

Psychological distress, killer lymphocytes and disease severity in HIV/AIDS

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Abstract

Immunochemical mechanisms that account for the association between psychosocial risk factors and increased susceptibility to faster progression of HIV/AIDS are largely unknown. This study used structural equation modeling to test the hypothesis that enumerative and functional alterations in killer lymphocytes mediate the relationship between higher levels of psychological distress (defined by perceived stress, anxiety and depressive symptoms) and greater HIV disease severity (defined by HIV-1 viral load and T-helper (CD4⁺) cell count), independent of standard demographic and various HIV-related covariates. Participants were 200 HIV-1 seropositive adults on combination antiretroviral therapy (ages 20–55 years; 67% men; 62% black; 84% AIDS). The data fit a psychoimmune model in which the significant relationship between higher distress levels and greater disease severity was mediated by diminished natural killer (NK) cell count and cytotoxic function, as well as increased cytotoxic (CD8⁺) T-cell activation. Overall the findings indicated that the psychoimmune model accounted for 67% of the variation in HIV disease severity. In contrast, the data did not support a reverse directionality mediation model, where greater HIV disease severity predicted greater distress as a function of killer lymphocyte status. In sum, the psychoimmune associations of the final model are physiologically consistent and suggest that distress-related alterations in killer lymphocyte immunity may play a role in the biobehavioral mechanisms linked with HIV-1 pathogenesis.

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1. Introduction

A growing body of evidence supports an association between specific psychological variables and greater sus-

ceptibility to HIV disease progression (Cole and Kemeny, 2001; Ironson et al., 2005b; Kemeny, 2003; Leserman, 2003b). However, the immunocellular mechanisms that mediate the relationship between psychological factors and indices of HIV disease severity remain largely unknown (Kemeny, 2003; Kopnisky et al., 2004; Segerstrom and Miller, 2004; Sloan et al., 2007). HIV infects immune cells with CD4 surface receptors, primarily T-helper cells, and disease progression is signaled by a T-helper cell count decrement, an HIV viral load expansion, and the occurrence of clinical symptoms of

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immunodeficiency (Vergis and Mellors, 2000). The severity of disease is indicated by the magnitude of viral load expansion and T-helper cell count depletion, and by the type of clinical conditions that have been diagnosed (Centers for Disease Control and Prevention, 1992). In HIV spectrum disease there may be a long interval, possibly 10–20 years, between initial infection and the onset of serious immunodeficiency symptoms during which HIV viral replication is largely restrained (Vergis and Mellors, 2000). During this period of clinical latency, millions of HIV virions may be produced and eliminated by the immune system daily (Ho et al., 1995). This rather remarkable feat is accomplished, in part, by the facilitation of cytotoxic or “killer” lymphocytes, including Natural killer (NK) cells and cytotoxic (CD8⁺) T-cells (Dines et al., 2004; Fortis and Poli, 2005; Gandhi and Walker, 2002; Levy et al., 2003; Whiteside and Herberman, 1994). Notably, poorer functional responsiveness of NK and cytotoxic T-cells has been associated with greater risk of progressive HIV immunodeficiency and mortality (Ullum et al., 1999).

Both NK and cytotoxic T-cells have the capacity to kill HIV-infected cells directly (Gandhi and Walker, 2002; Whiteside and Herberman, 1994). The NK cells, in addition to serving as the primary cytotoxic effectors of the innate immune system anti-viral response, are also instrumental in coordinating the adaptive immune system anti-viral response (Fortis and Poli, 2005; Whiteside and Herberman, 1994). The latter response may include the direct activation of cytotoxic T-cells via secretion of interferon (IFN)- γ , as well as the indirect action of soluble factors such as β -chemokines, which inhibit viral entry into T-helper cells by CCR5 coreceptor antagonism (Fortis and Poli, 2005; Kottlilil et al., 2003; Levy et al., 2003; Oliva et al., 1998). As HIV disease worsens, the number of NK cells decreases along with their cytotoxic function (NKCC), whereas the cytotoxic T-cell subset undergoes an expansion that is associated with an increase in cytotoxic T-cell activation, reflected by the expression of CD38 and HLADR cell surface antigens on these cells (Cole and Kemeny, 2001; Giorgi et al., 2002, 1993; Savarino et al., 2000; Sousa et al., 2002). Therefore, both NK cells and cytotoxic T-cells may influence HIV disease severity directly, via the ability to lyse and kill HIV-infected cells, and NK cells may also influence disease severity indirectly via chemokine-mediated viral replication inhibition and cytotoxic T-cell activation.

To date, there is a paucity of information regarding the immunocellular mechanisms that may mediate the relationship between psychological factors and indices of HIV disease severity. Several different psychosocial factors have been linked to accelerated HIV disease progression, including greater subjective life stress, pessimistic cognitive appraisals about disease progression and mortality, avoidant and emotionally inexpressive (Type C) coping strategies, persistent depressive symptoms, inhibited personality characteristics, and lower perceived social support (Cole et al., 1996; Cook et al., 2004; Ickovics et al., 2001; Ironson

et al., 2005a,b; Leserman et al., 2002; Reed et al., 1999; Solano et al., 2002). Studies have hypothesized that the linkage between psychological and immunological functioning is a result of a bidirectional communication between the central nervous system and immune effector cells, via sympathetic neurotransmitters, cytokines and hypothalamic–pituitary–adrenal hormones (Black, 1988; Schneiderman et al., 1999; Segerstrom and Miller, 2004). Findings from our laboratory studies of HIV-infected and seronegative persons have indicated that acute mental stress induces a mobilization of specific killer lymphocyte subsets and functions, but HIV-seropositive subjects displayed less stress-induced increase in NK cell numbers and NKCC, and greater mobilization of cytotoxic T-cells, particularly activated cytotoxic T-cells (Hurwitz et al., 2005). Assuming that these HIV serostatus response differences meaningfully reflect a disease-related difference in psychoneuroimmunological regulation, these findings would suggest that an association between greater perceived psychological distress and worsened HIV disease severity may be mediated directly or indirectly by differences in the regulation of NK and cytotoxic T-cells.

Previously, in HIV-infected but non-AIDS defined men and women, we have shown that HIV viral load moderates the relationship between psychological distress—measured by subjective perceptions of life stress, HIV/AIDS-related anxiety and depressive symptoms—and specific immunocellular subsets, namely T-helper memory cells and B-cells (Motivala et al., 2003). The present study sought to extend these findings by evaluating in AIDS-defined as well as pre-AIDS subjects the hypothesis that functional and enumerative alterations in killer lymphocytes mediate the relationship between higher levels of psychological distress and greater HIV disease severity, indexed by HIV-1 viral load and T-helper cell count. Because it is also plausible that HIV disease severity and related immunocellular alterations, such as killer lymphocyte activation and inflammatory cytokine production, can influence cognitive, affective, somatic and behavioral function (Brabers and Nonnett, 2006; Levy et al., 1989), this study also tested a reverse directionality hypothesis wherein variation in psychological distress was predicted by HIV disease-related alterations in killer lymphocytes. These hypotheses were evaluated using a structural equation modeling (SEM) approach, which controlled for numerous demographic and HIV-relevant variables.

2. Methods

2.1. Participants

Participants were drawn from the pre-intervention assessment of the Miami Selenium for Heart and Immune Health Trial (ISRCTN #22553118; Hurwitz et al., 2007). A convenience sample of HIV-1 seropositive men and women residing in Miami-Dade, Broward and Palm Beach counties of Florida were recruited between 2001 and 2005 using newspaper advertisement, flyer distribution at HIV/AIDS clinics and support groups, and physician and chain referrals. As described, the cohort

comprised individuals spanning several HIV exposure categories who manifested various stages of infection, from asymptomatic pre-AIDS to symptomatic AIDS (Hurwitz et al., 2007). Participant inclusion required: (1) written informed consent; (2) HIV-1 infection documentation; (3) age 18–55 years; (4) no medication or prior diagnosis for cardiovascular, diabetic, psychiatric, endocrine or other major systemic conditions; (5) no electrocardiographic evidence of myocardial infarction; (7) no gross neurocognitive dysfunction; (8) no surgery within 3 months of study entry; (9) premenopausal non-pregnant status; and (10) no concomitant participation in another clinical trial. For the present study, subjects were excluded if they were not being treated with a CART regimen consisting of at least two different antiretroviral medications.

2.2. Procedures

After telephone screening, persons who met study entry criteria were invited for a laboratory visit where study eligibility was confirmed. As previously described (Hurwitz et al., 2007), screening procedures included receipt of HIV serostatus documentation, and physical examination by a physician to evaluate physical health, personal and family medical history, HIV/AIDS clinical status, CD4 count history and time since HIV diagnosis. Current antiretroviral medication regimen and adherence to prescribed doses were documented during a structured interview (Chesney et al., 2000). A fasting intravenous blood sample was collected for standard chemistry analysis, as well as assessment of immunologic and virologic measures. A urinalysis was conducted to screen for alcohol and illicit substances (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, hallucinogens and opiates) and for pregnancy. In addition to surveying current cigarette use, the substance use disorder module of the structured clinical diagnostic interview was administered (SCID-1, v2.0; Spitzer et al., 1988). Gross neurocognitive dysfunction was assessed using the Folstein Mini-Mental State Exam (Folstein et al., 1975) and additional sociodemographic and psychosocial surveys were administered.

2.3. Measures

2.3.1. Psychological distress

As in our previous HIV research, psychological distress was operationalized using a set of self-report psychometric instruments (Motivala et al., 2003). Each instrument was selected to reflect a cognitive, affective and/or behavioral component of distress, including: (a) subjective appraisal of life stress [perceived stress scale (PSS)], (b) HIV/AIDS-related anxiety [impact of events scale (IES)] and (c) depressive symptoms [Beck depression inventory (BDI)] (Beck et al., 1961; Cohen et al., 1983; Horowitz et al., 1979). The PSS contains 14 Likert-scale items that assess the degree to which one appraises one's life as stressful (i.e., unpredictable, uncontrollable and overwhelming) during the past month. The IES is a 15-item, Likert-scale survey that assesses emotional disturbance and anxiety characterized by intrusive thoughts and intentional avoidance of distressing cues related to a traumatic experience, defined in this study as the diagnosis or threat of AIDS. The BDI is a 21-item survey that quantifies the severity of cognitive, affective, behavioral and somatic symptoms of depression during the past week. These three surveys are well established and have acceptable psychometric properties (Beck et al., 1961; Cohen et al., 1983; Horowitz et al., 1979).

2.3.2. Fasting blood measures

HIV-1 viral load and T-helper cell count were measured as standard pathophysiological indicators of HIV disease severity (Vergis and Mellors, 2000). HIV-1 viral load was determined using an 'nucleic acid amplification test with ultrasensitive and standard methods (AMPLICOR HIV-1 monitor test, v1.5; Roche Diagnostics, Branchburg, NJ; detection range, 50–750,000 HIV-1 RNA copies/ml). Lymphocyte subset counts, including T-helper cells (CD3⁺CD4⁺), NK cells (CD3⁺CD16⁺, CD3⁺CD56⁺), and activated cytotoxic T-cells (CD3⁺CD8⁺CD38⁺, CD3⁺CD8⁺HLADR⁺), were derived using the mean of two serially collected peripheral blood

samples analyzed with flow cytometry (Epics XL-MCL flow cytometer, Coulter, Hialeah, FL; inter-sample correlations, $r = .96$ to $.98$). NKCC was quantified as percent cytotoxicity of CD3⁺CD56⁺ NK cells against erythroleukemic (K562) tumor cells at an effector-to-target ratio of 1:1, as described (Fletcher et al., 1987).

2.4. Statistical analyses

SEM was used to analyze cross-sectional relationships between hypothesized latent constructs that represented the common variance among selected sets of indicator variables (Kline, 2005). The traditional two-step approach to SEM was used, in which confirmatory factor analysis (CFA) was performed to first establish construct validity and the pattern of factor correlations, followed by path analysis to test directional associations between validated latent variables (Kline, 2005). SEM was conducted using Mplus v3.13 (Muthén and Muthén, Los Angeles, CA) with the raw data input option. Selected software permitted simultaneous, unbiased parameter estimation of direct and indirect relationships between multiple predictor and criterion variables in a single model. In the SEM framework, direct associations are analogous to multiple regression coefficients, where parameter estimates reflect the relationship between a predictor variable and an outcome variable adjusting for all other predictor variables in the model (Kline, 2005). In contrast, indirect associations represent the product of two or more direct path coefficients that, in combination, form an indirect relationship between a predictor variable and an outcome variable. Significant indirect associations that involve a sequence of two or more direct paths linked together can be interpreted as support for the hypothesis that one or more intervening variables mediate the relationship between two other variables. The analysis provides a test of the total association of an independent with a dependent variable, which represents the sum of all possible direct and indirect pathways. In addition, the analysis provides an index of the proportion of variability explained (R^2) for each model factor by all pertinent predictor variables, including covariates.

All measured variables were screened for missing data and distributional assumptions. Transformations were applied where necessary to correct for deviation from normality. Missing data (<5% of measured variables) were handled using full information maximum likelihood estimation, which performs well under conditions of data missing at random (Enders and Bandalos, 2001). Parameter estimates, including factor loadings, path coefficients for direct, indirect and total associations, and residual error variance terms for criterion variables, were tested for statistical significance ($z = 1.96$, $\alpha = .05$, two-tailed). The following criteria were used as indices of acceptable model fit: (1) comparative fit index (CFI) $> .90$; (2) root mean square error of approximation (RMSEA) $< .06$; and (3) standardized root mean squared residual (SRMR) $< .08$ (Hu and Bentler, 1999; Kline, 2005).² To control for type I error associated with post hoc model modifications, estimated *a priori* path models were not modified (Green and Babyak, 1997). To assess psychoimmune associations independent of potentially confounding demographic characteristics and HIV-related influences, all SEM analyses employed the following covariates: age (years); sex (male = 1, female = 0); race (black = 1, other = 0); CART including protease inhibitor use (yes = 1, no = 0); and time since HIV diagnosis (months).

3. Results

3.1. Sample characteristics

Table 1 presents demographic characteristics for the study sample. Ages ranged from 20 to 55 years with a

² The χ^2 fit statistic has been used an indicator of model fit, but because it is vulnerable to inflation with sample sizes of 200 or more, it was not used herein (Hu and Bentler, 1999; Kline, 2005).

Table 1
Demographic and disease-related characteristics of the cohort ($N = 200$)

Measures	Mean	SD
Age (years)	41.2	6.9
Sex (%)		
Men	66.5	
Women	33.5	
Race/ethnicity (%)		
Black	61.5	
Hispanic	17.5	
Non-Hispanic white	9.0	
Other	12.0	
Body mass index (kg/m^2)	26.8	5.8
Socioeconomic status		
Education (years)	13.2	8.0
Household income (\$thousands/year)	9695.8	11242.9
Employment status (%)		
Employed	33.7	
Unemployed	28.2	
In school	6.0	
Other ^a	32.1	
HIV disease stage classification (%)		
Asymptomatic	12.6	
Symptomatic	3.5	
AIDS	83.9	
Time since HIV diagnosis (years)	9.3	5.4
Historical CD4^+ count nadir ($\text{cells}/\mu\text{l}$)	223.5	206.7
Undetectable HIV viral load (%) ^b	40.9	
Antiretroviral medication regimen		
Number of medications in regimen	3.39	0.81
CART with protease inhibitor (%)	53.8	
CART without protease inhibitor (%)	46.2	
Adherence (% doses taken/d)	96.8	11.8
Substance use history (%)		
Lifetime cocaine abuse/dependence	55.0	
Current cocaine use (% positive)	19.0	
Current cigarette smoker (% yes)	58.3	

Abbreviations: AIDS, Acquired Immunodeficiency Syndrome; CART, combination antiretroviral therapy.

^a Other main daily activities reported included volunteer work, family caretaking obligations, and attending medical appointments.

^b Below lower limit of assay sensitivity (i.e., 50 HIV-1 RNA copies/ml).

mean \pm SD of 41 ± 7 year. Approximately two-thirds of participants were men and more than three-quarters self-identified as racial or ethnic minorities. Mean body mass index indicated that on average the cohort was high normal to overweight. Participants were predominantly of lower socioeconomic status (SES) according to educational attainment, household income and employment. These characteristics are representative of HIV seropositive individuals living in South Florida (Florida Division of Disease Control, 2002) and were comparable to those reported in our previous studies (Brownley et al., 2001; Hurwitz et al., 2005; Motivala et al., 2003). Based on conventional criteria for HIV disease stage (Centers for Disease Control and Prevention, 1992), most study participants (84%) were classified as AIDS. Less than half of AIDS-defined participants reported current

immunodeficiency symptoms. Of the sample, 41% displayed an undetectable viral load (<50 HIV-1 RNA copies/ml). Over half the cohort was currently taking a CART regimen that included a protease inhibitor. Participants generally reported a high level of recent adherence to their antiretroviral regimen. Although over half the sample had a lifetime history of cocaine abuse or dependence, less than 20% of the participants reported current cocaine use. Over half of the participants identified themselves as current cigarette smokers. Table 2 provides descriptive statistics for measures of psychological distress, killer lymphocyte immunity and HIV disease severity used in the analyses; the correlation matrix is presented in Table 3.

3.2. Relationships among constructs: measurement model validation

Four latent factors were operationalized as follows: (1) psychological distress, indicated by PSS, IES and BDI total scores; (2) HIV disease severity, indicated by current HIV-1 viral load and T-helper cell count; (3) NK cell immunity, indicated by two NK cell subset counts ($\text{CD3}^-\text{CD16}^+$ and $\text{CD3}^-\text{CD56}^+$) and one measure of NK cell cytotoxicity (NKCC); and (4) cytotoxic T-cell activation, indicated by the percentage of $\text{CD3}^+\text{CD8}^+$ cells expressing CD38

Table 2
Means, standard deviations and factor loadings for modeling variables^a

Factors and Indicators	Mean	SD	Factor loadings ^c
Psychological distress			
Perceived stress scale (PSS)	21.9	7.5	0.81
Beck depression inventory (BDI)	8.0	8.5	0.71
Impact of events scale (IES)	16.1	16.0	0.57
Killer lymphocyte immunity			
Natural killer cells			
$\text{CD3}^-\text{CD16}^+$ count ($\text{cells}/\mu\text{l}$)	70.3	66.9	0.81
$\text{CD3}^-\text{CD56}^+$ count ($\text{cells}/\mu\text{l}$)	84.5	66.8	0.79
NKCC (% lysis of target cells)	15.8	11.6	0.27
Cytotoxic T-cell activation			
$\text{CD8}^+\text{CD38}^+$ (% CD8^+ cells)	28.3	13.8	0.77
$\text{CD8}^+\text{HLADR}^+$ (% CD8^+ cells)	21.2	12.4	0.68
HIV disease severity			
Viral load (copies/ml) ^b	16,282.1	71,755.0	0.68
CD4^+ T-cell count ($\text{cells}/\mu\text{l}$)	431.4	299.9	-0.72

Abbreviations: BDI, Beck depression inventory; HIV, human immunodeficiency virus; IES, impact of events scale; NKCC, natural killer cell cytotoxicity; PSS, perceived stress scale.

^a Non-transformed values were provided for ease of interpretation. Appropriate transformations resulted in all skewness values <2 and all kurtosis values <3 . Transformed variables were used in SEM analyses.

^b Lower bound detection limit of the HIV-1 viral load assay was 50 RNA copies/ml. A value of 49 was substituted for all values below this limit.

^c Beta (β) weights derived from confirmatory factor analysis of latent variables. All factor loadings were significant at $p < .001$, indicating acceptably strong correlations between each measured indicator and the corresponding latent construct.

Table 3
Correlations for variables used in modeling analyses

Variables	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. PSS	1.00														
2. BDI	.58**	1.00													
3. IES	.45**	.42**	1.00												
4. Viral load	.14*	.10	.22**	1.00											
5. CD4	-.16*	-.15*	-.18*	-.49**	1.00										
6. CD16	-.19**	-.04	-.03	-.16*	.20**	1.00									
7. CD56	-.21**	-.09	-.13 [†]	-.15*	.14*	.64**	1.00								
8. NKCC	.05	.21**	.15*	.03	-.02	.19**	.22**	1.00							
9. CD38	.12	.06	.10	.39**	-.47**	-.21**	-.16*	.00	1.00						
10. HLADR	.16*	.05	.17*	.42**	-.35**	-.20**	-.15*	-.15*	.53**	1.00					
11. Age	-.04	-.09	-.08	.03	.12 [†]	.04	.01	.02	-.03	-.02	1.00				
12. Sex	.06	.01	-.10	-.01	-.10	.11	.13 [†]	-.03	-.02	-.01	.32**	1.00			
13. Race	.22**	.20**	.13 [†]	.00	-.01	-.04	-.22**	-.07	-.05	.08	-.06	.26**	1.00		
14. PI use	-.04	-.10	.04	.13	-.30**	-.06	.09	.03	.18*	.16*	-.01	.04	-.00	1.00	
15. HIV dx	-.01	-.03	-.18*	.02	-.08	.01	-.05	-.03	.08	.15*	.23**	.15*	-.02	.13 [†]	1.00

Abbreviations: BDI, Beck depression inventory; CD4, CD3⁺CD4⁺ T-helper cell count; CD16, CD3⁻CD16⁺ NK cell count; CD56, CD3⁻CD56⁺ NK cell count; CD38, % of CD8⁺ T-cells expressing CD38 cell surface activation marker; HIV dx, time since HIV diagnosis in months; HLADR, % of CD8⁺ T-cells expressing HLADR cell surface activation marker; IES, impact of events scale; NKCC, natural killer cell cytotoxicity; PI, CART regimen including protease inhibitor(s); PSS, perceived stress scale; viral load, HIV-1 viral load.

* $p < .05$.
 ** $p < .01$.
[†] $p \leq .10$.

and HLADR markers. The CFA yielded a measurement model of good fit (CFI = .96, RMSEA = .06, SRMR = .06). Factor loadings shown in Table 2 revealed statistically significant correlations between each measured indicator and its respective latent factor, demonstrating construct validity for the latent variables. All factor loadings were >.50 except for NKCC on the NK cell immunity factor. Although NKCC did not load as strongly as the two NK cell subsets, NKCC was retained as an indicator of the NK cell immunity factor because of its highly significant factor loading ($p < .001$) and its functional importance in innate anti-viral defense.

The measurement model further revealed a pattern of significant correlations among latent variables. Specifically, the psychological distress factor was positively associated with cytotoxic T-cell activation ($r = .20, p < .05$) and HIV disease severity ($r = .29, p < .05$), and was negatively associated with NK cell immunity ($r = -.21, p < .05$). In addition, the HIV disease severity factor was strongly related to increased cytotoxic T-cell activation ($r = .80, p < .05$) and was negatively related to NK cell immunity ($r = -.29, p < .05$). Finally, NK cell immunity was negatively associated with cytotoxic T-cell activation ($r = -.31, p < .05$).

3.3. Predicting HIV disease severity: psychoimmune model evaluation

The psychoimmune factor correlations formed the basis for the path analytic modeling analysis in which greater distress was hypothesized to account for variation in HIV disease severity as a function of altered killer lymphocyte status (see Fig. 1A). Specifically, the analysis examined whether the relationship between psychological distress and HIV disease severity, independent of covariates, was

mediated by: (1) paths from distress to both NK cell immunity and cytotoxic T-cell activation; (2) paths from NK cell immunity and cytotoxic T-cell activation to HIV disease severity; and (3) a path from NK cell immunity to cytotoxic T-cell activation. Fig. 1B illustrates the psychoimmune mediation model, which provided acceptable fit to the data (CFI = .92, RMSEA = .06, SRMR = .06). A significant overall association was observed between higher distress level and greater HIV disease severity as a function of NK cell immunity and cytotoxic T-cell activation ($\beta = .31, z = 2.64, p < .05$). This overall association was accounted for by a combination of different direct and indirect associations between predictor and criterion variables in the model. Specifically, greater distress was directly related to both diminished NK cell immunity ($\beta = -.18, z = -1.98, p < .05$) and increased cytotoxic T-cell activation ($\beta = .20, z = 1.96, p < .05$). Cytotoxic T-cell activation, in turn, related directly to worsened HIV disease severity ($\beta = .68, z = 3.53, p < .05$). A trend toward a significant direct association was observed between diminished NK cell immunity and greater cytotoxic T-cell activation ($\beta = -.19, z = -1.67, p = .09$), even after accounting for the relationship between distress and cytotoxic T-cell activation in the model. The cumulative association of all mediating pathways taken together linking distress and HIV disease severity was significant ($\beta = .17, z = 2.03, p < .05$), whereas the strength of the direct association between distress and HIV disease severity was rendered non-significant ($\beta = .14, z = 1.43, p = .16$). In sum, these results are consistent with the hypothesis that intermediary factors, namely NK cell immunity and cytotoxic T-cell activation, substantially accounted for the covariation between higher distress level and greater HIV disease Severity.

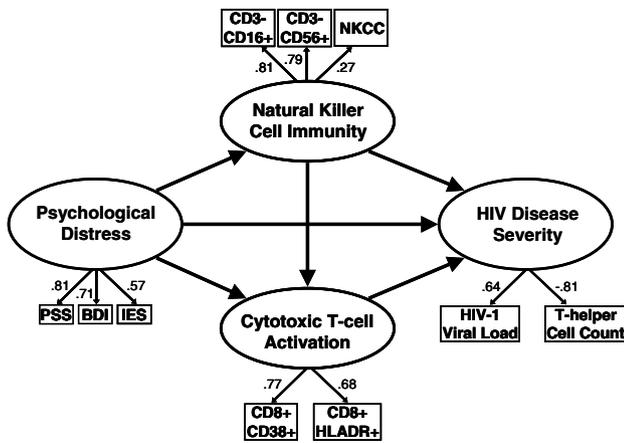
The model further revealed a significant total association between higher distress and greater cytotoxic T-cell activation, comprised of a direct path as well as an indirect path involving diminished NK cell immunity as a potential mediator ($\beta = .24, z = 2.30, p < .05$). However, the formal test of mediation linking distress to cytotoxic T-cell activation via NK cell immunity was not significant ($\beta = .03, z = 1.20, p > .10$). In addition, the direct association between diminished NK cell immunity and greater HIV disease severity was not significant. Table 4 presents the proportions of variance explained (R^2 values) for factors in the psychoimmune model. The unique variance accounted for by distress and distress-related alterations

in killer lymphocyte immunity independent of covariates were provided for the model both with and without the inclusion of covariates. As can be seen in Table 4, each factor had at least one significant or marginally significant covariate. For example, identifying ones' race as black was independently associated with higher levels of distress ($\beta = .29, z = 3.53, p < .05$) and more diminished NK cell immunity ($\beta = -.14, z = -1.82, p = .07$). Having a medication regimen including protease inhibitor(s) was associated with both greater HIV disease severity ($\beta = .19, z = 2.48, p < .05$) and greater levels of cytotoxic T-cell activation ($\beta = .24, z = 2.83, p < .05$). Conventional standards for variance accounted for in models with multiple predictors range from "small" (2–12%) to "large" ($\geq 26\%$; Cohen, 1988). The present model with covariates explained 10% of the variance in distress, 9% of the variance in NK cell immunity, 15% of the variance in cytotoxic T-cell activation and 67% of the variance in HIV disease severity. In sum, the psychoimmune model evaluation indicated that diminished NK cell immunity and heightened cytotoxic T-cell activation are possible mediators of the association between higher levels of psychological distress and worsened HIV disease severity.

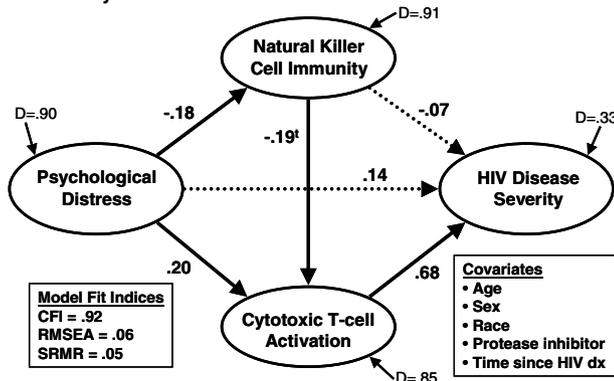
3.4. Predicting psychological distress: reverse directionality model evaluation

As shown in Fig. 1C, an alternative conceptual model was specified in which HIV disease-related alterations in killer lymphocyte factors were hypothesized to account for individual variation in psychological distress. This reverse directionality model estimated the same number of parameters as the psychoimmune model in panel B.

Panel A. Hypothesized Model



Panel B. Psychoimmune Model



Panel C. Reverse Directionality Model

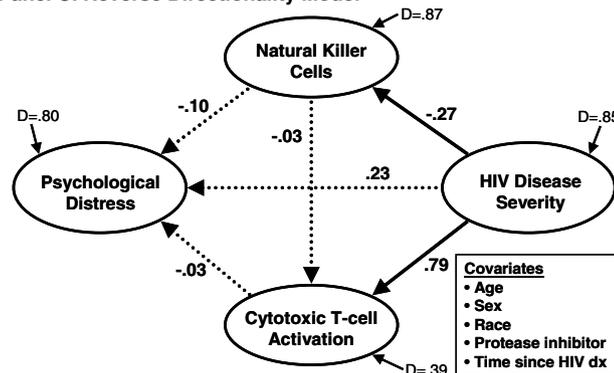


Fig. 1. (A) The hypothesized psychoimmune model including factor indicators in boxes and loadings are depicted. Abbreviations for factor indicators are defined in Table 2. Standardized solutions are presented for two alternative conceptual models. (B) The psychoimmune model in which psychological distress-related alterations in killer lymphocyte factors accounted for variation in HIV disease severity. (C) The reverse directionality model in which psychological distress was predicted by HIV disease severity and associated alterations in killer lymphocyte status. Ovals represent latent variables. Path coefficients are standardized regression β weights. Statistical tests were conducted for direct and indirect (mediational) pathways linking predictor and criterion variables. Solid arrows indicate statistically significant associations ($p < .05$). Dashed arrows indicate non-significant (ns) relationships ($p > .05$). Significance tests for the total association (i.e., the sum of all possible direct and indirect pathways) linking psychological distress and HIV disease severity factors were also obtained. Disturbance (D) terms are indicated reflecting the proportion of unexplained variance in a factor after accounting for all predictors and covariates in the model. Each factor specified in the path models was controlled using all covariates: age, sex, race, protease inhibitor use, and time since HIV diagnosis (see Table 4). To protect against type I error, a priori models were not modified to include (or exclude) any other model parameters, such as additional pathways or correlated residuals. The models provided acceptable fit without post hoc modification. Because each model estimated the same number of parameters, fit statistics were identical across models.

Table 4

Total variance (R^2) accounted for in the psychoimmune model factors with and without the inclusion of covariates

Psychoimmune model factors	R^2 with all covariates	R^2 with no covariates	Covariates				
			Age	Sex (men)	Race (black)	Protease inhibitor use	Time since HIV diagnosis
Distress	0.10	—	−0.10	0.14	0.29*	−0.06	−0.05
Natural killer cell immunity	0.09	0.04	−0.04	0.13	−0.14 [†]	0.06	−0.08
Cytotoxic T-cell activation	0.15	0.11	−0.04	−0.04	−0.09	0.24*	0.14
HIV disease severity	0.67	0.66	−0.12	0.15 [†]	−0.01	0.19*	−0.03

Standardized path coefficients (β s) for all covariates are displayed.

Abbreviations: HIV, human immunodeficiency virus; NK, natural killer.

* $p < .05$.[†] $p \leq .10$.

Thus, fit statistics were identical.³ The reverse directionality model indicated that greater HIV disease severity was directly associated with both diminished NK cell immunity ($\beta = -.27$, $z = -2.32$, $p < .05$) and increased cytotoxic T-cell activation ($\beta = .79$, $z = 3.62$, $p < .05$). Despite these two significant direct associations, no significant direct pathways to distress were found. Thus, findings from the reverse directionality modeling analysis did not support the alternative mediational hypothesis, in which HIV disease severity was posited to predict psychological distress.

4. Discussion

This study evaluated whether killer lymphocyte immunity may mediate the relationship between higher levels of psychological distress and greater HIV disease severity in a cross-sectional examination of a multiethnic community-based sample of pre-AIDS and AIDS-defined men and women on CART. The results confirmed that distress, conceptualized as perceived stress, anxiety and depressive symptoms, was positively related with HIV disease severity as a function of killer lymphocyte alterations. In contrast, the analyses did not support an alternative, reverse direction hypothesis that HIV disease-related alterations in killer lymphocyte immunity accounted for higher levels of distress. In sum, the major findings of the study were that the relationship between greater distress and HIV disease severity was linked with: (1) diminished NK cell immunity, indexed by fewer NK cells and decreased NK cytotoxic function; and (2) greater cytotoxic T-cell activation, indexed by a greater proportion of cytotoxic T-cells that display cell surface activation markers. Specifically, the results indicated that the role of NK cell immunity and cytotoxic T-cell activation in mediating the distress–disease severity relationship differed. The modeling analyses showed that while distress was directly related to both NK cell immunity and cytotoxic T-cell activation, only cytotoxic T-cell activation was related to disease severity. In addition, there was a trend toward a significant pathway

from NK cell immunity to cytotoxic T-cell activation. This model, which controlled for age, sex, race, CART regimens with or without protease inhibitor use, and HIV disease duration, accounted for 67% of the variance in HIV disease severity. Therefore, the study supports the possibility that psychological distress may influence HIV disease severity through innate and adaptive immunocellular mechanisms, independent of major demographic and HIV-related factors.

These findings are consistent with a recent meta-analysis showing that increased stressful life events are associated with diminished killer lymphocyte numbers in both HIV-infected and healthy seronegative individuals (Segerstrom and Miller, 2004). Similarly, in HIV-infected women, greater anxiety and depressive symptoms were associated with decreased NKCC, increased expression of the CD38 marker on cytotoxic T-cells, and greater HIV-1 viral load (Evans et al., 2002). The present study extends these data by documenting in a single, comprehensive model a moderate overall effect size for the sum of direct and indirect associations linking psychological distress to HIV disease severity via killer lymphocyte immunity. Taken together, these results provide empirical support for the suggestion that perceptions of distress in the course of HIV spectrum disease may account for individual variation in disease severity, indexed by HIV viral load and T-helper cell count, possibly through an interaction of NK and cytotoxic T-cell immunocellular processes. Notably, in the present study, the relationship between NK cell immunity and HIV disease severity became non-significant in the final model. Thus, NK cell immunity was not a direct mediator of the distress–disease severity relationship. In addition, although the model suggested that NK cell immunity via its relationship with cytotoxic T-cell activation indirectly mediated the relationship between distress and disease severity, analyses indicated that NK cell immunity was not a significant mediator of the distress–cytotoxic T-cell activation relationship. Therefore, it appears that NK cell immunity may play less of a mediational role than cytotoxic T-cell activation in HIV pathophysiology.

Comparable findings to the observed distress–HIV disease severity relationship have been reported in recent modeling investigations. For example, coping style, social

³ Because both conceptual models were saturated, i.e., each latent factor related to every other factor, the models could not be compared statistically (Kline, 2005).

support, religious involvement and spirituality have been shown to account for individual variation in distress level among HIV-infected men and women (Carrico et al., 2006; Llabre et al., 2006; Penedo et al., 2003; Prado et al., 2004; Weaver et al., 2005). The present study also extends previous reports by indicating that distress level may also be attributable, in part, to race. This finding is noteworthy because over the past decade, the proportion of HIV/AIDS cases is increasing in the black population despite a decreasing overall trend among other race/ethnic groups (Centers for Disease Control and Prevention, 2005). In addition, several psychosocial factors that contribute to distress, including negative mood state, avoidant coping and perceived social support, have been shown to account for individual variation in HIV-1 viral load through an association with poorer adherence to antiretroviral medication (Gonzalez et al., 2007; Weaver et al., 2005). One recent longitudinal investigation of a multiethnic sample of pre-AIDS, HIV-seropositive men and women found that after controlling for antiretroviral medication use, greater cumulative life event stress, avoidant coping, depression and hopelessness significantly predicted faster disease progression over a 2-year period, defined by CD4⁺ T-cell decline and HIV-1 viral load expansion (Ironson et al., 2005b). Dispositional characteristics and coping responses may also influence susceptibility to distress-related immune alterations and HIV disease progression. For example, plasma HIV viral load set-point was shown to be elevated eight-fold in socially inhibited individuals, who also showed poorer virologic and immunologic response to initiation of highly active antiretroviral therapy (Cole et al., 2003). Maladaptive Type C coping, which is characterized by under-recognition and -reporting of stress, needs and emotions, has been shown to predict faster transition from asymptomatic status to more advanced disease stages 6 and 12 months later (Solano et al., 2002; Temoshok et al., 2005, 2006). Finally, dispositional optimism has been associated with proactive health behavior, adaptive cognitive coping and less negative affect, which in turn related to slower HIV disease progression (Ironson et al., 2005a). Taken together, the present findings add to converging evidence that psychological distress and related psychosocial and demographic factors can explain individual variation in HIV disease severity and progression.

It is possible that autonomic biomediators may play a role in the observed psychoimmune associations. Considerable research has demonstrated that killer lymphocyte numbers and immunocellular activation state and cytotoxicity are modulated by sympathetic activation, primarily via β_2 -adrenergic cell surface receptors (Madden, 2003; Nance and Sanders, 2007; Padgett and Glaser, 2003). Others have shown that sympathetic activation, indexed by multiple non-invasive autonomic assessments, is linked with HIV disease severity indexed by higher HIV-1 viral load set-point and poorer immunologic and virologic responses to the initiation of treatment with antiretroviral therapy (Cole et al., 2003, 2001). In addition, *in vitro* stud-

ies have revealed that norepinephrine administration to HIV-infected peripheral blood leukocytes can enhance cellular vulnerability to infection by facilitating HIV-1 gene expression, inhibiting anti-HIV cytokine production, and up-regulating chemokine co-receptor expression necessary for HIV cell entry (Cole et al., 2001, 1998; Collado-Hidalgo et al., 2006). Thus stress-related sympathetic activation may influence HIV pathogenesis directly, via direct stimulation of HIV-1 replication, as well as indirectly through modulation of cellular immunity.

Alternatively, others have suggested that stress-related activation of neurogenic inflammation and counter-regulatory mechanisms of the hypothalamic–pituitary–adrenocortical axis may mediate psychoimmune relationships (Black, 2002; Leserman, 2003a,b). Acute arousal of negative affect, measured by subjective reports of perceived stress, anxiety, depression and anger, has been linked to higher proinflammatory cytokine levels (IL-1, IL-6 and TNF- α), which can facilitate HIV replication (Breen, 2002; Suarez et al., 2006, 2003). Another candidate biomediator is cortisol, which may become elevated in inflammatory conditions, such as chronic stress and/or viral infection (McEwen et al., 1997). Moreover, cortisol can directly stimulate HIV-1 replication, and may interact with viremia to inhibit killer lymphocyte cytotoxicity (Nair and Schwartz, 1995; Swanson et al., 1998). It has also been suggested that distress-related dysregulation of cytokine signaling may impact immunocellular activation and disease severity indirectly via disrupted intercellular communication among innate and adaptive killer lymphocytes (White-side and Herberman, 1994).

4.1. Study limitations

Although the study participants spanned several HIV exposure categories and manifested various stages of infection, from asymptomatic pre-AIDS to symptomatic AIDS, the present cohort comprised mostly low SES, AIDS-defined black men with a history of illicit substance use. Nevertheless, the cohort characteristics were representative of the local community. Study entry criteria, however, were stringent with regard to excluding those with comorbid systemic conditions, and psychiatric and immune complications. Hence, relatively normal psychological distress levels were reported. Thus, the present findings may not generalize to other populations with different demographic, psychological and disease-related characteristics. The modeling analysis employed several covariates, including age, sex, race, CART regimen and HIV disease duration, to control for potential confounding of these factors. Sample size considerations prohibited inclusion of other variables associated with HIV disease, including illicit substance use, co-infection with other viruses, and other subject-related variables.

The primary limitation of this study was its cross-sectional nature, wherein assigning cause and effect is not possible. However, the contrasting evaluations of the

psychoimmune and reverse directionality models were useful in elucidating model plausibility. The failure of the reverse directionality model to show that disease severity predicted distress suggests that the more primary pathophysiological linkage is likely to be from distress to immunity. Further research, however, is necessary to definitively reject the reverse directionality model and to evaluate whether disease severity is associated with other distress indicators or other psychosocial factors that may alternatively, independently or interactively influence distress as currently conceptualized. For instance, it is possible that increased distress levels may have been associated with clinical symptoms or knowledge of HIV disease severity or clinical sequelae (Gallego et al., 2000). Therefore, conclusions regarding the psychoimmune model viability must be restricted to the operational definitions of the constructs employed. In addition, future research may benefit from longitudinal analyses to address temporal causality issues and to explore candidate biobehavioral factors that may further explain psychoimmune mechanisms underlying individual variation in HIV/AIDS disease severity and progression.

5. Conclusions

The present findings support the psychoneuroimmunology hypothesis that psychological factors may account for individual variability in HIV disease severity in pre-AIDS and AIDS defined men and women treated with CART. The psychoimmune associations examined in this study reflect plausible biobehavioral influences underlying HIV-1 pathogenesis. In sum, the derived psychoimmune mediation model, when controlling for potential confounding demographic and HIV-related factors, was physiologically consistent and explained substantial covariation between higher levels of psychological distress and worsened HIV disease severity. The derived model showed that diminished NK cell immunity and elevated cytotoxic T-cell activation may be important factors mediating this relationship. However, while distress was directly related to both of these factors, only distress-related cytotoxic T-cell activation was directly linked to HIV disease severity. Therefore, it may be concluded that cytotoxic T-cell activation may play a more primary mediational role, a novel contribution to the literature. Thus, future investigations aimed at further elucidating the mechanisms by which distress affects killer lymphocyte immunity may be helpful in understanding variation in HIV disease pathophysiology.

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