 (2719)	1.22 (p = .23)		

[a]Multiple linear regression models were estimated, using HIV serostatus, family adversity, ANS reactivity, and all possible two way interactions as predictor of immune change scores.