CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Jan. 1996, p. 109–118 Vol. 3, No. 1
1071-412X/96/$04.00+0

Bereavement Is Associated with Time-Dependent Decrements in Cellular Immune Function in Asymptomatic Human Immunodeficiency Virus Type 1-Seropositive Homosexual Men

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Received 27 July 1995/Returned for modification 9 October 1995/Accepted 1 November 1995

Seventy-nine human immunodeficiency virus type 1 (HIV-1)-seropositive homosexual men participating in a longitudinal study of HIV-1 infection were assessed twice, 6 months apart, to investigate associations between bereavement and cellular immune function. Subjects were assessed by using a theory-driven model comprising life stressors, social support and coping style, and control variables. Natural killer cell cytotoxicity was decreased among the bereaved at both times. Lymphocyte proliferative response to phytohemagglutinin was decreased among the bereaved at the second time point but not at the first. These functional immune decrements are associated with increased neuroendocrine responses of the sympathetic adrenomedullary system as well as the limbic-hypothalamic-pituitary-adrenal axis. Implications for differential neuroendocrine responses over time are discussed. Active coping style was independently and positively related to both immune measures. The results imply that a bereavement support group intervention merits investigation for an effect on immunological measures and clinical progression of HIV-1 infection as well as grief resolution.

The first study demonstrating bereavement-related decrements in immune function was reported by Bartrop et al. (4) for loss of a spouse. Decrements in proliferative responses of lymphocytes to phytohemagglutinin (PHA) and concanavalin A, two T-cell mitogens, were found at 6 weeks postbereavement, in comparison with responses at weeks 1 to 3. Another prospective study (59, 63) replicated and extended these findings to decrements in proliferative response to pokeweed, a T-cell-dependent B-cell mitogen, as well. Return to baseline required from 4 to as long as 14 months, paralleling the time course observed in several studies demonstrating bereavement-associated decrements in health status (50, 55). Subsequent research by Linn et al. (41), among others (30, 70), showed that depressed mood, not bereavement itself, mediated these effects. Another independent, cellular immune function, natural killer cell cytotoxicity (NKCC), has been found to be decreased during anticipatory grieving and after a loss as well (29, 30).

Like studies of bereavement and immunity, studies of psychosocial factors (taken generally) and immune measures have not shown entirely consistent findings. In human immunodeficiency virus type 1 (HIV-1) infection particularly, studies have yielded conflicting results (18, 23, 25, 32, 51, 53, 56), sometimes with use of very similar methods and scales (10, 42). Several possibilities that may explain the variable relationships found in studies of psychosocial factors and immunity in the HIV-1 infected are the lack of (i) a theory-driven model guiding statistical hypothesis testing (24, 26); (ii) simultaneous controls for distress measures, in particular depressed mood (32), and other psychosocial factors potentially affecting immunity beyond those constituting the focus of investigation (e.g., other life stressors in a study of immunological measures associated with bereavement); (iii) a control for the timing of stressor occurrence (24, 26) in studies examining life stressors; (iv) consistent controls for other factors known to influence immune measures (e.g., alcohol use, recreational substance use, nutritional status, cigarette smoking, caffeine intake, sexual activity, and prescribed medication use) (23, 26, 34); (v) use of immune outcome measures other than those directly affected by the progression of HIV-1 infection (51, 53, 64); and (vi) controls for clinical stage of disease (26). This study attempted to address each of these issues.

MATERIALS AND METHODS

Subjects. The subjects were 79 asymptomatic, anti-HIV-1 antibody-seropositive homosexual men, previously aware of their serostatus, participating in a natural history study of HIV-1 infection at the University of Miami School of Medicine. Exclusion criteria were: (i) signs (other than persistent generalized lymphadenopathy) or symptoms referable to HIV-1; (ii) a CD4 cell count of >700 cells per mm^3; (iii) taking of antiretroviral medications (e.g., zidovudine, ddI, ddC, d4T, and 3TC) or immunomodulators or participation in an HIV-related drug trial at study entry; (iv) history of excessive alcohol or substance use; (v) history of severe head trauma; (vi) evidence of central nervous system disease; or (vii) history of a major psychiatric disorder. The bereaved group ($n = 25$) self-reported the loss to AIDS of a close friend ($n = 20$) or lover ($n = 5$) with a negative impact over the 6 months preceding the baseline assessment (time point 1 [*T1*]) on a survey of major life stressors (the *Life Experiences Survey* [57]; see below), generating the bereavement indicator (yes or no) used here. Participants were age 22 to 58 ($X = 33.0$, standard deviation [SD] = 7.4) at *T1*, well educated ($X = 14.6$ years, SD = 2.1), and largely employed, with a modal annual income of $15,000 to $20,000, and had a mean CD4 cell count of 524 cells per mm^3. Health examination verified the clinical stage as 1993 Centers for Disease Control and Prevention [CDC] stage A (asymptomatic) (1986 CDC stages II [$n = 51$] and III [$n = 28$]) (12, 13). Both Caucasian non-Hispanics ($n = 51$) and Hispanics

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($n = 28$) were represented and did not differ significantly in sociodemographic characteristics. Sixty-nine of the 79 subjects (87%) were available at the 6-month follow-up (*T2*): five refused, one missed his visit, and four were enrolled less than 6 months before study termination.

Psychological surveys. A description of the contents of the psychosocial battery follows and includes variables in addition to bereavement that have been previously demonstrated to be associated with immune measures for the purpose of documenting the specific effect of bereavement in statistical analyses in this report. Background life event stressors were measured as the number of major stressful life events (other than the loss) acknowledged as experienced over the prior 6-month interval (at any point) and rated as having a negative impact (on a five-point Likert scale from −2 to +2) on the *Life Experiences Survey* (57), a standardized checklist with minor adaptations for homosexual men with HIV-related concerns. As with bereavement itself, only stressors rated as negative in impact were included in this measure in order to specify those experiences found to be subjectively stressful. Social support was measured with a 20-item shortened version of the *Social Provisions Scale* (16), yielding perceived available support. Five theoretically derived subscales generated an equally weighted composite score for which Chronbach's α, a measure of internal consistency, was equal to 0.88. Dispositional coping was measured with the 57-item *COPE* (11). Factor analysis reduced the 13 theoretically derived, behaviorally specific coping styles to four coping predictors. Two were equally weighted composite scores: "active coping" ($\alpha = 0.87$)—five problem-focused scales (active coping, planning, suppression of competing activities, restraint coping, and seeking instrumental support) as well as three emotion-focused scales (seeking emotional social support, positive reinterpretation and growth, and acceptance)—and "disengagement/denial" ($\alpha = 0.75$)−behavioral disengagement, mental disengagement, and denial. The other two predictors were the "focus on and venting of emotions" subscale score ($\alpha = 0.68$) and the "turning to religion" subscale score ($\alpha = 0.89$). Psychological distress was measured by the total mood disturbance score from the *Profile of Mood States* (POMS) (45), a widely used 65-item mood adjective checklist, which also generated subscale scores for the specific mood states of depression and anxiety.

Viral serology. To confirm seropositive status at study entry, anti-HIV-1 antibody was assayed by enzyme-linked immunosorbent assay (Abbott) and confirmed by Western blotting (immunoblotting) (Hillcrest Laboratories, Cypress, Calif.).

Immune measures. Peripheral blood was drawn immediately prior to a resting period for a cold-pressor neuroendocrine reactivity study at *T1* (see below) and again at *T2*. Immune measures were not available simultaneously after the resting period. Recent evidence suggests that this might have an effect on immune associations because of changes in lymphocyte trafficking that may occur for several hours after mild, acute stressors (17). However, these effects are best documented for cell count and may be more pertinent to cell count than to cell function (controlled for cell count in this study).

NKCC. NKCC was assayed with a whole-blood chromium release assay as detailed by Baron et al. (3). The selection of a whole-blood assay is important in the context of the determination of immunological and psychosocial interaction (e.g., in bereavement). Use of whole blood, rather than density gradient-separated and washed mononuclear cells, permits determination of the functional attributes of the cells, both cytolytic and proliferative, in a milieu similar to that in which they exist in vivo. In contrast, density gradient centrifugation might change the relative ratios of certain lymphocyte subsets and artificially concentrate the mononuclear cells before assay. Washing clearly removes soluble factors present in whole blood that may inhibit or enhance NKCC, and effector cells may be lost (3). Maintaining an approximation of in vivo conditions is desirable in diseases characterized by lymphopenia, abnormal cellular distributions, and abnormal plasma proteins, as in HIV-1 infection. Another advantage of the whole-blood assay is that the cytolytic activity against K562 tumor cell targets is normalized to the number of cells of the natural killer (NK) phenotype that are present in the blood sample. Moreover, the whole-blood assay uses less blood than a mononuclear cell assay. NKCC results reported for HIV-1-infected individuals with the whole-blood assay have been shown to be comparable to those obtained by using density gradient-separated mononuclear cells (3).

The target cell line was the NK-sensitive erythroleukemic K562 cell line, which is kept under continuous culture in our laboratory. Target cells are labelled with ^{51}Cr (sodium chromate; NEN, Boston, Mass.) before use in the NKCC assay. The culture medium (CM) used was RPMI 1640 plus 15% fetal bovine serum and includes glutamine, penicillin, amphotericin B (Fungizone), nonessential amino acids, and sodium pyruvate. Briefly, whole-blood aliquots (150 μl) are dispensed in triplicate into 96-well flat-bottom tissue culture plates (Costar, Cambridge, Mass.). CM and Triton X-100 (Sigma, St. Louis, Mo.) are used as controls for spontaneous release and total release, respectively. Plasma controls are performed for each sample. ^{51}Cr-K562 cells (50 μl) are added at concentrations of 2, 1, 0.5, and 0.25×10^6/ml. The trays are covered, centrifuged at 400 × *g* (10 min, room temperature), and incubated in a humid environment at 37°C in 5% CO_2 for 4 h. Cold CM is added, the plates are centrifuged, and supernatant (100 μl) is withdrawn for measurement of radioactivity in a γ counter (LKB Instruments, Rockville, Md.). Percent killing at each of the four target cell concentrations is calculated by the following formula:

$$\% \text{ cytotoxicity} = \frac{\left\{(\text{ER} - b) \times \left[\frac{V_t - (V_b \times H)}{V_t}\right]\right\} - (\text{SR} - b)}{(\text{TR} - b) - (\text{SR} - b)} \times 100$$

where ER is the mean counts per minute (cpm) of experimental release of the specimen, SR is the mean cpm of spontaneous release, TR is the mean cpm of total release, V_t is the total volume in the well, V_b is the volume of blood in the well, H is the specimen hematocrit, and b is the instrument background. Percent cytotoxicity at each effector-to-target cell ratio, and the number of $CD56^+$ cells per unit of blood (determined by flow cytometry with phycoerythrin-conjugated anti-CD56 monoclonal antibody), are used in a linear regression equation to calculate NKCC, which is defined as percent killing of targets at an effector-to-target cell ratio of 1:1, where effector cells are defined as $CD56^+$ cells. Percent spontaneous release was <20%.

To avoid multiple statistical tests of the same immunological measure, only one other NKCC measure was selected for analysis here. A kinetic analysis of percent cytotoxicity was done in which the percent cytotoxicity determined by the ^{51}Cr release assay for the dilutions of target cells used was transformed to number of target cells killed in each dilution by using the formula $V = \%$ cytotoxicity $\times S$, where V (velocity) is the number of target cells lysed and S (substrate) is the number of target cells in the assay (3). The data were then fitted to a Michaelis-Menton kinetic equation as described by Cleland, using a computer program written in-house: $V = (V_{max} \times S)/(K_m + S)$, where V is the number of target cells killed, S is the number of targets in the assay, V_{max} is the number of targets killed when S is infinite, and K_m is the number of targets required for ½ V_{max}. Kinetic lytic units (KLU) were defined as the maximum number of targets lysed during the 4-h assay. KLU was chosen over other methods of expressing NKCC as the best measure approximating percent cytotoxicity on a 1:1 basis, as expressed above, and is the approximate equivalent of the number of target cells killed per effector cell in the 4-h assay.

Lymphocyte proliferation assay. The whole-blood lymphocyte proliferation assay has been described previously (19, 20). Briefly, heparinized blood is diluted 1:5 with CM (RPMI 1640 containing penicillin, streptomycin, and glutamine) and dispensed in 100-μl aliquots into triplicate 96-well U-bottom microculture plates (Costar). PHA (Wellcome Diagnostics, Dartford, England) is added at a final concentration of 10 μg/ml. The plates are incubated for 72 h (at 37°C) in a Napco 4200 water-jacketed incubator with CO_2. During the last 6 h of incubation, the culture wells are pulsed with 25 μl of [^{3}H]thymidine at 1 μCi per well ([^{3}H]thymidine; NEN) (specific activity, 2 to 10 Ci/mmol). Cultures are harvested onto glass filter paper disks (Titertek Cell Harvester; Flow Laboratories, McLean, Va.), the disks are immersed in scintillation fluid (Ecoscint; National Diagnostics, Highland Park, N.J.), and radioactivity is counted on a β-scintillation counter (LKB Instruments). Lymphocyte counts for each sample of blood were determined with a Coulter MaxM. Results are expressed (i) as a stimulation index calculated by division of the mean cpm of PHA-stimulated cultures by mean cpm of control unstimulated cultures and (ii) as net cpm (stimulated cpm − unstimulated cpm). The results are normalized to 100,000 lymphocytes. Results reported for HIV-1-infected individuals with the whole-blood assay have been shown to be more sensitive than those obtained with separated mononuclear cells, which may reflect a loss of cell number or populations having a stimulatory effect on PHA (e.g., polymorphonuclear leukocytes) during density gradient centrifugation or the effects of washing on soluble factors (19).

Flow cytometry. Cell types were enumerated by using flow cytometry with fluorescein isothiocyanate- or phycoerythrin-conjugated monoclonal antibodies as described by Klimas et al. (35). Absolute numbers of cell populations were determined by multiplying the total peripheral lymphocyte count by percent positive cells for each marker (i.e., CD4, CD8, and CD56).

Neuroendocrine measures. Peripheral blood for neuroendocrine measures was drawn as a baseline for a cold-pressor reactivity study conducted at *T1*, following a 30-min resting period with an indwelling venous catheter to control for physical activity before the draw as well as reactivity to the needle stick, generating the best approximation of in vivo neuroendocrine status. (Neuroendocrine measures were not available at *T2*.)

Plasma norepinephrine (NE) and epinephrine (E). Plasma NE and E were assayed by using a high-pressure liquid chromatography system with an electrochemical detector. Preservatives used were EDTA and sodium metabisulfite; dihydroxybenzylamine was used as an internal standard. The concentrations of NE and E were determined by using a ratio method on a preprogrammed data module. The intra-assay and interassay coefficients of variation ranged from 3 to 10% (37). Plasma cortisol was determined by using a solid-phase radioimmunoassay technique (DPC, Los Angeles, Calif.). The minimum detection limit is 0.2 μg of cortisol per dl. The interassay coefficient of variation is <6.5%, and the intra-assay coefficient of variation is between 2 and 8% (36).

Control variables for influences on immune measures other than psychosocial factors. Control variables for studies of behavior and immune measures in humans have been well described previously (34), along with their specific importance in HIV-1 infection (24, 26). For both immune outcomes, we controlled for age, ethnicity, years of education, current employment status, past and current use of recreational substances (alcohol, marijuana, cocaine, amphetamines, hallucinogens, and opioids), cigarette smoking, caffeine intake, sexual activity, macronutrient nutritional status (serum albumin, prealbumin, and retinol-binding protein), and prescribed medication use (e.g., antiretroviral drugs started

after study entry). Current alcohol use was noted as "regular" with a self-report of more than two to four drinks weekly; history of alcohol use was noted as "excessive" when so characterized by self-report. Controls for disease progression include 1986 CDC stage (II versus III) and the CD4 cell count. An index of neuropsychological impairment, a control for central nervous system disease progression, was generated with a 3-h test battery (66) from which the sum of all individual test *z* scores generated a cutoff score of 2 SD below the sample mean (identifying 5% of the sample) employed as a categorical indicator for impairment (yes-no).

We also controlled for dietary intake and levels of specific micronutrients in plasma (drawn simultaneously with immune measures). For NKCC, retinol, manganese, ω-3 fatty acids, and selenium intake and levels of vitamins A, B_6, and B_{12}, folate (and erythrocyte [RBC] folate), zinc (and RBC zinc), and iron in plasma were controlled. For lymphocyte proliferative response to PHA, we controlled for ω-3 fatty acid intake and levels of vitamins A, B_6, B_{12}, C, and E, folate (and RBC folate), zinc (and RBC zinc), and iron in plasma. Controls were selected on the basis of known associations with each immune measure (6). Dietary intake data were obtained from the Food Frequency Questionnaire obtained in a 15- to 20-min interview. While the Food Frequency Questionnaire does not yield precise milligrams of nutrients in individuals, it does permit classification of individuals into categories of intake that allow intergroup comparison (67). For macronutrients, serum albumin was obtained by standard rate nephelometry on a blood chemistry profile (SMAC-26; Technicon AU 5000; Technicon, Tarrytown, N.Y.) (5). Plasma prealbumin and retinol-binding-protein levels were measured by standard radial immunodiffusion (27). For micronutrients, vitamin B_6 (pyridoxine) levels were determined by an RBC transaminase assay (61). Zinc (and RBC zinc) and iron levels were determined by flame atomic spectrophotometry (47). Vitamin B_{12} (cobalamin) levels were established by radioisotope dilution assay (39). Folate levels in both plasma and RBCs were determined by a standardized microbiological assay employing *Lactobacillus casei* (60). Serum vitamin A (retinol), C (ascorbate), and E (tocopherol) levels were determined by high-performance liquid chromatography with the use of a standardized method (7, 40).

Statistical methods. Sociodemographic and psychosocial variables were compared by bereavement status by using *t* tests and χ^2 tests, where appropriate. The independent variable, the psychosocial model predictor of bereavement at *T*1, was represented as a categorical indicator (yes-no, over the prior 6 months). Psychosocial model variables affecting immunity used as controls for a specific bereavement effect here were number of background, negatively rated, stressful life events; perceived available social support; and the four coping styles noted above. The bereavement predictor and the negatively rated, stressful life event control at *T*1 were used as variables in both *T*1 and *T*2 immune outcome analyses, since both related to the occurrence of the loss itself. The remaining psychosocial variables (social support, active coping style, disengagement and denial, focus on and venting of emotions, and turning to religion) were used as concurrent control measures because of expected ongoing relationships with immune outcomes, independent of the loss. The bereavement predictor and all other psychosocial variables were used in *T*1 and *T*2 multiple regression models for both immune outcomes, with and without additional immune control variables, and were estimated by using the General Linear Models procedure within SAS (58). Interactions of bereavement status and other psychosocial variables were tested, with only significant interactions reported. All models were successfully confirmed on ranked values. Two steps determined inclusion of other control variables for immune effects as covariates (23). First, all such variables with a theoretical rationale for potentially affecting outcome ($n = 33$) were examined as control variables by using Spearman's rank order correlations. Those with relationships at the (conservative) level of $P < 0.20$ ($n = 8$) were then entered into a multiple regression analysis on the outcome. Only those retaining $P < 0.20$ (NKCC, $n = 5$ at *T*1 and $n = 4$ at *T*2; PHA, $n = 4$ at *T*1 and $n = 2$ at *T*2) were retained in the final multiple regression model as immune control variables with the psychosocial controls and the bereavement predictor. This method for controls allows testing of the hypothesis of whether bereavement specifically accounts for a decrement in NKCC and lymphocyte proliferative response to PHA (at *T*1 and *T*2) beyond other psychosocial variables previously demonstrated to have associations with changes in these immune measures in HIV-1-infected individuals as well as other populations (with life stressors showing associations with cellular immune decrements and social support and active coping style associated with increments) (23, 24, 26), according to our theoretical model (22).

RESULTS

There were no significant differences in sociodemographic characteristics by bereavement status (Table 1). Several significant differences in psychosocial measures by bereavement status, however, were observed (Table 2). The number of negatively rated stressful life events experienced (other than the index loss itself) was significantly higher for the bereaved within 6 months of their loss (*T*1, $P \leq 0.0001$) and remained higher over the following 6 months (*T*2, $P \leq 0.03$) than such stressor counts for the nonbereaved (and those reported for normative male samples) (57). At *T*2, the bereaved also showed a trend toward relying more heavily on focusing on and venting of emotions ($P < 0.09$). Psychological distress measures were not high compared with those of normative samples (45), and social support and coping measures were similar to values previously reported (23) and those obtained for normative samples (11, 16). Interestingly, there were no significant differences in overall psychological distress, depression or anxiety levels, social support, or other coping styles between the bereaved and the nonbereaved at either time point. There were no significant differences in NKCC (by percent cytotoxicity or KLU) or lymphocyte proliferative response to PHA (by stimulation index [SI] or net counts per minute) at *T*1 or *T*2 (Table 2). Though 1986 CDC stage (II versus III) was employed as a control for variability in immunological progression within the sample, the CD4 cell count itself was also examined. There was no significant difference in the CD4 cell count between the bereaved (*T*1, $X = 439$ cells per mm^3 [SD = 247]; *T*2, $X = 432$ cells per mm^3 [SD = 235]) and the nonbereaved (*T*1, $X = 564$ cells per mm^3 [SD = 322]; *T*2, $X = 513$ cells per mm^3 [SD = 290]) (*T*1, $t = 1.72$, $P = 0.09$; *T*2, $t = 1.12$, $P = 0.27$).

TABLE 1. Sociodemographic characteristics

Characteristic	Value for group (SD)		
	Bereaved	Nonbereaved	*P*
n	25	54	
Age (yr)	33.6 (8.3)	32.8 (7.0)	0.66
Education (yr)	15.1 (1.9)	14.3 (2.1)	0.14
Hispanic (%)	48.0	29.6	0.12
Caucasian (%)	52.0	68.5[a]	0.16
Relationship status (%)[b]:			
Single	56.0	53.7	0.85
Open relationship	4.0	9.3	0.36
Monogamous	28.0	33.3	0.64
Living arrangements (%)[c]:			
Living alone	36.0	25.9	0.37
Living with lover	36.0	25.9	0.37
Living with roommate	12.0	25.9	0.16
Living with parents	12.0	20.4	0.37
Modal income category ($)[d]	15,000–20,000	15,000–30,000	0.78

[a] There was one African-American subject, who was nonbereaved.
[b] Two of the nonbereaved and three of the bereaved were widowed, divorced, or legally separated.
[c] One of the nonbereaved and one of the bereaved reported "other" living arrangements.
[d] For the nonbereaved, there were 18 individuals in both the $15,000 to $20,000 and $20,000 to $30,000 categories.

The multiple regression results in Table 3 show a significant, negative effect of bereavement on NKCC at *T*1 ($P = 0.013$), with the negative effect of a history of excessive alcohol use and missing data for this variable controlled for, as well as a negative trend for selenium dietary supplementation. Bereavement and the other psychosocial model variables, taken together, accounted for 20.7% of the variance in NKCC at *T*1 without control variables and for 10.4% when variance due to controls was accounted for first. The unique variance attributable to bereavement alone was 6.9%. At *T*2, the bereavement effect on NKCC also was significant, though not of the same magnitude as that at *T*1 (Table 3; $\beta = -40.7$, $P = 0.04$). Here, bereavement and other psychosocial variables together accounted for 17.4% of the variance and continued to account for 14.7% of the variance when control variables were ac-

TABLE 2. Unadjusted means of variables by bereavement status

Variable	Time point	Mean value (SD) for group		P^a
		Bereaved	Nonbereaved	
Negative life stressor count	*T*1	9.8 (5.5)	5.1 (4.4)	0.0001***
	*T*2	8.0 (6.6)	4.2 (4.1)	0.03*
Social support	*T*1	62.8 (15.3)	66.6 (9.4)	0.26
	*T*2	67.1 (7.1)	65.7 (12.7)	0.80
Active coping	*T*1	102.5 (11.6)	102.3 (12.9)	0.96
	*T*2	102.4 (13.5)	97.6 (19.5)	0.23
Disengagement and denial	*T*1	22.5 (5.7)	21.9 (5.0)	0.61
	*T*2	21.7 (6.8)	21.3 (6.4)	0.83
Focus on and venting of emotions	*T*1	10.1 (2.5)	10.1 (2.5)	0.97
	*T*2	10.5 (2.8)	9.2 (2.6)	0.09+
Turning to religion	*T*1	9.8 (4.0)	8.6 (3.8)	0.20
	*T*2	9.2 (4.0)	8.2 (4.5)	0.41
POMS total mood disturbance score	*T*1	34.4 (31.3)	29.7 (30.1)	0.53
	*T*2	38.2 (38.9)	28.1 (30.6)	0.25
POMS depression/dejection subscale score	*T*1	13.0 (8.0)	10.3 (9.3)	0.21
	*T*2	13.4 (11.4)	10.5 (9.8)	0.30
POMS tension/anxiety subscale score	*T*1	14.6 (8.2)	12.9 (5.6)	0.33
	*T*2	14.4 (8.2)	11.5 (5.5)	0.16
NKCC, %	*T*1	16.7 (11.7)	15.4 (9.3)	0.58
	*T*2	14.3 (8.9)	14.4 (8.9)	0.96
KLU	*T*1	0.39 (0.21)	0.34 (0.23)	0.47
	*T*2	0.34 (0.26)	0.30 (0.23)	0.52
PHA SI[b]	*T*1	92.2 (91.8)	135.6 (163.1)	0.43
	*T*2	93.8 (109.8)	184.4 (510.9)	0.17
PHA net cpm[b]	*T*1	47,613 (35,920)	61,758 (50,851)	0.34
	*T*2	46,370 (26,499)	56,405 (28,947)	0.10+
History of alcohol use				
Excessive	*T*1	0.6 (0.5)	0.63 (0.49)	0.80
Missing data	*T*1	0.00 (0.0)	0.00 (0.0)	0.17
ω-3 fatty acid intake	*T*1	0.19 (0.13)	0.21 (0.25)	0.58
	*T*2	0.44 (0.75)	0.21 (0.26)	0.20
Retinol intake	*T*1	8,849 (8,800)	11,864 (12,025)	0.28
	*T*2	8,434 (10,078)	12,703 (15,789)	0.19
Selenium supplementation	*T*1	0.29 (0.46)	0.31 (0.47)	0.89
	*T*2	0.29 (0.46)	0.31 (0.47)	0.89
Plasma zinc level (μg/ml)	*T*1	0.90 (0.22)	0.94 (0.60)	0.69
	*T*2	0.85 (0.17)	0.87 (0.26)	0.75
Folate intake	*T*1	717 (447)	764 (447)	0.70
	*T*2	656 (393)	690 (343)	0.74

[a] For all tables, *P* values are indicated as follows: +, $0.05 < P \leq 0.10$ (trend); *, $0.01 < P \leq 0.05$; **, $0.001 < P \leq 0.01$; and ***, $P \leq 0.001$.
[b] The mitogen data were analyzed by using $\log_{10}$ transformations to normalize the distribution. The $\log_{10}$ values (and raw medians) of the SI at *T*1 are 1.7 (65.0) and 1.8 (83.9) for the bereaved and nonbereaved, respectively. At *T*2, they are 1.7 (59.5) and 1.9 (80.5). For the net counts at *T*1, they are 4.5 (40,503) and 4.6 (52,728) for the bereaved and nonbereaved, respectively. At *T*2, they are 4.6 (39,025) and 4.7 (48,272).

counted for first. The unique variance attributable to bereavement on NKCC at *T*2 was 6.3%. At *T*1, a positive relationship between active coping style and NKCC was found ($P < 0.005$), though this relationship was driven by the bereaved ($P \leq 0.01$).

The mean NK cell ($CD56^+$) count at *T*1 for the nonbereaved was 201 cells per mm^3 (SD = 137), while that for the bereaved was significantly lower at 138 cells per mm^3 (SD = 95) ($t = 2.30$, $P = 0.03$). At *T*2, there were no significant differences in mean NK cell count between the nonbereaved ($X = 176$ cells per mm^3; SD = 114) and the bereaved ($X = 182$ cells per mm^3; SD = 132) ($t = -0.17$, $P = 0.86$).

While there was no significant effect of bereavement on lymphocyte proliferative response to PHA at *T*1 (Table 3; $\beta = -0.09$, $P = 0.59$), a significant negative effect was found at *T*2, again with excessive alcohol use measures controlled for (Table 3; $\beta = -0.31$, $P \leq 0.03$) (Fig. 1). Psychosocial model predictors, taken together, accounted for 18.7% of the variance in response to PHA at *T*2 without control variables and continued to account for 17.8% when variance due to controls was accounted for first. The unique variance in response to PHA attributable to bereavement at *T*2 was 6.5%. Active coping style was also positively related to lymphocyte proliferative response to PHA at *T*2 ($P = 0.01$), with background stressful life events attending the loss controlled for, and accounted for 9.1% of the unique variance.

The summed $CD4^+$ and $CD8^+$ cell counts, an approximate index controlling for the effect of cell number on lymphocyte proliferative response to PHA, were 1,479 cells per mm^3 (SD = 663) at *T*1 for the nonbereaved and 1,252 cells per mm^3 (SD = 493) for the bereaved, a nonsignificant difference ($t = 1.53$, $p = 0.13$). At *T*2, the mean summed $CD4^+$ and $CD8^+$ cell counts were 1,318 (SD = 494) for the nonbereaved and 1,416 (SD = 624) for the bereaved, also a nonsignificant difference ($t = -0.70$, $P = 0.49$).

The post hoc examinations, with overall psychological distress (NKCC at *T*1: $\beta = 0.003$, $P = 0.94$; PHA response at *T*2: $\beta = -0.004$, $P = 0.11$) and the POMS subscale scores for depression (NKCC at *T*1: $\beta = -0.006$, $P = 0.97$; PHA response at *T*2: $\beta = -0.01$, $P = 0.15$) and for anxiety (NKCC at *T*1: $\beta = -0.07$, $P = 0.72$; PHA response at *T*2: $\beta = -0.008$, *P*

TABLE 3. Multiple regression models on NKCC and lymphocyte proliferative response to PHA at *T*1 and *T*2

Dependent measure[a]	NKCC, %				PHA SI[b]			
	Baseline (*T*1)		6 mo (*T*2)		Baseline (*T*1)		6 mo (*T*2)	
	β (SE)	*P*[c]	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*
Bereavement variables								
Bereavement indicator	−52.71 (14.02)	0.013**	−40.7 (19.6)	0.04*	−0.09 (0.17)	0.59	−0.31 (0.14)	0.03*
Nonbereaved × active coping interaction	−0.52 (0.20)	0.01**	−0.39 (0.19)	0.05*	—[d]		—	
Psychosocial model variables								
Negative life stressor count	0.10 (0.24)	0.69	0.16 (0.26)	0.55	0.01 (0.02)	0.77	0.02 (0.01)	0.23
Social support availability	0.12 (0.14)	0.42	−0.06 (0.14)	0.68	0.00004 (0.009)	0.99	−0.01 (0.01)	0.36
Active coping style	0.51 (0.14)	0.005**	0.37 (0.17)	0.04*	−0.001 (0.007)	0.84	0.01 (0.005)	0.01**
Disengagement and denial	0.37 (0.25)	0.14	−0.21 (0.20)	0.31	0.02 (0.03)	0.31	−0.002 (0.01)	0.83
Focus on and venting of emotions	−0.78 (0.52)	0.14	−0.48 (0.57)	0.40	0.01 (0.02)	0.73	0.01 (0.03)	0.82
Turning to religion	−0.12 (0.30)	0.69	−0.01 (0.30)	0.98	−0.004(0.02)	0.83	−0.01 (0.01)	0.51
Alcohol use controls								
History of alcohol use								
Excessive	−6.46 (2.36)	0.008**	−13.67 (6.73)	0.05*	−0.17 (0.34)	0.62	−0.27 (0.12)	0.20
Missing data	−11.22 (5.77)	0.06+	−3.18 (2.51)	0.21	−0.27 (0.15)	0.08+	−0.43 (0.33)	0.03*
Nutritional controls								
ω-3 fatty acid intake	6.24 (4.91)	0.21	—		—		—	
Retinol intake	0.00013 (0.0001)	0.22	0.00004 (0.0001)	0.63	—		—	
Selenium supplementation	−4.37 (2.50)	0.09+	−3.37 (2.74)	0.23	—		—	
Folate intake	—		—		0.0002 (0.0002)	0.15	—	
Plasma zinc level	—		—		0.22 (0.13)	0.11	—	
Full regression information								
Intercept	12.56 (14.02)	0.37	31.9 (8.4)	0.0004	1.30 (0.91)	0.16	1.44 (0.41)	0.001
R^2	0.40		0.25		0.16		0.26	
Model *F*	3.15	0.002	1.4	0.20	1.05	0.42	2.23	0.04
n	75		64		73		66	

[a] All measures are from concurrent time points except the bereavement indicator and negative life stressor count, which are always at *T*1 assessment (see Materials and Methods).
[b] Log_{10} transformed.
[c] See Table 2, footnote *a*. Excludes full regression information.
[d] —, variable not included in the analysis; hence, no p value is presented.

= 0.52 controlled for), showed no effect on the results with NKCC or with lymphocyte proliferative response to PHA. Including the type of loss (partner versus close friend) in the model also had no effect on these results (NKCC at *T*1: β = −2.58, *P* = 0.62; PHA response at *T*2: β = 0.09, *P* = 0.73). This was also true when controls for disease progression—baseline CD4 cell count, 1986 CDC stage (II versus III), and neuropsychological impairment—were included. Furthermore, none of these analyses had an effect on the relationship of any of the other regression variables (psychosocial, alcohol, or nutrition)

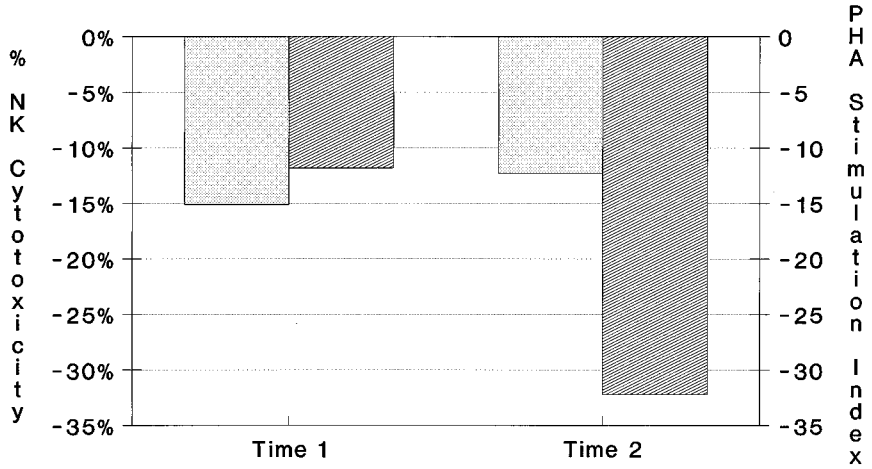

FIG. 1. The differential effect of bereavement on NKCC and lymphocyte proliferative response to PHA for the bereaved versus the nonbereaved is shown for the four linear regression models (percent NKCC and PHA SI at *T*1 and *T*2). These are adjusted values from the regression equations and do not represent actual values for these immune measures. The values presented account for the effects of the control variables used, which are required to properly depict these associations. That is, these adjusted values from the regression models depict what the effect of bereavement would be on the respective immune measure if all the other psychosocial model and control variables could be held constant. ▨, NKCC; ▨, PHA.

TABLE 4. Means of neuroendocrine variables by bereavement status

Variable[a]	Mean value (SD) for group		P^b
	Bereaved	Nonbereaved	
Σ (NE + E)	457.0 (268.7)	315.8 (166.5)	0.02*
Log_{10} (NE + E)	2.60 (0.24)	2.44 (0.25)	
NE	382.3 (251.9)	299.2 (193.1)	0.09+
Log_{10} NE	2.51 (0.25)	2.40 (0.28)	
E	35.2 (26.9)	32.5 (26.6)	0.82
Log_{10} E	1.31 (0.59)	1.27 (0.56)	
Cortisol	12.7 (4.7)	10.3 (3.3)	0.04*

[a] Σ (NE + E), NE, and E are represented in picograms per milliliter and are analyzed using a log_{10} transformation to achieve normality. Cortisol is represented and analyzed in micrograms per deciliter.
[b] See Table 2, footnote *a*.

with the immune outcome measures. The negative impact required for losses examined here acted as a control for losses not deemed to be subjectively stressful; an analysis including all losses as a qualification for the bereavement indicator (n = 30) was conducted and did not change the pattern of these results.

The neuroendocrine results for this sample showed a trend for the level of NE to be higher among the bereaved than the nonbereaved (P = 0.09) (Table 4). When NE and E were summed, the bereaved had significantly higher levels (P = 0.02). Cortisol levels were higher among the bereaved than the nonbereaved (P = 0.04). Results were confirmed by using the Wilcoxon rank sum test. There were no significant Pearson product moment correlations between these three neuroendocrine measures (available at $T1$ only) and percent NKCC or lymphocyte proliferative response to PHA (log_{10}SI): r(NE-NKCC) = 0.13, P = 0.27; r(E-NKCC) = 0.08, P = 0.54); r(cortisol-NKCC) = 0.02, P = 0.88; r(NE-PHA) = 0.01, P = 0.94; r(E-PHA) = 0.07, P = 0.58; r(cortisol-PHA) = −0.20, P = 0.12. However, in a multiple regression analysis of NE, E, and cortisol, a significant negative association of cortisol with concomitant (i.e., $T1$) lymphocyte proliferative response to PHA (log_{10} SI) was found (β = −2.08, P = 0.04).

DISCUSSION

When appropriate controls were used, this study confirmed prior bereavement findings showing that both NKCC and lymphocyte proliferative response to PHA are decreased following a loss of a significant other, and this study extends these findings to the specific setting of HIV-1 infection. In order to interpret the effect of reduced NKCC on a per-cell basis (as determined here), one must also evaluate a possible independent effect on NK cell count. Since NK cell counts for the bereaved were significantly lower than those for the nonbereaved at $T1$, it may be inferred that the effect of bereavement on NKCC was not counterbalanced by an effect on count but, instead, that the decrement in NKCC observed may actually have been compounded by a decrease in count. However, additional statistical controls, such as those used here for the planned regression model tests of associations with NKCC and lymphocyte proliferative response to PHA at $T1$ and $T2$, would be required before this inference could be confirmed. Moreover, no effect on NK cell count was observed for bereavement at $T2$, though a decrement in function on a per-cell basis was still observed. Three caveats regarding the measure of NK cell count used here, identification by anti-CD56 monoclonal antibody alone, should be pointed out. First, NK cells are identified as $CD3^-$ as well as $CD56^+$. The combined use of monoclonal antibody markers against CD3 and CD56 would delineate a $CD3^-$ $CD56^+$ cell population which makes up most, though not all, of the NK cell population (38). At the time this study was conducted, we did not routinely use dual monoclonal antibody marker identification for the NK cell phenotype. This method would also identify a small population of $CD3^+$ $CD56^+$ cells (normally <2%) which otherwise would have been enumerated as NK cells. We do not expect that this small population of cells would have affected the study results here. In fact, this cell population has recently been suggested to have NK functional activity against the K562 cell line at intermediate levels that can be augmented by interleukin 2, although antibody-dependent cell-mediated cytotoxicity (ADCC) and lymphokine-activated killer cell activities are not exhibited and these cells are incapable of producing gamma interferon. Moreover, it has been preliminarily suggested that these cells (identified as $CD3^{bright+}$ $CD56^+$) may increase in number in HIV-1-infected individuals, may demonstrate non-major histocompatibility complex-restricted cytotoxicity, and could represent a part of the immunological response (perhaps genetically based) to HIV-1 infection (65). Hence, these cells may prove relevant in future studies of psychosocial factors and immunological changes in HIV-1 infection. A second, lesser caveat involves the fact that anti-CD56 and anti-CD16, another NK cell monoclonal antibody marker, largely, but not entirely, overlap in identification of NK cells in the peripheral blood. This may be particularly relevant in HIV-1 infection, since CD16 is responsible for mediating ADCC of normal NK cells and a decline in ADCC has been observed with increasing clinical progression of HIV-1 infection. ADCC has been shown to be mediated by gp120/41-specific antibody and has recently been demonstrated to be positively correlated with NKCC in HIV-1-infected individuals (1). Though a controversial issue, it might be important to identify CD16 as well as CD56 on NK cells in studies of psychoneuroimmunological associations. A third issue was the choice of percent cytotoxicity expressed on a per-cell basis. KLU was chosen as an alternative expression that was related to our method for expression of percent cytotoxicity and was found to show similar mean differences between the bereaved and the nonbereaved at baseline; to avoid increasing the experiment-wide error estimate, only one index of NKCC was employed in the regression models at $T1$ and $T2$.

Analogously, for lymphocyte proliferative response to PHA, the total T-lymphocyte count is relevant. The summed $CD4^+$ and $CD8^+$ cell count was employed as an approximate index for this count and is used as internal quality control for this count in our laboratory. However, it should be noted that this approximation may be less accurate with HIV-1 infected individuals than with non-HIV-1-infected individuals because of an increase in the frequency of $CD3^+$ $CD4^-$ $CD8^-$ cells, possibly related to premature release of immature cells from the thymus into the peripheral blood. In HIV-1-infected individuals, this index was not significantly different at $T1$ or $T2$ for the bereaved versus the nonbereaved. Regression models controlling for the CD4 cell count as well as the summative index of the total T-lymphocyte count were conducted and showed the expected significant association of these counts with this immune function. However, there was no change in the overall pattern of regression model variable results reported here. (This was also true when the CD4 cell count was used as a

more refined control for clinical progression in regression models on NKCC, though no significant association of the CD4 cell count with NKCC was observed.) Another issue regarding interpretation of lymphocyte proliferative response involved the choice to employ the SI measure, although release from unstimulated cultures has been demonstrated to be unusually high in HIV-1-infected individuals (596 cpm in HIV-1-positive individuals versus 314 cpm in HIV-1-negative controls) (unpublished data). However, net counts per minute was also examined and was found to show similar mean differences between the bereaved and the nonbereaved at baseline; to avoid increasing the experiment-wide error estimate, only one index of lymphocyte proliferation in response to PHA was employed in the regression models at *T*1 and *T*2. Hence, it may be concluded from the foregoing that the effect of bereavement on lymphocyte proliferative response to PHA at *T*2 was an accurate reflection of this immune function not likely to be affected by cell count in either direction, thus representing a solely functional change.

The experiment-wide error rate accounting for the four regression models conducted in this study was 18.6% with an individual test α of 0.05. To maintain an experiment-wide error rate of 0.05, the models would have to be used with an α of 0.013. The model for the effect of bereavement on NKCC at *T*1 remains significant at this level. When nonsignificant control variables were deleted from the other three models, the bereavement effect on lymphocyte proliferative response to PHA at *T*2 surpassed this α level ($P = 0.002$). The regression model for the effect on NKCC at *T*2 approached significance at this level ($P = 0.03$). To our knowledge, this is the first study of bereavement and immunity in which mood states (depression, anxiety, and overall distress) and other relevant psychosocial factors (negatively rated stress-ful life events, social support availability, and coping style) were simultaneously controlled for. Importantly, we have found that depressed mood, anxious mood, and overall distress did not account for the effects of the bereavement itself, regardless of the perceived impact of the loss or the type of loss. Controls for other effects on these immune measures were also important. Negative associations for the effect of alcohol use on NKCC and lymphocyte proliferative response to PHA have been reported previously outside HIV-1 infection and have also been demonstrated to be of empirical importance in studies relating psychosocial factors to immune measures in HIV-1 infection (23, 24, 26). Though moderate selenium supplementation has been associated with enhanced cellular immune function, in this study it was most likely a marker for excessive multivitamin and mineral supplementation, which has been previously associated with impaired immune function (6, 23). One prior study reported no bereavement effect on immune measures in HIV-1-seropositive individuals (32). However, that study did not report on NKCC, did not employ the psychosocial model variables described above (e.g., active coping style) as controls for a specific effect of bereavement, examined only losses of close friends, and utilized frozen peripheral blood samples, all of which could have contributed to the lack of an observed bereavement effect on lymphocyte proliferative response to PHA. Hence, after accounting for each of these factors in this study, it can be concluded that the stressor of bereavement itself does appear to be most likely to be responsible for this association, as originally suggested by the work of Bartrop et al. (4). Future studies should examine the role of caregiving prior to loss (9, 21) and of complicated bereavement reactions (52).

Our theoretically driven model also demonstrated that active coping style was positively associated with both functional immune measures. For NKCC, this association was driven by the bereaved. For lymphocyte proliferative response to PHA, the effect of active coping was equally prominent among the bereaved and the nonbereaved. Though prior research in bereavement-related psychosocial adaptation focused on the need to vent emotions, the present results suggest that venting emotions may be less important than using active coping styles, such as planning a strategy to deal with the stressor and carrying through such actions, not only for successful psychosocial adaptation to loss (31, 68) but also for its physiological sequelae (23, 25).

The time course of the immunological changes following bereavement depends on which immune measure is observed. NKCC decrements occur within 6 months—prior to decrements in proliferative response to PHA—and persist (though diminished) up to a year, when decrements in proliferative response to PHA were first observed. This time course is supported by bereavement studies with healthy, non-HIV-1-infected subjects, in which NKCC decrements have been found to actually precede the loss itself (29), while lymphocyte proliferative response decrements to PHA occurred after a 6-week delay following the loss (4) but not prior to loss (62) or even three weeks afterward (4). Of note, Irwin et al. (29) found that NKCC decrements following the loss were associated with increased serum cortisol level, but this relationship was not observed for NKCC decrements in anticipation of loss. This suggested that other neuroendocrine mechanisms, such as the sympathetic adrenomedullary system, might be playing a role, in particular the locus ceruleus, a central noradrenergic input for activity of this system with fibers projecting into the parvocellular division of the paraventricular nucleus of the hypothalamus, which may interact with the limbic-hypothalamic-pituitary-adrenal (LHPA) axis in determining bereavement-associated functional immune decrements.

Our results lend support to both neuroendocrine systems being involved in NKCC decrements within the first 6 months of a loss. Interestingly, while an elevated level of cortisol is present at this time, no decrement in lymphocyte proliferative response was yet observed, although a negative association between cortisol level and lymphocyte proliferative response to mitogen has been long recognized (14). This relationship has not always been demonstrated, however, in studies of psychosocial factors and immunity. The analysis described here using multiple regressions of NE, E, and cortisol on lymphocyte proliferative response to PHA was required in order to delineate this association at *T*1. This suggests that previous studies of this relationship might have been affected by the concomitant influences of other neurohormones and that such effects should be controlled for in future studies by using multiple regression controlling for other neurohormones, rather than simple correlation coefficients, to interpret effects. Since LHPA axis effects are known to be of long duration following bereavement, it might be suggested further that an inverse relationship with cortisol level might still be observed at *T*2 and beyond. It has also been suggested that the inverse relationship between cortisol level and lymphocyte proliferative response to PHA may not be observed in response to stressors because of the effect of HIV-1 infection itself on the LHPA axis (2), although that study did not focus on bereavement and did not employ a resting period prior to obtaining a blood sample for neuroendocrine measurements. Twenty-four-hour urinary measures (including 3-methoxy-4-hydroxyphenylethylene glycol, the primary central metabolite of NE, e.g.) are more likely to reflect ongoing neuroendocrine status in response to such a "field" stressor. Thus, both the cross-sectional and the longitudinal effects of neuroendocrine changes presumed to underlie bereavement-associated immunological decrements remain to be conclusively determined.

There is some evidence of the potential for clinically significant consequences of these immunological associations for progression to AIDS and death. In healthy subjects, the immunological decrements observed following bereavement may relate to the increased morbidity and mortality (as much as 10-fold) from a variety of illnesses that have been reported in the year following bereavement, especially in men, though this remains to be adequately demonstrated (50, 55). Regarding the clinical implications of the immune measures assessed in this study, lymphocyte proliferative response to PHA may be of greater import in the asymptomatic stages (as it predicts decreases in the CD4 cell count) while NKCC may be of greater import in AIDS (when the CD4 cell count has already decreased substantially). Moreover, an immune measure specifically related to long-term survivorship, the cytotoxic T-lymphocyte count, has also been negatively related to life stressors in HIV-1 infection (18). One of the putative neuroendocrine mediators found in elevated levels in this study, cortisol, has recently been proposed to be of central importance in clinical progression of HIV-1 infection due to suppression of production of Th1 cytokines (46), enhancement of production of Th2 cytokines (46), induction of apoptosis (programmed cell death) and, more directly, through increased replication of HIV-1 (15). This has been supported by a recent study documenting that cortisol together with HIV-1 envelope peptides produced a decrement in vitro for NKCC not observed with either one alone, i.e., a synergistic effect (48), which may dictate an interaction with a requisite level of HIV-1 viral load in order for cortisol to demonstrate an inhibitory effect. Thus, an effect of cortisol on NKCC in the present study may have been absent because of the early clinical stage of the subjects, who presumably have a low level of viral load, or alternatively may have been masked by the lack of a control for viral load in the sample. Though one study has suggested that no related clinical health effects of psychosocial stressors are observed in HIV-1 infection (33), it has also been reported that decreased use of active coping style (8), increased negative expectations (54), and increased use of denial (28) are related to increased clinical progression of HIV-1 disease.

While early in the epidemic it appeared that multiple losses were associated with increased distress and associated complications (43), subsequent longitudinal research on the same cohort has not confirmed this finding (44). However, despite a lower-than-expected incidence of complicated bereavement reactions, multiple losses nevertheless are associated with high-er levels of grief and a greater sense of subjective threat over time (44, 49). Thus, multiple losses—a frequent characteristic of bereavement in the AIDS epidemic—may be important to investigate regarding unresolved grief, associated immunological changes, and clinical progression of HIV-1 infection.

Bereavement support group interventions may prove to mitigate the neuroendocrine changes associated with bereavement as a stressful life event as well as the immunological decrements demonstrated here and, putatively, clinical progression of HIV-1 disease as well, independent of level of psychological distress following a loss. Regarding such interventions, this work suggests the value of a therapeutic focus on encouraging active coping (putatively, with controllable aspects of the loss) as well as fostering such coping with other types of life stressors generally. Active coping style here also includes seeking instrumental and emotional social support to cope adaptively with stressful life events. The lack of separate relationships with immune measures observed for the perceived social support variable may reflect the need for measures sensitive to other aspects of social support, such as support actually available and received, its sufficiency, and participation in larger organizations (network level of support). It may be inferred that psychotherapeutic group interventions to increase accuracy of stressor appraisal might also prove salutary to cellular immune function, since stressor appraisal is likely to have effects on the coping strategy selected, which in this study was demonstrated to have associations with cellular immune function. Psychotherapeutic interventions aimed at improving accuracy of stressor appraisal (e.g., stress management training) and use of active coping strategy (e.g., cognitive behavioral therapy) currently are available and are of proven clinical value for mental health. Since bereavement support group techniques typically focus on resolution of the grieving process alone (69), these results have implications for expanding the focus of bereavement support group interventions so that attempts to integrate foci on active coping styles (including seeking social support, e.g.) and accurate appraisal of stressor burden directed to adaptive coping are actively incorporated to prevent severe psychological distress and possibly to enhance cellular immune function and deter the clinical progression of HIV-1 infection as well. Though such interventions must still be considered investigational for immunological and related clinical health effects, they may nevertheless be considered indicated clinically at this time for psychological and quality-of-life outcomes, which may also affect clinical health status directly by improving adherence with primary medical care regimens.

ACKNOWLEDGMENTS

We acknowledge the support of NIH grants MH48628, MH48628S, and P50MH43455.

We also acknowledge Robert Morgan for his helpful supervision of database construction and management; Jose Szapocznik and Carl Eisdorfer for their administrative support of the study generating this report; Michael Uselmann, Alicia Capitaine-Ceballos, Victoria Dickman, Ken Christianson, and Janet Yodsnukis for their efforts in data collection and subject management; and Gladys Chayeb for help in manuscript preparation. Finally, we acknowledge the community agencies caring for the HIV-1 infected in Miami, Fla., for referring subjects to this study.

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