Offspring of parents who were separated and not speaking to one another have reduced resistance to the common cold as adults

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Exposure to parental separation or divorce during childhood has been associated with an increased risk for physical morbidity during adulthood. Here we tested the hypothesis that this association is primarily attributable to separated parents who do not communicate with each other. We also examined whether early exposure to separated parents in conflict is associated with greater viral-induced inflammatory response in adulthood and in turn with increased susceptibility to viral-induced upper respiratory disease. After assessment of their parents' relationship during their childhood, 201 healthy volunteers, age 18–55 y, were quarantined, experimentally exposed to a virus that causes a common cold, and monitored for 5 d for the development of a respiratory illness. Monitoring included daily assessments of viral-specific infection, objective markers of illness, and local production of proinflammatory cytokines. Adults whose parents lived apart and never spoke during their childhood were more than three times as likely to develop a cold when exposed to the upper respiratory virus than adults from intact families. Conversely, individuals whose parents were separated but communicated with each other showed no increase in risk compared with those from intact families. These differences persisted in analyses adjusted for potentially confounding variables (demographics, current socioeconomic status, body mass index, season, baseline immunity to the challenge virus, affectivity, and childhood socioeconomic status). Mediation analyses were consistent with the hypothesis that greater susceptibility to respiratory infectious illness among the offspring of noncommunicating parents was attributable to a greater local proinflammatory response to infection.

parental divorce | childhood adversity | adult health | cold susceptibility | inflammation

The past half century has witnessed sweeping cultural changes in the acceptability of parental separation and divorce (1). Consequently, the number of youth whose parents do not live together has increased markedly, with more than one million children in the United States alone being exposed to parental separation each year (2). These observed changes to the traditional family environment evoked questions and concerns among clinicians, social scientists, and public policy makers about the impact parental separation may have on a child's adjustment and well-being. To understand this issue better, numerous studies have documented negative psychosocial outcomes among children whose parents separated, including psychological distress, internalizing and externalizing behaviors, educational deficits, persistent family conflict, household economic disadvantage, and exposure to other adverse childhood experiences (for reviews, see refs. 3 and 4). Moreover, studies also revealed that the consequences of parental separation during early life extend into the adult years. For example, adults who experienced the dissolution of their parents' relationship during childhood are more likely to report lower psychological well-being, higher rates of psychiatric morbidities, and poorer adult relationships (for reviews, see refs. 5 and 6).

Less is known about how childhood parental separation impacts physical health across the life span, although available data suggest adverse consequences. Youth who experience parental separation report more somatic complaints (7), are at increased risk for developing asthma (8), and are more likely to be hospitalized for infectious illnesses (9). Additionally, adults whose parents separated during childhood self-report more medical complaints and poorer perceived health (10) and are more likely to endorse having previously experienced both myocardial infarction (11) and stroke (12). Furthermore, a study using data from a large cancer registry found increased risk for some types of cancer among adults who experienced childhood parental separation (13). Finally, mortality studies document increased risk for premature death, especially from cardiovascular disease, among adults who experienced childhood parental separation (14–16). Taken together, these findings support the notion that childhood parental separation may have a lasting impact on lifespan physical health. However, this work is largely reliant on survey data in which health status is determined via self-report. This reliance on self-reporting makes it difficult to disentangle the extent to which childhood parental separation is associated with objectively determined health outcomes versus the subjective perception of health. Moreover, although data from the cancer registry and mortality records are consistent with an association between childhood parental separation and objectively determined risk for disease in adulthood, the underlying pathophysiological processes remain unclear.

One possibility is that childhood parental separation may increase adult risk for morbidity and mortality in part through stress-related perturbation of immunologic processes. Psychological

Significance

Adults whose parents separated during childhood are at increased risk for poorer health, although the underlying mechanisms remain unclear. Furthermore, increasing evidence suggests that aspects of the family environment following parental separation better predict a child's adjustment than the separation itself. Using a viral challenge study, we found that adults whose parents separated but remained on speaking terms during childhood were no more likely to develop a cold when exposed to a cold-causing virus than adults from intact childhood families. However, adults whose parents separated and did not speak to each other during childhood were more than three times as likely to develop a cold following viral exposure. This increased risk was attributed to heightened inflammation in response to infection.

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stress is associated with dysregulation of immunomodulating systems such as the hypothalamic–pituitary–adrenal axis and the sympathetic–adrenal–medullary system, which are responsible for coordinating the body's response to threat (17). In turn, dysregulation of these systems via psychological stress can engender a proinflammatory phenotype and increase the risk for a broad range of diseases (18). Relatedly, childhood adversity potentiates heightened levels of inflammatory molecules during adulthood (19). Additionally, the illnesses (e.g., asthma, cancer, and cardiovascular disease) disproportionally reported by individuals who experience childhood parental separation are all shaped by ongoing inflammatory activity (20). Taken together, these lines of evidence provide theoretical scaffolding for an immunologic link between childhood parental separation and poorer health outcomes during adulthood.

Research directly connecting parental separation during childhood to deleterious immune outcomes is sparse, although there are some data consistent with this possibility. For example, one study found heightened concentrations of inflammatory molecules among young children whose parents had recently separated (21). Additionally, when comparing responses to a threatening laboratory speech task by young adults whose parents had separated during childhood and individuals from intact families, another study found lower output of the antiinflammatory regulatory hormone cortisol among individuals whose parents had separated (22). Furthermore, in a large longitudinal study that followed participants from birth, researchers documented elevated levels of the inflammatory marker C-reactive protein among individuals in their mid-40s who had experienced parental separation during childhood compared with those who had not (23). However, these studies did not directly evaluate whether parental separation during childhood actually increases disease susceptibility through inflammatory mediators.

Another outstanding issue in the literature is that, although research generally shows a negative association between childhood parental separation and adult health and well-being, the effect sizes tend to be modest, and there is substantial variability in individual outcomes (5, 6, 24). Indeed, increasing evidence highlights that family environment and the quality of parents' relationship with each other following separation may be more important predictors of childhood adjustment than parental separation per se (4). In particular, having parents who can communicate and cooperate in jointly attending to a child's care following separation may protect against adverse psychosocial outcomes in the years following the separation (25–28). Furthermore, compared with individuals who report poorer childhood family environments, individuals who report more family cohesion and less parental conflict following childhood parental separation endorse fewer health complaints and show decreased risk of morbidity and mortality during adulthood (29, 30). These findings indicate that not all forms of childhood parental separation are equally detrimental and suggest that separations marked by conflict and lack of effective communication related to parenting may be particularly impactful to a child's adjustment and subsequent adult health.

In the current project we sought to address these issues using data obtained from 201 healthy adult men and women who participated in a viral challenge study. In this study, participants who were selected for "good health" by medical history, physical examination, and biological profiles answered questions regarding their parents' relationship during childhood. They then were quarantined in a hotel for 6 d. On the first day they were administered nasal drops containing rhinovirus 39 (RV39), a virus that causes the common cold, and then were monitored for 5 d for the development of viral infection and objective physical markers of having a cold. Monitoring included daily collection of nasal secretions for viral isolation and assessing local proinflammatory cytokine production. Assessments of objective cold markers included daily measurements of the individual's total mucus weight and nasal mucociliary clearance function, a marker of nasal congestion. The primary outcome was the development of the common cold. The criteria used to determine cold status were based on methods validated and used extensively in previous viral challenge studies over the past two decades (e.g., refs. 31–33). Specifically, individuals were considered to have a cold if they were infected with the challenge virus and showed increased expression of at least one of two objective markers of illness.

We evaluated whether the status of the parental relationship during the individual's childhood was associated with the individual's differential risk for developing a cold after exposure to the challenge virus. Individuals were sorted into one of three groups capturing childhood parental-relationship status: those who reported that their parents remained together throughout their childhood (the reference category; $n = 109$), those who reported that their parents separated but stayed on speaking terms during childhood ($n = 41$), and those who reported that their parents separated and were not on speaking terms during childhood (parents lived apart and never spoke, hereafter "PLANS"; $n = 51$). We additionally tested whether any observed associations could be accounted for by individual differences in various potentially confounding variables including a set of demographic and study-related covariates referred to as the "standard covariates," i.e., age, sex, race, education attainment, body mass index (BMI), season of the year during which the trial was conducted, and baseline immunity to RV39 (31); personality characteristics that could bias recall (positive and negative affectivity); and childhood socioeconomic status (SES) indicators (childhood parental education and homeownership). We predicted that individuals reporting PLANS would show the highest risk for developing a cold after exposure to the challenge virus.

We also assessed two physiologic pathways through which childhood parental separation might increase susceptibility to developing a cold following exposure to a challenge virus. It is possible that parental separation heightens cold risk by increasing individuals' likelihood of becoming infected after exposure to the challenge virus. Alternatively, childhood parental separation may increase the expression of illness markers in response to infection. To evaluate these possibilities, we examined if childhood parental separation predicted infection in the full sample or markers of illness among individuals who became infected. Finally, the severity of illness markers expressed in response to infection are driven by local proinflammatory cytokine production (34). As such, we also assessed whether the production of nasal proinflammatory cytokines mediated an observed association between parental separation and illness.

Results

Descriptive Information. Participants in this study were an average of 29.76 y old (SD = 10.85). The sample contained 118 (59%) men and 83 (41%) women; 138 (69%) of the participants identified as white; and 63 (31%) of the participants self-identified as nonwhite; 83% of nonwhite individuals identified as black. Ninety-two participants (46%) reported that their parents had separated or divorced during their childhood. Of these, 51 (25%) participants reported PLANS. One hundred forty-nine participants (74%) met criteria for developing an infection with RV39, and 60 (30%) participants met the criteria for a cold (i.e., infection and sufficiently severe objectively assessed markers of illness). For full descriptive information on all of the variables in this study, see *Materials and Methods* or Tables S1 and S2.

Does the Status of the Parental Relationship During Childhood Predict Colds During Adulthood?

Standard covariate-adjusted model. We first used logistic regression to predict cold risk following exposure to the challenge virus as a function of the two childhood parental-relationship status dummy variables indicating having parents who were separated but on speaking terms or PLANS. Participants who reported that their parents were not separated during childhood served as the reference category. We also adjusted the model for the seven standard covariates. Evidence supported an association between childhood parental-relationship status and cold risk [χ^2 with two degrees of freedom, hereafter "$\chi^2(2)$" = 10.489, $P = 0.005$] (Fig. 1). Specifically, individuals whose parents separated but remained on speaking terms during childhood were no more

likely to develop a cold than individuals whose parents remained together during childhood [odds ratio (OR) = 0.839, $P = 0.716$, 95% CI = (0.326, 2.162)]. However, compared with individuals whose parents were together during childhood, those who reported PLANS were more than three times as likely to develop a cold [OR = 3.265, $P = 0.004$, 95% CI = (1.462, 7.290)].

Alternative explanations. We next examined whether the association between childhood parental-relationship status and cold risk remained after controlling for the standard covariates as well as individual differences in two personality characteristics associated with recall biases, positive and negative affectivity. Including positive and negative affectivity in the model did not attenuate the association between childhood parental-relationship status and cold risk [$\chi^2(2) = 9.664$, $P = 0.008$]. As in the previous analyses, those whose parents separated but remained on speaking terms were not at increased risk for developing a cold [OR = 0.938, $P = 0.898$, 95% CI = (0.356, 2.474)], whereas individuals who reported PLANS remained more than three times as likely to develop a cold [OR = 3.260, $P = 0.004$, 95% CI = (1.445, 7.357)].

Next, in a separate model, we tested whether the association between childhood parental-relationship status and cold risk could be attributed to individual differences in childhood SES. In this model, we adjusted for the standard covariates as well as the two indicators of childhood SES, parental education and homeownership. The association between childhood parental-relationship status and cold risk remained evident in this model [$\chi^2(2) = 8.987$, $P = 0.011$]. Once again, individuals whose parents were separated but remained on speaking terms did not show increased cold risk [OR = 0.772, $P = 0.617$, 95% CI = (0.280, 2.128)]. However, the association between reporting PLANS and cold risk persisted [OR = 3.091, $P = 0.011$, 95% CI = (1.295, 7.377)].

Was the Association Between PLANS and Colds Explained by Infection Status or Markers of Illness? Participants were considered to have a cold if they were both infected with the challenge virus and expressed sufficiently severe objective markers of illness.

Infection status. We used logistic regression to test whether the two childhood parental-relationship dummy variables predicted becoming infected after exposure to the challenge virus. Adjusting for the standard covariates, we did not find evidence for an association [$\chi^2(2) = 2.121$, $P = 0.346$].

Markers of illness. We next used logistic regression to test whether childhood parental-relationship status was associated with markers of illness among individuals infected with the challenge virus (as evidenced by meeting the criteria for having a cold once infected). After adjusting for the standard covariates, childhood parental-relationship status was associated with illness markers among infected individuals [$\chi^2(2) = 6.918$, $P = 0.031$]. Compared with individuals whose parents remained together throughout childhood, those whose parents separated but remained on speaking terms did not show differential markers of illness [OR = 0.865, $P = 0.785$, 95% CI = (0.306, 2.449)]. Conversely, individuals who reported PLANS were more likely to show sufficient markers of illness to meet criteria for a cold [OR = 2.957, $P = 0.017$, 95% CI = (1.210, 7.230)].

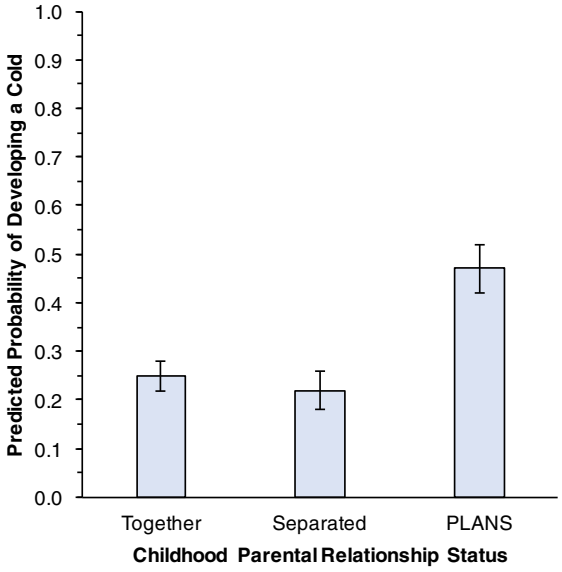

Fig. 1. Predicted probability of developing a cold as a function of whether participants reported that, during their childhoods, their parents were not separated or divorced (Together; $n = 109$), were separated or divorced but were still on speaking terms (Separated; $n = 41$), or were separated or divorced and not on speaking terms (PLANS; $n = 51$), adjusted for the seven standard covariates. Error bars represent 95% CIs for the mean predicted probabilities of developing a cold.

Was Increased Illness Among Individuals Reporting PLANS Mediated by Local Proinflammatory Cytokine Levels? Because individuals whose parents separated but remained on speaking terms did not differ in clinical illness risk from those whose parents remained together, we collapsed these two groups for the mediation analyses. We evaluated whether the association between PLANS and increased illness was mediated by local levels of the proinflammatory cytokines TNF-α, IL-1β, and IL-6. Because of strong correlations among the three cytokines, we constructed a single composite nasal inflammation variable by calculating and then summing *z*-scores for TNF-α, IL-1β, and IL-6 (e.g., ref. 35). Adjusting for the standard covariates, we found that reporting PLANS predicted higher composite nasal inflammation [regression coefficient (b) = 0.943, $P = 0.023$, 95% CI = (0.130, 1.755)]. We then used bootstrapping techniques (36, 37) to assess the indirect effect of the composite inflammation variable on the association between reporting PLANS and the criteria for a cold. Adjusting for the standard covariates, we found evidence that an increase in the composite inflammation variable mediated the association between PLANS and greater risk for clinical illness [$b = 0.401$, 95% CI = (0.001, 1.079)].

Finally, as an alternative test of mediation of the association between PLANS and clinical illness, we fit a structural equation model in which the three cytokines were loaded onto a single latent factor for inflammation. In this model, the observed variable PLANS predicted the latent factor for inflammation, which in turn predicted whether the individual met the criteria for a cold. After adjusting for the standard covariates, the model fit the data well [$\chi^2(18) = 24.676$, $P = 0.134$; standardized root mean square residual (SRMR) = 0.024; confirmatory fit index (CFI) = 0.978; root mean square error of approximation (RMSEA) = 0.043; 90% CI for RMSEA = (0.000, 0.082); $P(\text{RMSEA} \leq 0.05) = 0.568$]. For the individual regression paths, there was a positive association between PLANS and the latent factor for inflammation [$b = 2.404$, $P = 0.051$, 95% CI = (−0.009, 4.817)] as well as between the latent factor for inflammation and colds [$b = 0.033$, $P < 0.001$, 95% CI = (0.019, 0.047)]. There also was evidence for a direct association between PLANS and colds [$b = 0.160$, $P = 0.016$, 95% CI = (0.031, 0.289)]. A graphical depiction of this model is shown in Fig. S1.

Discussion

In this study, we found that individuals whose parents separated during childhood but remained on speaking terms were no more likely than those whose parents remained together to develop a cold during adulthood in response to exposure to a challenge virus. However, individuals who reported PLANS were more than three times as likely to develop a cold during adulthood after exposure to the challenge virus as individuals from intact families. Moreover, this finding persisted even after analyses were adjusted for a variety of potentially confounding variables.

Our finding that PLANS but not parental separation with continued communication between parents predicted increased cold risk converges with literature showing that adverse family processes related to parental separation are more potent predictors of negative outcomes than parental separation alone.

Indeed, on a conceptual level, it is likely that PLANS reflects a number of deleterious childhood family dynamics occurring before and after parental separation. For example, having parents who are separated and not on speaking terms with each other suggests high levels of acrimony in the childhood family environment. Although conflict and rancor in the family environment are associated with negative outcomes among youth regardless of whether their parents are separated (28), such conflict is also an important pathway through which parental divorce may negatively impact individuals both during childhood and in their adult years (38). Additionally, parents who are not on speaking terms might be more prone to recruit the child to serve as an intermediary or attempt to get the child to take sides against the other parent. These behaviors in turn are associated with increased distress and poorer adjustment among youth (39, 40). Furthermore, cooperative communication between parents regarding childcare is associated with better psychosocial and behavioral outcomes among youth and may protect individuals against at least some of the negative consequences of parental separation (25, 28, 41). However, if a child's parents are not speaking to each other, there is no possibility for such collaboration in attending to the child's needs. Thus individuals whose parents are separated and not on speaking terms during childhood may be exposed to ongoing family processes that interfere with effective parenting, that convey threat, and that increase stress. Importantly, this type of environment can stymie a child's ability to adjust to parental separation (24, 42) and can increase the risk for future morbidity (43, 44).

We also evaluated two physiological processes through which PLANS may have increased cold risk. To meet the criteria for having developed a cold after exposure to a challenge virus, individuals must both become infected with the virus and exhibit excessive objective markers of illness related to the infection. Expression of these illness markers is driven largely by local proinflammatory activity (34). Although we did not find evidence that PLANS predicted infection, it did predict clinical illness among infected individuals. Furthermore, the association between PLANS and clinical illness was mediated by local proinflammatory cytokine expression as evinced by a model evaluating the indirect effect of the composite inflammation variable and by a structural equation model in which the three individual cytokines were loaded onto a latent factor for inflammation. These findings corroborate and extend previous studies documenting poorer regulation of inflammation and increased levels of inflammatory molecules among adults who experienced childhood adversity related to parental separation (e.g., refs. 22, 23). Mechanistically, these findings are also consistent with more general models of stress-induced reductions in immune cells' sensitivity to the inhibitory effects of glucocorticoids (e.g., ref. 45). Glucocorticoids play a pivotal role in regulating the immune system's inflammatory response to viral infection. However, stressful experiences marked by long-term threat can result in local immune cells becoming desensitized to regulation by glucocorticoids. This resistance to glucocorticoids is in turn associated with increased production of proinflammatory cytokines in response to infection and greater risk for developing a cold following a viral challenge (33). Additionally, it is possible that experiences such as PLANS may modulate the regulation of autonomic nervous system processes, resulting in exaggerated sympathetic versus parasympathetic effects on the system, which in turn are associated with increased inflammation and morbidity (46).

This study has several strengths. Most notably, the unique viral-challenge paradigm provides a sensitive assessment of immunocompetence by measuring host resistance to a challenge with a common viral pathogen. Furthermore, the prospective nature of the study enabled us to rule out the possibility that the cold itself influenced participants' reports of their parents' relationship during childhood. Additionally, the 11 covariates were chosen to eliminate the possibility that the associations we found were attributable to their impact on both parental-relationship status and cold susceptibility. Nonetheless, there are some potential limitations to consider. First, the viral-challenge paradigm would be extremely difficult to implement in a longitudinal prospective study, and hence data regarding childhood parental separation and PLANS were obtained retrospectively. Although we statistically controlled for individual differences that could bias recall, it is always possible that some other unmeasured characteristic could account for our findings. Second, although the strict recruitment criteria for this sample allow us to rule out a number of potential third-variable explanations (e.g., medical and psychiatric illnesses, prescription medication use), they also restrict our ability to examine other potentially deleterious life outcomes that might correlate with PLANS in a more heterogeneous sample. Furthermore, the age range of our sample was somewhat restricted. Thus future studies will be needed to examine if our findings replicate in other populations, especially those that are older and less healthy. Third, to determine cold status, we relied on both evidence of infection and objective markers of illness. Although there is substantial evidence for the validity of this approach (e.g., refs. 31–33), these illness markers do not cover the range of indicators used in the self-reported symptom measures. As such, it is possible that the objective markers are not tapping all manifestations that physicians and patients may view as colds (for further information regarding subjective symptoms, please see *SI Text* and Table S3). Fourth, when examining residual versus fitted value and quantile– quantile (Q–Q) plots, there was some evidence that the portion of our mediation model predicting the composite inflammation variable may not have met the underlying assumptions of normality of errors and homoscedasticity of residuals. Although deviations from normality are less of a concern because of the use of nonparametric bootstrapping, heteroscedasticity of the residuals could bias the SEs and thus affect inferences. Indeed, when applying a power transformation to the composite inflammation variable or when analyzing the original data using heteroskedasticity-consistent-3 robust SEs, the mediation findings were attenuated. Thus the mediation findings may be less robust and should be replicated. Finally, our assessment of mediation by cytokines was correlational, precluding a causal interpretation.

Notwithstanding these limitations, this study provides theoretical insights regarding the association between childhood parental separation and adult health. Using a prospective viral challenge paradigm, we found that individuals who reported that their parents had separated or divorced during childhood but remained on speaking terms were no more likely to develop a cold after exposure to a challenge virus than individuals from intact families. However, individuals whose parents were separated and not on speaking terms during childhood were more than three times as likely to develop a cold following exposure to the challenge virus, highlighting poorer immunocompetence among these individuals. Furthermore, this association appeared to be attributable to increased expression of illness markers among infected individuals and was mediated by heightened levels of local proinflammatory cytokines. Given the wide prevalence of parental separation and divorce and limited intervention resources, these findings have important implications for better understanding who is at increased risk for deleterious health consequences following parental separation.

Materials and Methods

Participants. Participants for this project were from a study of how socioenvironmental factors influence host resistance to an infectious illness. Through the auspices of the NIH, these data (and data from four other viral-challenge studies conducted by our group) are available online (www.commoncoldproject.com). For this project, 212 healthy volunteers, age 18–55 y, were recruited between 2007 and 2011 from the greater Pittsburgh area. Individuals were excluded from participation if they were not deemed to be in "good general health" as determined through a medical history, physician-conducted physical examination, and clinical profiles via urinalysis, complete blood cell count (CBC), and analysis of blood chemistry. Specifically, volunteers were excluded if they had a history of psychiatric illness, major nasal or ontological surgery, asthma, or cardiovascular disease; had abnormal urinalysis, CBC, or blood enzymes; were pregnant or currently lactating; were seropositive for HIV; were taking regular medications other than oral contraceptives (women only); or if they reported experiencing

cold/flu symptoms within 30 d of quarantine. To maximize infection rates, volunteers were also excluded from the study if a blood sample taken 4 wk before the physical examination revealed antibody titers ≥4:1 for RV39. Each participant received $1,000 as an honorarium for taking part in the study. The Institutional Review Boards at Carnegie Mellon University and the University of Pittsburgh approved this study, and all participants provided informed written consent. Only individuals with complete data regarding childhood parental-relationship status were included in the current analyses, providing a final sample size of 201.

Procedures. Upon being enrolled into the study, participants filled out questionnaires regarding demographic (including age, sex, race, and education attainment) and psychosocial (including trait positive and negative affectivity) factors. Approximately 8 wk after this assessment, participants provided a sample of blood that was used to quantify prechallenge (baseline) antibody titers to RV39. At this session, they also answered questions regarding their childhood SES and parental relationships. Two to three days later, participants were quarantined in a hotel for 6 d. On the afternoon of the first day, participants were administered nasal drops containing RV39. On all 6 d of quarantine participants were monitored for the development of viral infection and objective markers of illness. This monitoring included taking daily collections of nasal secretions for viral isolation via culture and for assessing proinflammatory cytokine production and daily measurements of individuals' total mucus weight and nasal mucociliary clearance function. Four weeks later, participants provided a final blood sample that was used to test again for antibodies to RV39. Individuals also participated in two laboratory stress-reactivity sessions that were unrelated to the current research question. Relevant to the current project, during these sessions individuals' height and weight were measured via a balance scale and stadiometer, and these values were used to calculate their BMI. The BMI was highly stable between the two reactivity sessions ($r = 0.991$, $P < 0.001$). Therefore we averaged the two measurements for use in analyses.

Measures.

Parental relationships during childhood. Childhood parental separation or divorce status was ascertained by asking participants if their parents had separated or divorced during the participant's childhood or adolescence. Participants who responded affirmatively were asked how old they were when their parents separated, and individuals indicating they were ≤18 y old were considered to have experienced childhood parental divorce or separation. Additionally, participants were asked "how often did your parents argue" in reference to when they were age 5, 10, and 15 y separately. Responses included "all of the time," "most of the time," "some of the time," "never," and "not sure." Participants could also respond with "my parents lived apart and never spoke" (PLANS). We then calculated a binary variable indicating whether the parents of each participant had ever lived apart and not spoken during the individual's childhood. For this variable, we assigned participants a value of 1 if they indicated PLANS at age 5, 10, or 15 y. We assigned a value of 0 to all other responses, including "not sure." Across the three ages, 42 responses (7.0%) were "not sure." We then constructed two dummy codes indicating parental separation/divorce either with communication or PLANS. All participants who reported PLANS also reported contact with each parent for at least one of the three ages queried. Individuals whose parents remained together throughout childhood served as the reference category.

Objective disease outcomes. The primary outcome for this project was the development of a common cold. An individual's cold status was determined based on both evidence of viral infection and sufficiently severe objective markers of clinical illness (31). Viral infection occurs when a virus is able to invade a host and replicate. Viruses such as RV39 that replicate in the upper respiratory system can be detected in nasal secretions. Therefore samples of participants' nasal secretions were collected each day during quarantine via a saline wash of the nasal passages. These samples were centrifuged at 2,060 × *g* for 15 min to remove mucus, and debris and a 1.35-mL aliquot of the resulting lavage fluid was mixed with 0.45 mL of viral collection broth. They then were stored in a freezer at −70 °C until they were cultured for RV39 following standard protocols (47). Additionally, the immune system responds to viral infection by producing antibodies to the virus. An increase in these virus-specific antibodies can be used as a marker of infection. Thus, virus-specific antibody titers were assessed in serum via blood draws 2 or 3 d before and 28 d after exposure to RV39 (47). Individuals were considered to be infected if the virus could be detected in culture in any of the five postchallenge days or if a fourfold or greater rise in virus specific antibody titers was observed between the prechallenge and 28-d postchallenge exposure assessments (31).

Two objective markers of upper respiratory illness were also assessed: nasal mucus production and mucociliary clearance function. To determine daily mucus production, used facial tissues were collected in sealed plastic bags each day. Daily mucus weight was subsequently obtained by weighing the bags containing the soiled tissues and subtracting the predetermined weight of the tissues when clean and the bags. Mucociliary clearance function is an indicator of how effectively cilia located in the nasal passageways clear mucus through the nose toward the throat. Mucociliary clearance function was measured by administering a sweetened-dyed solution into the anterior portion of the nose and timing how long it took for the participant to be able to taste the solution (48). Baseline-adjusted daily scores were generated for both mucus weight and clearance function. To generate these scores, values obtained the day before the administration of the viral challenge were subtracted from each of the five daily postchallenge scores (31). Finally, the variable for total mucus weight was generated by summing the average adjusted daily weights across each of the five postchallenge quarantine days (mean = 9.98, SD = 17.32). Likewise, the variable for average mucociliary clearance function was computed by taking the mean of the adjusted daily clearance function scores over the five postchallenge quarantine days (mean = 3.78, SD = 3.93).

Participants met criteria for having a cold if they were (*i*) infected with the challenge virus and (*ii*) showed increased expression of illness markers as evidenced by either a total baseline-adjusted mucus weight of at least 10 g or an average baseline-adjusted nasal mucociliary clearance time of at least 7 min (31).

Nasal inflammatory markers. The inflammatory cytokines IL-1β, IL-6, and TNF-α were quantified in nasal secretions using commercially available ELISAs (Endogen). These assays were performed in duplicate following the manufacturer's instructions. The minimum detection thresholds for the assays ranged from 0.02 to 0.04 pg/mL, and the inter- and intra-assay variabilities were less than 10%. The nasal cytokine responses to viral exposure were calculated as the log-transformed area under the curve (AUC) for the five postexposure days adjusted for the baseline day. Participants' levels of IL-1β (mean = 31.09, SD = 41.82), IL-6 (mean = 58.83, SD = 80.00), and TNF-α (mean = 5.95, SD = 9.72) in nasal secretions were highly intercorrelated [r(IL-1β, IL-6) = 0.65, $P < 0.001$; r(IL-1β, TNF-α) = 0.59, $P < 0.001$; r(IL-6, TNF-α) = 0.53, $P < 0.001$]. As such, we computed a composite measure of nasal inflammation for use in data analyses by *z*-scoring participant values on each of the cytokines and then summing them (for an example of another study that has used this approach, see ref. 35). Three individuals were missing cytokine data. Therefore analyses involving the cytokine data had a sample size of 198.

Standard covariates. We included the following seven covariates in all adjusted analyses: self-reported age, sex, race (coded as white or not-white because of the small number of nonblack racial groups represented), educational attainment (in years; mean = 14.10, SD = 1.91), objectively assessed BMI (calculated as kilograms per square meter; mean = 27.35, SD = 6.36), season of the year (via two dummy codes to represent winter, $n = 55$; spring, $n = 67$; and summer, $n = 79$; spring was the reference category), and baseline immunity to RV39 (coded as ≥4:1, $n = 41$; or <4:1, $n = 160$). These covariates were chosen a priori mirroring previous analyses by our group using data from viral challenge studies (e.g., ref. 31).

Psychological covariates. Negative affectivity has been linked to recall of negative experiences, whereas positive affect has been linked to recall of positive experiences (49). To control for the potential confounding of trait affectivity in our analyses, we evaluated neuroticism and trait positive and negative affect.

Neuroticism was assessed using the International Personality Item Pool (IPIP) Big-Five Factor Markers (50). For this measure, participants indicated how accurately each of 10 statements indicating emotional stability (e.g., "I seldom feel blue") described them in general on a scale from 0 (very inaccurate) to 4 very accurate). Item responses were reverse scored and summed so that higher values indicated higher neuroticism (mean = 15.31, SD = 7.72). Internal consistency for this scale was satisfactory (Cronbach's $\alpha = 0.82$).

Trait positive and negative affect were assessed using the Positive and Negative Affect Schedule-Expanded Form (51). In this measure, participants rated the extent to which 10 positive and 10 negative mood-related adjectives described how they generally felt on a scale from 0 (very slightly or not at all) to 4 (extremely). The two sets of 10 items were each summed to give total scores for positive (mean = 34.96, SD = 6.91) and negative affect (mean = 16.53, SD = 5.66). Internal consistency for both domains was satisfactory (Cronbach's $\alpha = 0.86$ for both domains). Trait negative affect and neuroticism were strongly correlated ($r = 0.691$, $P < 0.001$). Therefore we *z*-scored and summed the trait negative affect and neuroticism variables to create a composite measure of negative affectivity for use in analyses.

Childhood SES. We assessed two indicators of each participant's childhood SES during the first 18 y of life: parental education and homeownership. To obtain parental education, participants were asked to recall which of nine levels of education (for details, see Table S1) each parent had achieved by

the time the participant was 5, 10, and 15 y old, and the maximum value from either parent was used. Because of small cell sizes for some of the nine categories, we collapsed parental education into four categories for analysis (high school or less, $n = 70$; some college, $n = 40$; college degree, $n = 46$; graduate/professional degree, $n = 45$). Parental homeownership was assessed by asking participants to indicate for each year of the first 18 y of their lives whether their parents owned their own home. This variable was expressed as the percentage of years that participants endorsed their parents owning a home (mean = 68.98, SD = 37.92).

Data Analyses. The data analyses reported here were all preplanned for this project. Analyses were conducted using the software package SPSS (52). For logistic regressions we report Wald tests of the childhood parental-relationship status dummy variables (with individuals whose parents remained together during childhood serving as the reference category) distributed as χ^2 with two degrees of freedom and the OR. For linear regressions we report the unstandardized regression coefficients (*b*). For these tests, we present the two-tailed *P* values as well as the 95% CIs for the ORs and regression coefficients.

To test simple mediation of the association between PLANS and clinical illness by the composite inflammation variable, we used the PROCESS macro for SPSS (37) to obtain an estimate of the indirect effect along with a corresponding bootstrapped 95% CI. Following recommendations described by Biesanz, et al. (36), this CI was constructed using the percentile bootstrap based on 50,000 resamples. We also fit a structural equation model using the Lavaan package for R (53, 54) as an alternative evaluation of whether nasal inflammation mediated the association between PLANS and clinical illness. In this model, we used the observed variable PLANS to predict a latent factor for nasal inflammation based on the three cytokine variables, which in turn predicted the observed variable for colds. We allowed the nasal cytokine error terms to covary with each other and adjusted the model for the standard covariates. The model was evaluated using the maximum likelihood estimator and probit link function. We present the χ^2 test for model fit, as well as the fit indices SRMR, CFI, and RMSEA. For RMSEA, we provide the 90% CI as well as the *P* value for testing the hypothesis of close fit (RMSEA ≤ 0.05). We also report unstandardized regression coefficients (*b*) and corresponding two-tailed *P* values and 95% CIs.

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