Early childhood stress is associated with elevated antibody levels to herpes simplex virus type 1

Elizabeth A. Shirtcliff[a,1], Christopher L. Coe[b], and Seth D. Pollak[c,1]

[a]Department of Psychology, University of New Orleans, 2006 Geology/Psychology Building, New Orleans, LA 70148; [b]Harlow Center for Biological Psychology and Department of Psychology, University of Wisconsin, 22 North Charter Street, Madison, WI 53715; and [c]Waisman Center and Department of Psychology, University of Wisconsin, 1500 Highland Avenue, Madison, WI 53705

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It is well known that children need solicitous parenting and a nurturing rearing environment to ensure their normal behavioral development. Early adversity often negatively impacts emotional and mental well-being, but it is less clearly established how much the maturation and regulation of physiological systems is also compromised. The following research investigated the effect of 2 different types of adverse childhood experiences, early deprivation through institutionalization and physical abuse, on a previously unexplored outcome: the containment of herpes simplex virus (HSV). The presence of HSV-specific antibody in salivary specimens was determined in 155 adolescents, including 41 postinstitutionalized, 34 physically-abused, and 80 demographically-similar control youth. Across 4 school and home days, HSV antibody was higher in both postinstitutionalized and physically-abused adolescents when compared with control participants. Because the prevalence of HSV infection was similar across the groups, the elevated antibody was likely indicative of viral recrudescence from latency. Total secretory Ig-A secretion was associated with HSV, but did not account for the group differences in HSV-specific antibody. These findings are likely caused by a failure of cellular immune processes to limit viral reactivation, indicating a persistent effect of early rearing on immune functioning. The fact that antibody profiles were still altered years after adoption into a more benevolent setting with supportive families suggests these results were not caused by contemporaneous factors, but rather reflect a lingering influence of earlier life experiences.

child development | early experience | health | immune | risk

One of the most significant contributions of psychological research during the last century was the unequivocal demonstration of the extent to which early caregiving influences the behavioral development of children (1). The prevailing view today emphasizes the essential role of solicitous and responsive parenting; we look back in disbelief at historical recommendations to limit time holding an infant because the young baby would become spoiled and desire cuddling (2). Pioneering studies on the hospitalism syndrome and later ones on the biological bases of attachment definitively ended such erroneous notions in the scientific realm (3, 4), but many children throughout the world continue to experience inadequate care. More than 1.5 million children in the United States are victims of substantiated child maltreatment annually, and Americans adopted >20,000 children from other countries in the past year, most of whom had begun their lives in institutional/orphanage settings (5). In this article, we report on the potential lingering consequences of such adverse rearing experiences across development, focusing on biobehavioral plasticity through infection and containment of herpes viruses.

The early rearing environment exerts powerful effects on children's cognitive and emotional development (6–9). Numerous studies in nonhuman animals have also shown that the early rearing environment impacts the maturation of many physiological systems (10–12), including the immune system (13, 14). The development of immune processes in children must be responsive to environmental stimulation and demands early in postnatal life and thus immunity shares many parallels with the effects of experience on brain development. For example, antigenic priming, exposure to infectious pathogens, and behavioral interactions between the infant and the caregiver are all required for immune responses to mature normally (15–17). Experiential factors such as breastfeeding, the occurrence of involuntary separations from parents, and early weaning all can contribute to long-term vulnerabilities to disease later in adulthood (18–22). Similarly, studies of children have shown that family functioning and early life events can influence the frequency of respiratory illness and immune functioning (23–26). In addition, retrospective surveys of adults who had experienced abuse as children indicate that they are more likely to develop chronic pain conditions and gastrointestinal disorders (27–30) and evince a physiological bias toward proinflammatory responses (31, 32).

The present experiment investigated whether an altered regulation of immunity might be evident in young adolescents who had experienced adverse rearing backgrounds. Specifically, we studied a group of postinstitutionalized adolescents who had experienced early caregiving deprivation before adoption into a more normative family context (an "early adversity" group) and a sample of adolescents who had experienced substantiated physical abuse and were still residing within their families of origin (a "current adversity" group). We tested the competence of the adolescents' immune system functioning by determining whether these adolescents showed signs of a reactivation of a latent virus that is typically maintained in a quiescent state by the immune system.

Most individuals have been exposed to several different herpes viruses [e.g., Epstein–Barr virus (EBV), herpes Zoster, cytomegalovirus]. After a period of viremia during the primary infection, immune responses drive the virus into quiescence and most herpes viruses remain latent within nerves. In the case of HSV type 1, the initial infection usually occurs via exposure to virus-shedding family members or in school settings, resulting in infections in ≈20% of children (33). By young adulthood the prevalence of infection reaches >60% (34, 35). Whereas most herpes viruses reactivate only if the host becomes markedly immune suppressed, HSV recrudesces more frequently and is manifest symptomatically as a cold sore. Several pioneering studies documented an association between stress, virus reoccurrence and the appearance of overt clinical symptoms (36–40).

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[1]To whom correspondence may be addressed. E-mail: eshirtcl@uno.edu or spollak@wisc.edu.

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Viral reactivation is caused, in part, by a transient reduction in the cellular immune defenses that should respond to any incipient viral replication. When the virus is unchecked, a rise in antibody titers occurs (even in the absence of overt physical symptoms) as the person's humoral immune responses are elicited (41, 42). Thus, elevated HSV antibodies can serve as an indirect functional measure of a lapse in cellular immune competence (43). Stressful life events have been found to reliably increase the levels of herpes antibody circulating in the blood stream (32, 41, 44). However, the need to collect blood samples poses ethical and practical concerns for evaluations of children, especially those from disturbed family backgrounds.

To overcome this obstacle when assessing sensitive populations, we developed an innovative approach to quantify salivary levels of HSV antibody (45). We also measured the level of total antibody in saliva because the mucosal surface of the oral cavity responds to many pathogens. Thus, we considered the possible differences in HSV antibody in the context of any nonspecific changes in total antibody secretion [secretory Ig-A (sIgA)]. Previous studies had documented that acute stressful events lower total sIgA secretion; in contrast, our a priori prediction was that adolescents who experienced chronic adversity would have elevated levels of HSV-specific antibody, indicating a longer-term impaired immune competence (46, 47).

The present study compared 2 groups of adolescents from disturbed rearing backgrounds. First, we examined adolescents who had experienced a circumscribed period of early deprivation within institutionalized/orphanage settings, but whose adversity was resolved by adoption into a benevolent family setting in the United States. These adolescents are referred to as "postinstitutionalized." Second, we examined adolescents who had experienced more sustained adversity throughout their childhoods; these youngsters had substantiated reports of physical abuse during childhood and continue to reside with their parents. Although interventions from social service agencies have hopefully improved their family conditions, these adolescents continue to live in difficult and stressful circumstances. Studies of child maltreatment always involve numerous risk factors that tend to co-occur within an adverse family setting. To help control for the many possible confounding environmental factors (e.g., poverty, current stress exposure) a reference comparison group was recruited from normative families similar on the basis of age, gender, and socioeconomic status. We also statistically controlled for co-occurring risk factors and other sources of individual differences as detailed in *SI Text*.

These data revealed that a profile of high HSV antibody serves as a sentinel marker of a history of adverse experience during formative development. The elevated antibody levels in the postinstitutionalized adolescents indicate that this biological alteration continues to linger even after the resolution of the period of inadequate care and emotional neglect. Consistent with studies of nonhuman animals, these findings indicate that supportive early rearing conditions are critical for the normal biobehavioral development of children.

Results

Both the postinstitutionalized and physically abused adolescents had elevated HSV levels when compared with the controls, $F(2,109) = 5.57$, $P < 0.005$ (Fig. 1). Despite the improved family conditions for many years, those who had experienced the early institutional rearing and neglect still exhibited high HSV-sIgA in the elevated range as did those who continued to reside in familial settings with an abusive caregiver ($P > 0.8$).

Overall, the HSV-sIgA and total-sIgA measures were positively associated ($r = 0.61$, $P < 0.001$). Importantly, additional analyses verified that the elevated HSV-sIgA levels were not caused by general difference in antibody secretion. After controlling for total sIgA levels, the group differences in herpes antibody remained significant ($P < 0.03$).

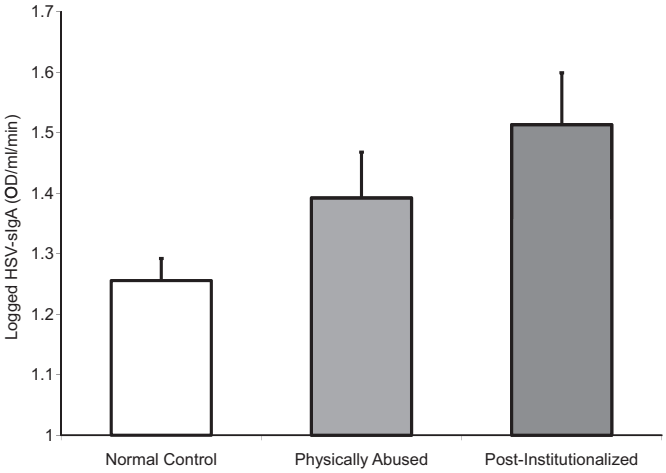

Fig. 1. HSV-sIgA levels were significantly higher in the postinstitutionalized and physically abused adolescents than in age-matched participants who had normative rearing backgrounds.

Because of the many possible explanatory factors, the potential influence of several other variables was also considered. Antibody differences related to the adverse rearing histories remained significant after accounting for age, gender, race, country of origin, familial income, parent education level, child body mass index, child and parent current mental health status, chronic medical conditions, and current level of stress ($P < 0.02$). None of these factors was individually associated with HSV-sIgA level ($P > 0.14$).

Another potential concern was that the findings were driven by elevated antibody levels on a single day rather than providing a more stable index of immune competence. As shown in Fig. 2, secreted HSV-sIgA and total sIgA levels were quite stable when adolescents provided the saliva on school days and on weekend days at home. The stability across days was likely caused in part by our use of daily pools in the assays, which had been combined together from 6 passive drool specimens collected each day.

One additional explanation was that higher HSV exposure rates accounted for the group differences. To test this possibility, the HSV antibody values were categorized dichotomously into negative (optical density values of 0–0.24) and positive (optical density values > 0.25). Seventy-one of the adolescents (66%) were classified as having tested HSV-positive. Neither physically-abused [$\chi^2(1) = 0.60$, $P = 0.32$], nor postinstitutionalized adolescents [$\chi^2(1) = 2.2$, $P = 0.14$], were more likely than the controls to have been classified as HSV-positive. Group differences persisted when analyses were limited to just the subset of youth with positive HSV scores [$F(2,71) = 8.52$, $P < 0.0001$]. Not only did the maltreated youths have higher antibodies than control youth ($P < 0.009$), but the postinstitutionalized adolescents had higher antibodies than the physically-abused adolescents ($P = 0.015$), further underscoring the deleterious consequences of adverse early rearing environments.

Conclusions

The results reported here document that a stressful early childhood history affects the long-term functioning of the immune system, specifically evinced by a secretion of higher levels of HSV-sIgA into saliva. This finding is in keeping with other studies indicating that stressful life events in adults impair immune function (48), including an elevation of other herpes-specific antibodies in circulation, such as EBV (42, 49, 50). The findings are particularly noteworthy because of the clear dem-

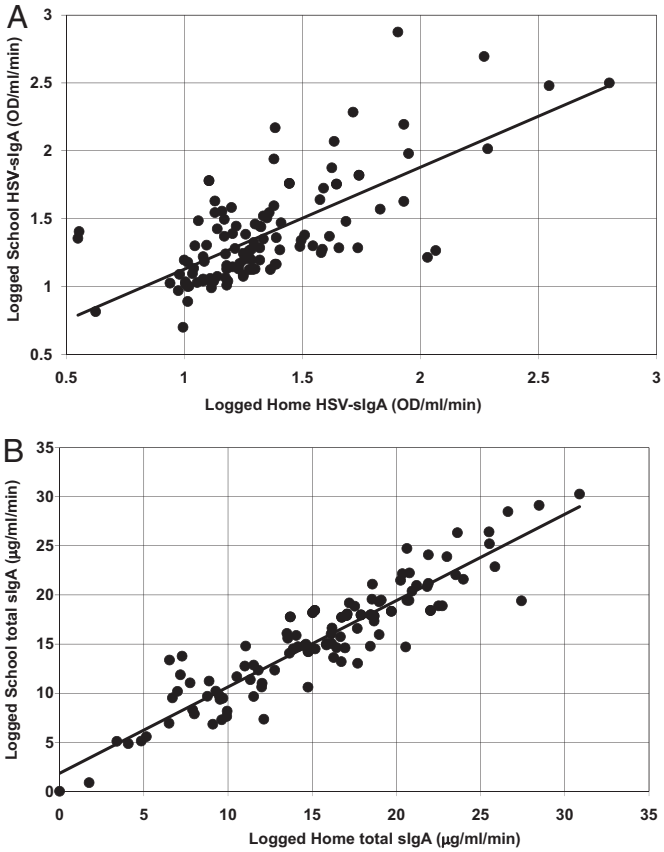

Fig. 2. Levels of secreted HSV-sIgA (*A*) and total sIgA (*B*) were stable across 4 days of collection, including 2 weekend days when the adolescents were at home and 2 school days. Intercorrelations across days ranged from 0.47 to 0.74, $P < 0.0001$, and $\alpha = 0.88$ for HSV-sIgA and from 0.52 to 0.68, $P < 0.001$, and $\alpha = 0.86$ for total sIgA.

onstration that these effects linger even after the resolution of the period of childhood adversity. Although other studies have shown immune consequences of stressors (51, 52), the present study is unique in demonstrating these effects with a pediatric population. In the case of the postinstitutionalized adolescents, they had been adopted into more benevolent family conditions by 2.8 years of age on average. Thus, for many, the period of adversity had been over for nearly a decade before the current assessment.

These immune findings concur with a growing body of literature indicating that there are not only emotional scars and cognitive deficits associated with adverse and inadequate parental care and nurturance, but also an impact on physiological systems and physical health (53, 54). Children from institutional settings suffer from many immune challenges early in development (55), but much less is known about their health after they have been reared subsequently in more benevolent settings. Similarly, abused children exhibit poor health early in development (56), which often persists through adolescence (57, 58) and into adulthood (59, 60). Several papers have recently reported abnormal cellular and humoral immune responses in adults who retrospectively report abuse histories (31, 45).

One alternative explanation for our findings is that institutional and abusive family settings may have resulted in higher levels of infection during childhood. There are many reports of high rates of pediatric illness in orphanages, which may still be evident at the time of adoption (55, 61, 62). In addition, children from poor backgrounds may experience a differential exposure to infectious pathogens, as may those from larger families or in childcare/school settings (34, 63–65). Yet, our analyses indicated a similar overall prevalence of HSV infection across the 3 groups, which at 66% is in keeping with reports of levels of herpes infections in adult populations. This prevalence rate of HSV suggests that differences in levels of antibody more likely reflect functional differences in immune competence rather than differences in rates of exposure in postinstitutionalized or physically-abused youth. This impairment was particularly striking in those postinstitutionalized youth who were found to be HSV-positive, underscoring the idea that institutionalization increases viral recrudescence, not just exposure.

The high antibody levels in the 2 groups who had experienced caregiving adversity were relatively stable across days, evident both on school days and weekend days at home. Evidence for elevated HSV antibodies across several days (which were not usually consecutive) suggests that these youth may be chronically trying to keep the virus from emerging from a latent state. This stability also supports the conclusion that the findings were not secondary to contemporaneous factors and likely reflects the methodological rigor of generating daily pools from 6 specimens each day. This approach diminished the influence of the moment-to-moment variation that occurs in secreted antibody. The overall levels of total sIgA are also indicative of other infectious agents in the oral cavity, including the bacteria associated with gum disease and thus can vary with current events and infections (66, 67). For example, stressful family functioning can be associated with streptococcal infections and the frequency and severity of upper respiratory infections, at least in younger children (18, 24, 25, 46).

Our general conclusion is that early life events are critical for creating the healthy foundation on which both emotional and physical well being is established. Although the adolescents in our study varied greatly in the duration and type of adversity they had experienced, which is a common limitation of studies of at-risk children and adolescents, the HSV-antibody effects were quite consistent. Other alternative explanations may emerge and at this stage we have not delineated all of the pathways and processes that could account for the observed group differences. Although a reactivation of HSV does not have serious clinical ramifications per se, there is a growing belief that the body resources needed to restrain multiple herpes viruses from recrudescing can exert a wear and tear on immune reserves over the course of the life span (68). When normal rearing conditions have been compromised, there may be a reduced capacity to retain herpes viruses in the latent, quiescent state. One additional benefit of good parenting and normal rearing conditions may thus be an enhanced ability to contend with exposure to infectious pathogens, including the ones that linger after the initial infection. In this manner, the susceptibility of the immune system to early caregiving experiences reveals an important aspect of developmental plasticity: Environmental factors promote the normal maturation of the human brain.

Methods

The participants were 155 adolescents (80 male, 75 female), 9–14 years of age (mean = 11.2 years), who were recruited from the surrounding community through local advertisements. Half of the controls were demographically similar to the physically-abused adolescents, and the remaining controls were demographically similar to the postinstitutionalized adolescents in age ($P < 0.42$), gender ($P < 0.48$), and socioeconomic status ($P < 0.28$) (see Table 1). Potential subjects were excluded if they had congenital abnormalities indicative of fetal alcohol exposure, were taking steroidal medications, or had braces or retainers, which might result in blood or bacterial contamination of the saliva. Informed consent and assent were obtained from all parents and adolescents, respectively. All procedures were approved by the University of Wisconsin Institutional Review Board.

Adolescents were classified as having had a history of physical abuse if there were substantiated Child Protective Service reports from Dane County, Wis-

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Table 1. Demographic information

Measure	Control	Physically abused	Postinstitutionalized	Total
N	80	34	41	155
Gender, male/female	40/40	22/12	18/23	155
Race, % white	58	26*†	89*†	57
Age, years	11.2 (0.19)	11.5 (0.31)	11.0 (0.26)	11.2 (0.14)
Body mass index percentile	72.4 (2.9)	84.6 (3.9)†	60.2 (3.8)†	71.3 (2.1)
Socioeconomic status	43.6 (1.9)	31.2 (2.3)†	49.7 (1.7)†	41.8 (1.3)
Recent life stress	15.9 (0.44)	19.6 (0.58)*	18.5 (0.69)*	17.4 (0.33)
Lifetime stress	3.4 (0.23)	6.8 (0.32)*†	4.6 (0.34)*†	4.5 (0.19)
Depressive symptoms	5.3 (1.0)	5.4 (1.1)	6.2 (1.4)	5.5 (0.68)
Anxiety symptoms	10.6 (1.3)	10.6 (1.3)	11.8 (1.9)	10.9 (0.88)
Chronic medical conditions	0.80 (0.13)	0.89 (0.25)*†	1.8 (0.28)*†	1.1 (0.12)
Global physical health	1.4 (0.19)	2.7 (0.44)†	1.3 (0.37)†	1.7 (0.17)
Logged HSV-sIgA	1.3 (0.04)	1.4 (0.08)*	1.5 (0.09)*	1.3 (0.03)
Logged sIgA	3.0 (0.14)	3.1 (0.20)	3.5 (0.27)	3.1 (0.11)

Values are mean ± SEM.
*Values are significantly different from family-reared control group, $P < 0.05$.
†Values are significantly different between the physically abused and postinstitutionalized adolescents, $P < 0.05$.

consin Department of Human Services or if parents self-reported physical abuse of their children through the Conflict Tactics Scale Parent-Child Version ($n = 34$, including 22 males) (69). Postinstitutionalized adolescents had resided in Romanian ($n = 17$), Russian ($n = 13$), other Eastern European ($n = 5$), or Chinese ($n = 6$) orphanages for an average of 2.8 years (range 0.5 to 7 years) from shortly after birth ($n = 41$, including 18 males). These adolescents had resided in their adoptive homes for 3.5–13 years before participation in this study, which ensured that any signs of HSV activation were not caused by recent stress of transitioning into the adoptive home.

Measurement of Immune Competence. Saliva was collected by passive drool at 6 prespecified time points per day across 4 days (70). Collection times were confirmed unobtrusively by using polypropylene cryovials with caps that encoded the closure time (Aardex). Samples were stored initially in home freezers until all 24 were collected, then shipped overnight on ice packs to the laboratory, and stored at −60 °C in an ultracold freezer. To generate a representative daily pool, equal volumes of the 6 samples were combined for that day.

sIgA Assays. Specific salivary antibody against HSV was determined by an ELISA*. Microtiter plates were coated with HSV type 1 (inactivated strain F; ATCC) (Trinity Biotech), which bound HSV antibody, and then the bound complex was detected with goat anti-human sIgA. After addition of alkaline phosphatase substrate (Sigma), the color reaction was stopped with sodium hydroxide at 30 min and read on a Dynatech plate reader at 405 nm. Values are shown on relative scales as optical density units because there are no commercially available IgA standards for HSV antibody. Total sIgA concentrations were determined with a commercial kit (Salimetrics). Antibody levels were adjusted for salivary flow rate (μg/mL per min) (71). Overall, saliva flow was moderately correlated with the total sIgA [$r(157) = 0.24$, $P = 0.003$], and it was nonsignificantly associated with HSV-sIgA [$r(158) = 0.11$, $P = 0.15$]. Antibody values were averaged across the 4 days, and a natural log (+1) transformation established normal distributions.

Co-Occurring Risk Factors. The Hollingshead 4-factor index of social position was used to quantify socioeconomic status. To determine the current levels of stress exposure, adolescents and their parents were interviewed separately by using the Episodic Life Stress Interview (72). Weight/height-squared (kg/m^2) was used to calculate body mass index and then transformed into a percentile for age based on 2000 Centers for Disease Control guidelines. The Revised Children's Manifest Anxiety Scale provided a general measure of anxiety symptoms (73). The Children's Depression Inventory (74) provided a standardized score for depressive symptoms.

See *SI Text* for additional information.

Statistical Analyses. Cronbach's α and Pearson correlations were used to examine stability in antibody levels across days. Group differences were tested by ANOVA, with the immune measures as the dependent variables and group as the between factor. Explanatory or mediating variables were included later as covariates in an ANCOVA model. Differences in the prevalence of HSV-positive status were compared by Pearson χ^2 in a 2 × 3 contingency table.

*Because of the methodological challenges of research with at-risk adolescents, we anticipated that some participants might not collect and return all saliva samples. Therefore, we collected samples from each participant during their initial laboratory visit so that any subjects who did not return a complete set of samples could be compared with those who did. As expected, physically abused adolescents were more likely to have missing home samples [$\chi^2(2) = 10.42$, $P < 0.005$]. However, there was no evidence that participants who failed to collect all home samples differed systematically from those participants who fully complied. All analyses were repeated by substituting the lab-day values for missing values. Postinstitutionalized and abused adolescents still had significantly higher HSV-sIgA than normal controls [$F(2,151) = 4.73$, $P < 0.01$]. All other findings also remained unchanged.

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1. Bowlby J (1988) *A Secure Base: Parent–Child Attachment and Healthy Human Development* (Routledge, London).
2. Watson JB (1928) *Psychological Care of Infant and Child* (Norton, New York).
3. Spitz RA (1945) Hospitalism: An inquiry into the genesis of psychiatric conditions in early childhood. *Psychoanal Study Child* 1:53–74.
4. Ainsworth MDS (1985) Attachments across the life span. *Bull NY Acad Med* 61:792–812.
5. U.S. Department of Health and Human Services (2001) *Child Maltreatment: Reports from the States to the National Child Abuse and Neglect Data System* (U.S. Government Printing Office, Washington, DC).
6. Rutter M, O'Connor TG (2004) Are there biological programming effects for psychological development? Findings from a study of Romanian adoptees. *Dev Psychol* 40:81–94.
7. Cicchetti D, Lynch M (1995) in *Developmental Psychopathology*, eds Cicchetti D, Cohen DJ (Wiley, New York), pp 32–71.
8. Tarullo AR, Gunnar MR (2006) Child maltreatment and the developing HPA axis. *Horm Behav* 50:632–639.
9. Gunnar M, Morison SJ, Chisholm K, Schuder M (2001) Salivary cortisol levels in children adopted from Romanian orphanages. *Dev Psychopathol* 13:611–628.
10. Levine S (2005) Developmental determinants of sensitivity and resistance to stress. *Psychoneuroendocrinology* 30:939–946.
11. Ladd CO, *et al.* (2000) Long-term behavioral and neuroendocrine adaptations to adverse early experience. *Prog Brain Res* 122:81–103.
12. Meaney MJ, Szyf M (2005) Maternal care as a model for experience-dependent chromatin plasticity? *Trends Neurosci* 28:456–463.
13. Ader R (1983) Developmental psychoneuroimmunology. *Dev Psychobiol* 16:251–267.
14. Surtees P, *et al.* (2003) Adverse experience in childhood as a developmental risk factor for altered immune status in adulthood. *Int J Behav Med* 10:251–268.

15. Coe C, Lubach GR, Schneider ML, Dierschke DJ, Erschler WB (1992) Early rearing conditions alter immune responses in the developing infant primate. *Pediatrics* 90:505–509.
16. Levine S, Coe C, Wiener SG (1989) in *Psychoneuroendocrinology*, eds Brush FR, Levine S (Academic, New York), pp 341–377.
17. Bach JF (2002) The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med* 347:911–920.
18. Friedman SB, Glasgow LA (1966) Psychologic factors and resistance to infectious disease. *Pediatr Clinics North Am* 13:315–335.
19. Ader R (1965) Effects of early experience and differential housing on behavior susceptibility to gastric erosions in the rat. *J Comp Physiol Psychol* 60:233–238.
20. Kruschinski C, *et al.* (2008) Postnatal life events affect the severity of asthmatic airway inflammation in the adult rat. *J Immunol* 180:3919–3925.
21. Solomon GF, Levine S, Kraft JK (1968) Early experience and immunity. *Nature* 220:821–822.
22. Labbok MH, Clark D, Goldman AS (2004) Breastfeeding: Maintaining an irreplaceable immunological resource. *Nat Rev Immunol* 4:565–572.
23. Boyce WT, *et al.* (1995) Psychobiologicreactivity to stress and childhood respiratory illness: Results of two prospective studies. *Psychosomat Med* 57:411–426.
24. Boyce WT, Jemerin JM (1990) Psychobiological differences in childhood stress response: I. Patterns of illness and susceptibility. *Dev Behav Pediatri* 11:196–206.
25. Boyce WT, *et al.* (1993) Immunologic changes occurring at kindergarten entry predict respiratory illnesses after the Loma Prieta earthquake. *J Dev Behav Pediatr* 14:296–303.
26. McDade TW, *et al.* (2000) Epstein–Barr virus antibodies in whole blood spots: A minimally invasive method for assessing an aspect of cell-mediated immunity. *Psychosom Med* 62:560–567.
27. Drossman DA (1997) Irritable bowel syndrome and sexual/physical abuse history. *Eur J Gastroenterol Hepatol* 9:327–330.
28. Drossman DA, Talley NJ, Leserman J, Olden KW, Barreiro MA (1995) Sexual and physical abuse and gastrointestinal illness. Review and recommendations. *Ann Intern Med* 123:782–794.
29. Green CR, Flowe-Valencia H, Rosenblum L, Tait AR (2001) The role of childhood and adulthood abuse among women presenting for chronic pain management. *Clin J Pain* 17:359–364.
30. Davis DA, Luecken LJ, Zautra AJ (2005) Are reports of childhood abuse related to the experience of chronic pain in adulthood? A meta-analytic review of the literature. *Clin J Pain* 21:398–405.
31. Danese A, Pariante CM, Caspi A, Taylor A, Poulton R (2007) Childhood maltreatment predicts adult inflammation in a life-course study. *Proc Natl Acad Sci USA* 104:1319–1324.
32. Glaser R, Kiecolt-Glaser JK (1997) Chronic stress modulates the virus-specific immune response to latent herpes simplex virus type 1. *Ann Behav Med* 19:78–82.
33. Tunback P, *et al.* (2003) Prevalence of herpes simplex virus antibodies in childhood and adolescence: A cross-sectional study. *Scand J Infect Dis* 35:498–502.
34. Siegel D, *et al.* (1992) Prevalence and correlates of herpes simplex infections. The population-based AIDS in Multiethnic Neighborhoods Study. *J Am Med Assoc* 268:1702–1708.
35. Xu F, *et al.* (2006) Trends in herpes simplex virus type 1 and type 2 seroprevalence in the United States. *J Am Med Assoc* 296:964–973.
36. Schmidt DD, Zyzanski S, Ellner J, Kumar ML, Arno J (1985) Stress as a precipitating factor in subjects with recurrent herpes labialis. *J Fam Pract* 20:359–366.
37. Luborsky L, Mintz J, Brightman VJ, Katcher AH (1976) Herpes simplex virus and moods: A longitudinal study. *J Psychosom Res* 20:543–548.
38. Cushing H (1905) Surgical aspects of major neuralgia of trigeminal nerve: Report of 20 cases of operation upon the Gasserian ganglion with anatomic and physiological notes on the consequence of its removal. *J Am Med Assoc* 44:1002.
39. Warren SL, Carpenter CM, Boak RA (1940) Symptomatic herpes, a sequela of artificially induced fever. *J Exp Med* 71:155.
40. Perna FM, Antoni MH, Kumar M, Cruess DG, Schneiderman N (1998) Cognitive-behavioral intervention effects on mood and cortisol during exercise training. *Ann Behav Med* 20:92–98.
41. Glaser R, Kiecolt-Glaser J (1994) in *Human Herpesvirus Infections*, eds Glaser R, Jones J (Dekker, New York), pp 245–270.
42. Glaser R, Kiecolt-Glaser JK (2005) Stress-induced immune dysfunction: Implications for health. *Nat Rev Immunol* 5:243–251.
43. Cohen S, Herbert TB (1996) Health psychology: Psychological factors and physical disease from the perspective of human psychoneuroimmunology. *Annu Rev Psychol* 47:113–142.
44. Bonneau RH, Hunzeker JT (2007) in *Psychoneuroimmunology*, ed Ader R (Elsevier, San Diego), pp 1077–1096.
45. Garcia-Linares MI, Sanchez-Lorente S, Coe CL, Martinez M (2004) Intimate male partner violence impairs immune control over herpes simplex virus type 1 in physically and psychologically abused women. *Psychosom Med* 66:965–972.
46. Drummond PD, Hewson-Bower B (1997) Increased psychosocial stress and decreased mucosal immunity in children with recurrent upper respiratory tract infections. *J Psychosom Res* 43:271–278.
47. Sanchez-Martin JR, *et al.* (2001) Social behavior, cortisol, and sIgA levels in preschool children. *J Psychosom Res* 50:221–227.
48. Herbert TB, Cohen S (1993) Stress and immunity in humans: A meta-analytic review. *Psychosom Med* 55:364–379.
49. Freeman ML, Sheridan BS, Bonneau RH, Hendricks RL (2007) Psychological stress compromises CD8+ T cell control of latent herpes simplex virus type 1 infections. *J Immunol* 179:322–328.
50. Kemeny ME, Cohen F, Zegans LS, Conant MA (1989) Psychological and immunological predictors of genital herpes recurrence. *Psychosom Med* 51:195–208.
51. Esterling BA, Kiecolt-Glaser JK, Glaser R (1996) Psychosocial modulation of cytokine-induced natural killer cell activity in older adults. *Psychosom Med* 58:264–272.
52. McKinnon W, Weisse CS, Reynolds CP, Bowles CA, Baum A (1989) Chronic stress, leukocyte subpopulations, and humoral response to latent viruses. *Health Psychol* 8:389–402.
53. Carlson M, Earls F (1997) Psychological and neuroendocrinological sequelae of early social deprivation in institutionalized children in Romania. *Ann N Y Acad Sci* 807:419–428.
54. Gunnar M, Bruce J, Grotevant HD (2000) International adoption of institutionally reared children: Research and policy. *Dev Psychopathol* 12:677–693.
55. Johnson DE, *et al.* (1992) The health of children adopted from Romania. *J Am Med Assoc* 268:3446–3451.
56. Flaherty EG, *et al.* (2006) Effect of early childhood adversity on child health. *Arch Pediatr Adolesc Med* 160:1232–1238.
57. Lansford JE, *et al.* (2002) A 12-year prospective study of the long-term effects of early child physical maltreatment on psychological, behavioral, and academic problems in adolescence. *Arch Pediatr Adolesc Med* 156:824–830.
58. Hussey JM, Chang JJ, Kotch JB (2006) Child maltreatment in the United States: Prevalence, risk factors, and adolescent health consequences. *Pediatrics* 118:933–942.
59. Felitti VJ, *et al.* (1998) Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults. The Adverse Childhood Experiences (ACE) Study. *Am J Prev Med* 14:245–258.
60. Batten SV, Aslan M, Maciejewski PK, Mazure CM (2004) Childhood maltreatment as a risk factor for adult cardiovascular disease and depression. *J Clin Psychiatry* 65:249–254.
61. Coe CL, Lubach GR (2003) Critical periods of special health relevance for psychoneuroimmunology. *Brain Behav Immun* 17:3–12.
62. Albers LH, Johnson DE, Hostetter MK, Iverson S, Miller LC (1997) Health of children adopted from the former Soviet Union and Eastern Europe. Comparison with preadoptive medical records. *J Am Med Assoc* 278:922–924.
63. Melchior M, Moffitt TE, Milne BJ, Poulton R, Caspi A (2007) Why do children from socioeconomically disadvantaged families suffer from poor health when they reach adulthood? A life-course study. *Am J Epidemiol* 166:966–974.
64. Flohr C, Pascoe D, Williams HC (2005) Atopic dermatitis and the ''hygiene hypothesis'': Too clean to be true? *Br J Dermatol* 152:202–216.
65. Satterthwaite D (1993) The impact on health of urban environments. *Environ Urban* 5:87–111.
66. Nikfarjam J, *et al.* (2004) Oral manifestations in selective IgA deficiency. *Int J Dent Hyg* 2:19–25.
67. Teeuw W, Bosch JA, Veerman EC, Amerongen AV (2004) Neuroendocrine regulation of salivary IgA synthesis and secretion: Implications for oral health. *Biol Chem* 385:1137–1146.
68. Lang A, Brien JD, Messaoudi I, Nikolich-Zugich J (2008) Age-related dysregulation of CD8+ T cell memory specific for a persistent virus is independent of viral replication. *J Immunol* 180:4848–4857.
69. Straus MA, Hamby SL, Finkelhor D, Moore DW, Runyan D (1998) Identification of child maltreatment with the parent–child conflict tactics scales: Development and psychometric data for a national sample of American parents. *Child Abuse Neglect* 22:249–270.
70. Shirtcliff EA, Granger DA, Schwartz E, Curran MJ (2001) Use of salivary biomarkers in biobehavioral research: Cotton-based sample collection methods can interfere with salivary immunoassay results. *Psychoneuroendocrinology* 26:165–173.
71. Kirschbaum C, Hellhammer D (1992) in *Assessment of Hormones and Drugs in Saliva in Biobehavioral Research*, eds Kirschbaum C, Read GF, Hellhammer D (Hogrefe & Huber, Gottingen, Germany), pp 67–76.
72. Rudolph KD, *et al.* (2000) Toward an interpersonal life-stress model of depression: The developmental context of stress generation. *Dev Psychopathol* 12:215–234.
73. Reynolds CR, Richmond BO (1978) What I think and feel: A revised measure of children's manifest anxiety. *J Abnormal Child Psychol* 6:271–280.
74. Kovacs M (1985) The children's depression inventory (CDI). *Psychopharmacol Bull* 21:995–998.