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Modulation of Human Natural Killer Cell Activity by Exposure to Uncontrollable Stress[1]

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Changes in natural killer cell (NK) activity and proportions of circulating T and NK lymphocyte subsets were assessed in adult males immediately after exposure to controllable or uncontrollable stress (noise) as well as 24 and 72 h later, in order to track the time course of the effects of stress. The role of control-relevant personality variables as moderators of the stress–immunosuppression relationship was considered. Subjects who perceived they had control over the noise as well as no-noise "control" subjects showed no reduction in NK activity. By contrast, subjects who perceived that they had no control over the stressor showed reduced NK activity immediately after the conclusion of the first 20-min stress session, and the reduced NK activity was found as long as 72 h later. Optimism and one's desire to be in control enhanced the negative impact of uncontrollable noise on NK activity. No differences between conditions were found on number of NK cells or a variety of T cell subsets. The results suggest the importance of perceived control in moderating the short- and long-term effects of stress on NK activity.

Stress has been shown to suppress immune activity in both animals (Shavit, Lewis, Terman, Gale, & Liebeskind, 1984) and humans (Kiecolt-Glaser, Fisher, Ogrocki, Stout, Speicher, & Glaser, 1987; Antoni, Schneiderman, Fletcher, Goldstein, Ironson, & Laperriere, 1990). However, it is still difficult to specify the conditions under which stress has these effects, leading to controversies in the literature (Irwin & Hauger, 1988; see Geiser, 1988, for a review). A more specific model identifying these critical conditions is needed to further examine the effects of stress on the immune system. Three parameters of stress-induced immunosuppression that need clarification are the timing and duration of effects, the role of controllability over the stressor, and the impact that individual difference variables may have on the stress–immunosuppression relationship.

Timing may be a critical factor in the immune response to a stressor. For example, stress may induce transient depression of immune function, followed by return to baseline and then even an overshoot to higher than baseline levels, or the opposite may be true—initial enhancement followed by suppression (Dorian, Garfinkel, Keystone, Gorcynski, Darby, Garner, 1985). Thus, depending on when

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the response is measured in relation to the occurrence of the stressor, immunosuppression, no effect, or even enhancement is possible (Palmblad, 1981; Lysle, Lyte, Fowler, & Rabin, 1987; Sklar, Bruto, & Anisman, 1981). To plot a temporal curve of the impact of a stressor on a measure of the immune system, the present study included multiple poststressor measures. Unless more is known of the time course of stress-induced immunosuppression, the application of findings of stress studies to disease models may be limited.

A second factor that may be crucial in the stress–immunosuppression relationship is the control an individual has over the stressor. The effects of controllability have been widely studied in animal models. Most studies have relied on the triadic-yoked design (Seligman, 1975). In this design, one group is exposed to a stressor (e.g., shock) from which it can escape after learning the correct response, a yoked group receives shock of identical frequency and intensity without the possibility of escape, and a third group receives no shock. Reduced lymphoproliferative responses to the mitogens PHA and ConA (Laudenslager, Ryan, Drugan, Hyson, & Maier, 1983), decreased NK cell cytotoxicity (Shavit et al., 1984), enhanced tumor growth (Sklar & Anisman, 1979), and impaired tumor rejection (Vistainer, Volpicelli, & Seligman, 1982) have been shown in animals exposed to uncontrollable but not controllable stress. Some failures to replicate these findings have been reported (see Maier & Laudenslager, 1988), though recent studies continue to find that uncontrollable, but not controllable stress, has immunosuppressive consequences (e.g., Weiss, Sundar, & Becker, 1989).

Studies in humans also suggest that control might be important in moderating the immunological effects of stress. For example, low perceived control over a significant life stressor has been shown to predict blunted T cell responses to a mitogenic (PHA) and antigenic challenge (mixed lymphocyte culture reactivity) in older people (Rodin, 1986). Similarly, individuals whose explanatory style leads them to see events in their environment as uncontrollable have lower ratios of helper to suppressor T cells and reduced lymphoproliferative responses to PHA (Kamen, Rodin, Seligman, & Dwyer, 1991). Efforts have begun recently to look at more short-term, acute stress, such as might be manipulated in the laboratory, so that stress and control could be experimentally varied rather than studied in a correlational fashion. In a recent laboratory investigation, Weisse, Pato, McAllister, Littman, Breier, Paul, and Baum (1990) reported decreased lymphocyte proliferation to ConA and PHA in subjects who had control over a stressful task—findings that are inconsistent with evidence from animal studies. Clearly, greater clarification of the role that control over a stressor has on immune responses in humans is needed.

A third factor that has not been adequately addressed in previous work in this area is the role of individual differences. In humans, one type of trait variable that may be significant is personality. While personality traits have been correlated with immune functioning in general (Cohen & Williamson, 1991; Jemmott & Locke, 1984; Kiecolt-Glaser & Glaser, 1988), only recently has research begun to explore personality variables as moderating the impact of uncontrollable stress on the immune system. For example, Kamen (1989), studying the effects of uncontrollable noise, found that people with a more optimistic explanatory style, as measured by the Attributional Style Questionnaire (Seligman, Abramson, Semmel, & von Baeyer, 1979), showed a significantly reduced response to delayed cutaneous hypersensitivity testing to tetanus, mumps, and candida challenges.

This suggests that control-relevant individual differences may be important in moderating an individual's response to uncontrollability.

Personality traits that relate to desire for or feelings about control may operate in at least two important ways when a person is exposed to a controllable or uncontrollable stressor. First, how uncontrollable the stressor seems may be determined by certain personality variables (e.g., a desire for control in general). Second, control-relevant individual differences may affect one's efforts to exercise control (Cohen, Evans, Stokols, & Krantz, 1986). For instance, Kamen (1989) found that high "optimism" was associated with a lower rate of responding during an uncontrollable task.

The primary immunologic parameter investigated in the present work was natural killer (NK) cell activity, which has been shown to be highly responsive to psychological stress in both field (Irwin, Daniels, Smith, Bloom, & Weiner, 1987; Levy, Herberman, Simmons, Whiteside, Lee, McDonald, & Beedle, 1989; Kiecolt-Glaser, Speicher, Holliday, & Glaser, 1984) and laboratory studies (Naliboff, Benton, Solomon, Morley, Fahey, Bloom, Makinodan, & Gilmore, 1991). NK cells are large granular lymphocytes that are activated in a nonantigen-specific manner and are unique in that they can recognize, bind, and lyse a variety of target cells without prior exposure to the target cell or antigen. Recently, Whiteside and Herberman (1989) reviewed evidence for the association of low NK activity with the metastatic spread of cancer (Lotzova, Savary, & Herberman, 1987), with increased frequency of acute viral infections (Welsh, 1981), and with certain autoimmune diseases (Gonzales-Amaro, Alcocer-Varela, Martinez-Cordero, & Alarcon-Segovia, 1984). Whiteside and Herberman (1989) point out that most studies have found that stress affects the activity of NK cells rather than the absolute number of cells circulating in peripheral blood. We predicted the same outcome in the present work.

To summarize, the present study was undertaken with three goals in mind: (1) to examine the effect on NK activity of control over a stressor in an experimental laboratory model in humans, (2) to identify individual difference variables (personality traits relevant to control) that might moderate the stress–immunosuppression relationship, and (3) to plot a temporal curve of the immune response to the stressor. The hypothesis tested was that uncontrollable stress would result in greater decreases in NK activity than controllable stress, that the number of NK cells would not be differentially affected, and that personality traits related to issues of control would interact with stressor controllability to reduce NK activity.

METHOD

Subjects

One hundred and five males between the ages of 18 and 26 responded to a local community advertisement for subjects interested in a study of "Personality and Health." (This study was approved by the Human Investigation Committee of the Yale University School of Medicine.) These age and sex exclusion criteria were applied to minimize the between-subject variability in immune function that might result from sex-related hormone and age differences (Ratliff-Crain, Temoshok, Kiecolt-Glaser, & Tamarkin, 1989). Telephone screening of these respondents

was undertaken to exclude those with health-related problems. First, respondents were ineligible to participate due to current use of psychotropic medication (n = 3), cigarette smoking (n = 12), or a history of immune-mediated disease or recent or current infectious illness (n = 17). A checklist of symptoms of respiratory illness was also administered. Nine respondents who had previous experience with "learned helplessness" theory (either through participation in research or through academic course content) were also excluded from the study. Following all exclusion procedures, 64 subjects were tested, 16 per group. Fifty-five of these subjects had all immunologic measures complete for all time points and were included in the final analyses.

Procedure

Following the in-depth screening, subjects were scheduled for three appointments over a 4-day period. All subjects were assigned randomly to begin participation either at 8:00 AM, 10:00 AM, or 12:00 noon. Condition assignment was randomly distributed among the three times. The time at which the subject was studied was kept constant over the 4-day period. Subjects were asked to report to the laboratory each morning after eating a standard breakfast, restricted in fat and caffeine.

On Day 1, the study requirements were explained in detail, eligibility criteria were confirmed, the symptom checklist regarding symptoms of respiratory illness was given again, and the consent form was signed. Subjects were then allowed to relax for 15 min before a baseline blood sample was drawn. After another 15-min rest, the first stress session (described below) was conducted. Each session lasted 20 min. Immediately after session one was completed, a blood sample was taken and the subject was allowed to rest for 30 min. A second stress session then followed, and a third blood sample was obtained at its conclusion.

On the second day the subject again reported to the laboratory after eating the recommended breakfast. Sleep history and health symptoms were taken and a 24-h post-training blood sample was drawn. A packet of questionnaires was then administered. On the third day of the study (72 h postbaseline), subjects returned to the lab after eating the standard breakfast. They reported on their sleep history and health symptoms, and a fifth blood sample was drawn. Subjects were then fully debriefed and were paid $100 for their participation.

Stressor Task

Subjects were assigned at random to one of four conditions, with 16 tested per group: Escapable Noise (EN), Inescapable Noise/Response (IN/R), Inescapable Noise/No Response (IN/NR), No Noise (NN).

Subjects in the Excapable Noise and the Inescapable Noise/Response conditions received the following instructions:

> "From time to time a loud tone will come on for a while. When that tone comes on, there is something you can do to stop it. If the blue light goes on following the termination of the tone, then your response turned it off. If the red light goes on, then your response did not terminate the tone, but rather it went off automatically according to a preprogrammed schedule. Taking the earphones off or dismantling the apparatus is not the way to stop the tone. Please stay seated."

Subjects in the Inescapable Noise/No Response condition were told the following:

"From time to time, a loud tone will come on. Please sit and listen to it."

Subjects in the No Noise condition were asked to sit quietly alone in an adjacent room and read the magazines provided or other reading material they had with them. Subjects in this condition were run at the same times of day as subjects in other conditions, completed the questionnaires and had blood drawn at the same time intervals.

Subjects assigned at random to the stressor conditions listened to the tones through Koss Model Pro 4X Plus headphones. There were 45 trials, and the intertrial interval of noise ranged from 10 to 25 s, with a mean of 14 s, while maximum noise duration (without a correct response) was 5 s.

Subjects in the Escapable Noise condition could terminate the noise if they pressed the button on a box in front of them four times within the 5-s duration of the noise. If they were successful on any given trial, the blue light illuminated; if they were unsuccessful, the red light illuminated. For subjects in the Inescapable Noise/Response condition, all trials were followed by illumination of the red (failure) light, regardless of what response they made. The length of the tone sequence for a subject in both the Inescapable Noise/Response and Inescapable Noise/No Response conditions was yoked to a subject in the Escapable Noise condition, thereby making the duration of exposure to the noise equivalent in all three groups.

Apparatus

A black spring-loaded button housed in a 12 × 8-in. wooden box served as the method by which subjects were able to stop an intermittent noise. Five inches and equidistant from the button were located two 24-V lights—one red, one blue. This apparatus was connected to relays controlling the termination of the noise. The noise was a 90-dB, 3000-Hz tone emanating from an Eico audio generator (Model 377). These parameters were calibrated (General radio Model 1565-B sound level meter) after every 10th subject to assure equivalence between subjects in stimulus intensity.

Measures

Immune measures. Five blood samples were collected for each subject over the 4-day period: three on the first day, one on the second day, and one on the fourth day. The sequence of blood draws was intended to track the course of the immune recovery that occurred poststress. Venipuncture was performed by an experienced nurse, using aseptic technique, with 20 ml of blood drawn into heparinized tubes. Immediately following blood drawing, the specimens were placed in shipping containers, stored at ambient temperature, and shipped by next-day air service to the Immunologic Monitoring and Diagnostic Laboratory at the Pittsburgh Cancer Institute. The specimens were always received and processed within 24 h of being drawn. Prior to this study the laboratory conducted an extensive pilot study to compare samples of immediately processed blood versus samples from the same individual shipped overnight and found differences in NK activity of ±10%. This is not different from daily intraassay differences experienced in this lab.

Following Ficoll–Hypaque centrifugation, mononuclear cells were recovered, washed, resuspended in sterile RPMI 1640 medium supplemented with 2 m*M* glutamine, antibiotics, and 5% (v/v) fetal calf serum, and placed at 4°C overnight. On the following morning, the cells were washed, counted in a trypan blue dye, and assayed for NK activity in a 4-h ^{51}Cr-release assay exactly as described earlier (Whiteside, Bryant, Day, & Herberman, 1990) and for surface markers by two-color flow cytometry.

Briefly, the NK cell assay was performed using K562 as the target, a chronic myelogenous leukemia cell line, maintained in RPMI medium supplemented with 10% (v/v) fetal bovine serum (FBS). Tumor cells were subcultured as needed, and only cultures in the log phase of growth were used for cytotoxicity assays. K562 targets were labeled with 100–150 μCi of sodium ^{51}Cr for 1–2 h at 37°C. Cells were then washed four times in a tissue culture medium (TCM), consisting of RPMI 1640, supplemented with 5% (v/v) fetal bovine serum (FBS), 2 m*M* L-glutamine, penicillin (100 U/ml), streptomycin (100 mg/ml), all from GIBCO (Grand Island, NY). Cells were resuspended in fresh medium, counted, and aliquoted at 5×10^3 targets/well into 96-well U-bottom plates (Costar), into which the effector cells (PBMC) had been previously added at the predetermined concentrations. The effector: target cell ratios ranged from 50:1 to 6:1. Plates were centrifuged at 65*g* for 5 min and incubated in 5% CO_2 in air at 37°C for 4 h, after which medium was harvested from each well using a Skatron supernatant harvesting apparatus (Skatron, Sterling, VA). All determinations were done in triplicate. Radioactivity was counted in a gamma-counter and percentage specific lysis was determined according to the formula:

$$\frac{\text{Mean experimental cpm} - \text{mean spont. release cpm}}{\text{Mean maximal cpm} - \text{mean spont. release cpm}} \times 100.$$

Lytic units were calculated according to the formula of Pross, Baines, Rubin, Shragge, and Patterson (1981). One lytic unit was defined as the number of effector cells, out of 10^7 effector cells, that was required to kill 20% of the 5×10^3 target cells.

The daily variability of the NK cell assay was monitored by using cells from three normal individuals whose peripheral blood mononuclear cells were cryopreserved in large quantities and defrosted daily immediately prior to the NK cell assay. Effector cells with low, medium, and high levels of NK cell activity were cryopreserved in quantities sufficient for 3 to 4 months when used in daily NK cell assays. In addition, each NK cell assay included cells obtained freshly from a normal volunteer. On the basis of over 500 NK assays in this laboratory, mean NK activity for normal donors was 170 LU/10^7 cells, with ranges of 70 and 230 LU/10^7 cells defining the lower and upper limits, respectively, of the interquartile range. The daily variability in the NK cell assay was determined with frozen and fresh normal control cells during the period of this study.

In the present study, all values were within the established normal range, so there was no evidence that the handling and delay between sample collection and assay adversely affected the samples or measurements of NK activity.

Two-color flow cytometry was performed to determine the phenotype of circulating T lymphocytes and NK cells. Peripheral blood mononuclear cells were separated on Ficoll–Hypaque gradients, washed in medium, counted in trypan

blue dye, and adjusted to a concentration of 5×10^5/ml in PBS–0.1% (v/v) sodium azide. Next, 0.2 ml of this cell suspension were incubated with 5 μl of fluorescein- or phycoerythrin-labeled monoclonal antibodies for 30 min at 4°C. The cells were then washed three times in PBS sodium azide and fixed in 2% (w/v) paraformaldehyde solution in PBS. Two-color analysis was performed on FACScan (Becton-Dickinson FACS System, Mountain View, CA). The monoclonal antibodies employed included the following specificities: Leu4 (anti-CD3), Leu2a (anti-CD8), Leu3a (anti-CD4), Leu19 (anti-CD56), anti-IL2R (anti-CD25), Leu11a (anti-CD16), and Leu12 (anti-CD19). As controls, mouse isotypes IgG1 and IgG2a were used in all experiments. Cells in the "lymphogate" were always stained with anti-CD45 (FITC) and antiCD14 (PE) monoclonal antibodies to estimate the percentage of all leukocytes and monocytes, respectively. The "lymphogate" typically contained >95% lymphocytes. All antibodies were purchased from Becton Dickinson.

Behavioral measures. Three different measures of behavior were computed for each subject. One measure was the total number of responses made during the 45 presentations of noise (Kamen, 1989). The minimum number of responses to successfully terminate the noise in all 45 trials was 180. A second measure, the total number of responses made between trials, assessed whether subjects tried other strategies to avoid the noise if they were unable to terminate it during its presentation. A third measure, the mean latency from the onset of noise to the subject's first response, served as an indicator of involvement in the task. That is, longer latencies may suggest a lower amount of effort toward the task.

Psychological measures. Four measures were chosen on the basis of their relevance to the concept of control. It was reasoned that individual differences in attitude toward and feelings about control and self-efficacy might be a moderating factor in subjects' responses to an uncontrollable stressor. Each measure used has a reported internal consistency and test–retest reliability of over .70. The following questionnaires were administered to each subject: (1) Attributional Style Questionnaire (ASQ; Seligman et al., 1979), which assesses how people explain positive and negative life events. Respondents were presented with 12 hypothetical bad events involving themselves and were asked to rate the cause of this event in terms of internal (self) versus external control, stability versus instability, and globality versus specificity; (2) Life Orientation Test (LOT; Schier & Carver, 1985) consists of eight items which measure an individual's expectations that he or she is able to get good as opposed to bad outcomes; (3) Self-Control Schedule (SCS; Rosenbaum, 1980), which is a 36-item scale that measures an individual's tendency to apply self-control methods to the solution of behavioral problems; and (4) Desire for Control Scale (DC: Burger & Cooper, 1979), which consists of 20 items scored on 7-point Likert scales that tap an individual's level of motivation to control a variety of events in one's life.

Statistical Methods

In order to assess differences among groups in immune response over time, the primary analysis used was a repeated measures analysis of covariance (ANCOVA) of lytic units (LU) with basal values as the covariate. Analyses of lytic units were performed on logarithmic transformations in order to normalize data distribution, as suggested by Whiteside et al. (1990). Planned contrasts were then performed to assess the specific differences among conditions. Finally, re-

gression analyses exploring possible personality influences on immunological response were performed.

RESULTS

NK Cell Activity

NK activity measured at baseline in LU, as well as at four different effector to target (E:T) ratios, was not significantly different among the four conditions studied (Table 1). All measured NK activity was within normal control values established by the laboratory (see Methods). However, given the large between-subjects variance and the interest in identifying between-condition differences after exposure to stress, a repeated-measures analysis of covariance, using the baseline LU as the covariate, was used to test the primary hypothesis.

The overall ANCOVA revealed a significant effect for condition ($F(3,50) = 6.20$, $p < .001$) with no significant effect for time and no interaction of time by condition. Post hoc contrasts (with Bonferroni corrections) indicated that while the Escapable Noise, Inescapable Noise/Response, and No Noise conditions did not differ significantly form one another overall, NK activity for the Inescapable Noise/No Response subjects was significantly lower compared to the other three conditions (all p's $< .001$).

The mean log lytic units of NK activity for each condition at all five time points are shown in Fig. 1. It can be seen that while there were no significant differences between groups prestress, the Inescapable/No Response group differed significantly from the other three groups overall.

Lymphocyte Subset Enumeration

In addition to NK activity, proportions of total T lymphocytes (CD3+), CD8+ and CD4+ T lymphocyte subsets, and proportion of NK cells (CD56+) were determined at baseline and all time points after the stressor. These are presented in Table 2. As expected, no statistically significant differences were found be-

TABLE 1
Means (and Standard Deviations) of Percentage Specific Lysis by Condition at Baseline

	Condition			
NK E:T ratios	EN ($n = 15$)	IN/R ($n = 14$)	IN/NR ($n = 14$)	NN ($n = 12$)
50:1	48.8	47.6	41.8	41.3
	(16.8)	(18.0)	(13.0)	(15.2)
25:1	34.2	32.9	26.0	25.4
	(15.6)	(16.5)	(8.3)	(13.3)
12:1	21.0	21.2	14.4	15.1
	(13.0)	(12.4)	(4.7)	(7.3)
6:1	12.8	11.9	7.9	8.4
	(10.1)	(7.7)	(2.9)	(5.1)
Lytic Units ($LU20/10^7$ cells)	177.1	185.1	115.5	123.5
	(153.9)	(141.5)	(47.4)	(76.2)

Note. No significant differences among groups were found ($p > .10$) and all values are within normal limits for NK activity as defined for normal adults in this laboratory.

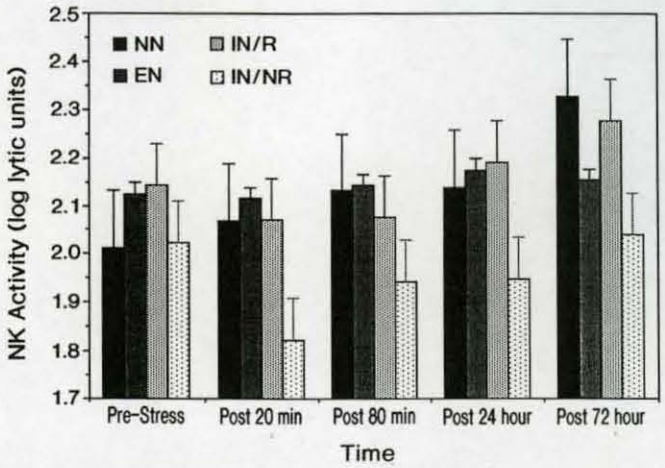

FIG. 1. Natural killer (NK) cell activity of peripheral blood mononuclear cells expressed in mean log lytic units. The data over time for the four groups of subjects studied are shown.

tween mean percentages over time or among conditions for CD8+ (T suppressor), CD4+ (T helper), or for CD56+ (NK cell) subsets. There was an unexpected significant decrease in the mean percentage of CD3+ T lymphocytes at 24 h after the stressor ($p < .01$) which occurred in all groups but EN, and which probably reflected a decrease in percentages of CD4+ T cells.

Table 3 provides the means for other blood measures taken. Previous research has been inconsistent in showing an impact of stress on these measures and so the lack of significant differences between conditions in the present study was not unexpected.

Behavioral Data

A MANOVA of the behavioral responses of the subjects who had the response key present—those in the Escapable Noise and Inescapable Noise/Response experimental groups—was performed. The overall effect for condition was highly significant after both the first stressor ($F(1,29) = 5.28$, $p < .05$) and the second stressor ($F(1,29) = 19.43$, $p < .001$) sessions. As shown in Table 4, subjects in the Escapable Noise condition demonstrated learning over time as they began to make more correct responses during the noise presentation from the first to second trial. Subjects in the Inescapable Noise/Response condition appeared to be still searching for a response that would terminate the noise. At Time 1 they pressed about equally during the noise presentation and between trials, leading to significant differences between them and subjects assigned to the Escapable Noise condition in between-trial responding. In the second stressor period, they further decreased their number of responses during the noise, but continued to make a greater number of responses between trials. This suggests that subjects in the Inescapable Noise/Response condition may not have experienced helplessness because they were still attempting to gain control by trying another strategy when they found that responding during the task did not serve to terminate the noise. No statistically significant differences were found between experimental groups on latency to respond to the noise.

TABLE 2
Means for T Lymphocytes and NK Cells (Standard Deviations) across All Five Time Points by Condition

	Condition			
	EN (n = 15)	IN/R (n = 14)	IN/NR (n = 14)	NN (n = 12)
Total T cells (CD3+)				
Baseline	73.4 (5.4)	74.4 (7.7)	73.2 (8.5)	72.7 (5.2)
Post first stressor				
20 min	73.3 (5.5)	73.5 (7.1)	72.5 (10.1)	75.1 (8.4)
80 min	74.9 (5.4)	74.2 (6.2)	74.1 (6.8)	73.3 (8.8)
24 h	73.2 (6.5)	71.0 (7.5)	69.9 (10.1)	69.3 (5.2)
72 h	73.9 (6.4)	70.5 (7.4)	71.4 (10.5)	71.0 (8.6)
Suppressor cells (CD8+)				
Baseline	27.9 (6.4)	29.9 (5.1)	28.3 (6.9)	26.5 (5.4)
Post first stressor				
20 min	27.7 (5.9)	28.5 (5.3)	28.1 (6.6)	25.3 (5.6)
80 min	28.9 (6.3)	29.6 (5.3)	29.3 (5.9)	26.5 (4.6)
24 h	28.3 (7.7)	29.6 (5.1)	28.7 (7.6)	24.4 (5.9)
72 h	30.6 (7.7)	29.1 (6.2)	28.3 (7.4)	24.6 (8.0)
Helper cells (CD4+)				
Baseline	45.5 (5.6)	44.5 (8.5)	44.9 (7.0)	46.2 (4.7)
Post first stressor				
20 min	45.6 (6.4)	45.0 (7.8)	44.4 (7.3)	49.9 (5.8)
80 min	46.1 (5.9)	44.6 (7.3)	44.8 (5.6)	46.8 (7.6)
24 h	44.9 (8.4)	41.4 (8.8)	41.2 (7.9)	44.9 (5.2)
72 h	43.3 (7.9)	41.4 (8.2)	43.1 (6.8)	46.4 (5.0)
NK cells (CD56+)				
Baseline	13.5 (5.6)	13.0 (6.6)	10.3 (4.8)	12.3 (3.8)
Post first stressor				
20 min	13.5 (7.5)	11.6 (5.2)	11.6 (8.7)	11.3 (6.6)
80 min	11.9 (5.0)	12.1 (4.1)	10.7 (5.8)	13.5 (6.3)
24 h	15.8 (8.8)	17.3 (8.6)	12.1 (5.9)	14.8 (7.0)
72 h	16.4 (6.0)	16.0 (6.4)	12.6 (5.1)	14.1 (7.5)

TABLE 3
Means (and Standard Deviations) for Blood Measures Collected at Baseline between Conditions

	Condition			
	EN (n = 15)	IN/R (n = 14)	IN/NR (n = 14)	NN (n = 12)
White blood cell count	497.1 (119.0)	424.3 (119.8)	481.3 (123.0)	520.3 (139.9)
Percentage monocytes	5.2 (1.7)	6.1 (2.0)	5.8 (2.0)	6.9 (1.7)
Percentage lymphocytes	34.8 (8.2)	38.9 (9.5)	35.3 (8.2)	33.2 (8.6)

Personality Data

As would be expected by random assignment of subjects to condition, there were no significant differences among groups on any of the personality measures. The hypothesis suggests that control-relevant personality traits may moderate immunologic response to a stressor as a function of whether it is controllable. Therefore, only subjects exposed to the noise were used to test this hypothesis. A hierarchical regression analysis was conducted to test for the effects of personality in interaction with condition on NK activity immediately after the stressor, as well as at all subsequent time points. In step one, we entered as control variables the effects of baseline log lytic units at Time 1, the main effects for experimental group, and the main effects for personality. In step 2, the interaction terms between personality and experimental group were entered. There were significant interactions between condition and scores on the Desire for Control scale ($F = 3.16$, $p < .05$) and the Life Optimism tests ($F = 2.97$, $p < .05$) immediately after the stressor. There were no significant interaction effects at later time points.

For illustrative purposes, Fig. 2 shows subjects' scores on the Desire for Control scale plotted against the adjusted log lytic units immediately after the stressor. Regression lines for each condition are also shown. It can be seen that the higher one's desire to have control, the greater the suppression in NK activity after exposure to the uncontrollable situation (Inescapable Noise/No Response).

TABLE 4
Behavioral Measures (and Standard Deviations) for Both Stressor Periods for both Escapable Noise (EN) and Inescapable Noise/Response (IN/R) Conditions

	Stressor time 1		Stressor time 2	
	EN	IN/R	EN	IN/R
Total number of responses during noise	103 (66)	82 (76)	152 (48)	55* (55)
Total number of responses between trials	38 (26)	82* (50)	45 (39)	90* (73)
Mean latency (s/trial) to first response	0.8 (0.4)	0.7 (0.4)	0.8 (0.5)	0.6 (0.4)

Note. Asterisks indicate a significant difference ($p < .05$) between conditions within the given stressor period.

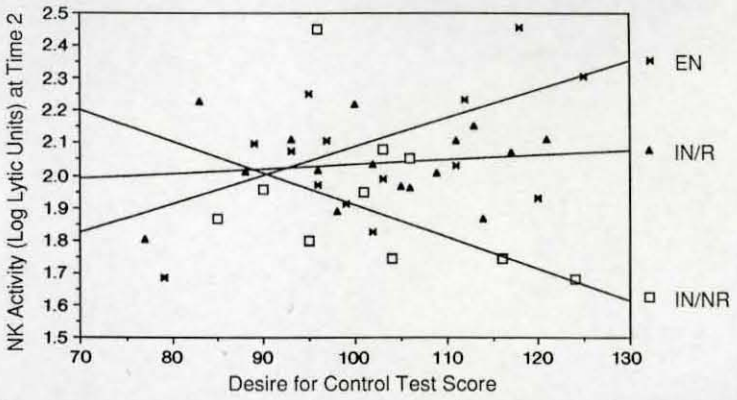

FIG. 2. Scatterplot and regression lines for each experimental group. Scores on the Desire for Control scale are plotted on the *X*-axis while log lytic units immediately after the first stressor period are plotted along the *Y*-axis.

DISCUSSION

Several studies have observed reduced immune responses associated with chronic stress (e.g., Kiecolt-Glaser, Glaser, Shuttleworth, Dyer, Ogocki, & Speicher, 1987) or acute stress such as examinations (Kiecolt-Glaser et al., 1984). However, in order to elaborate a clear causal relationship and to specify the variables responsible for the stress–immunosuppression relationship, controlled laboratory investigations are also needed.

In the present study it was found that subjects exposed to uncontrollable stress without any available behavioral response exhibited reduced NK activity immediately after 20 min of exposure to the stressor as well as up to 72 h later. For these subjects, no response keys were present and they did not try any observable type of response to terminate the noise. Although the immediate clinical significance of the reduced NK activity is not known, the duration of such an effect is noteworthy given the short exposure to the uncontrollable noise (two sessions of 20 min each). The current findings suggest that the timing of the interaction between uncontrollable stress and exposure to a pathogen needs to be examined more systematically.

It is possible that the reduced NK activity seen at 24 and 72 h poststress represents, in part, a conditioned response rather than sustained reduction due to the stressor on Day 1. Substantial evidence exists in the animal literature that immune responses can be modified by classical conditioning (e.g., Ader & Cohen, 1985; Gorczynski, 1987). While subjects were tested on Days 2 and 4 in a different room with no noise apparatus present, the same nurse drew the blood and the room was in the same research unit where the initial noise study occurred. Thus, the possibility of a conditioned response exists. Distinguishing effects due to conditioning from those due to long-term reduction would be important to explore in future studies. Additionally, given the extensive research on cortisol (e.g., Hanson, Larson, & Snowdon, 1976) and catecholamines (e.g., Weiss, 1970; see Dantzer, 1989, for a review) as mechanisms for the effects of control on immune function, future research on humans would do well to assess these endocrine responses simultaneously with immune measures such as NK activity.

A second finding worthy of further exploration is the difference in NK func-

tional data between the subjects exposed to noise without a behavioral response available compared to subjects in the inescapable noise condition where the response keys were present. The latter group of subjects never found the correct response but had behavioral options since the response keys were available. Correctly recognizing that their responses *during* the noise were ineffective in stopping the noise, these subjects appeared to be trying to escape the noise by responding more during the intertrial intervals. It may have been harder to learn that this effort was ineffective, since the range of times to noise onset (the intertrial interval) was larger (10–25 s) than the range of noise exposure times (0–5 s). Since they appeared to believe that they had behavioral options and they continued to exercise them, it is important to note this group had levels of NK activity that were as high as subjects in the Escapable Noise condition. The reduced NK levels only for subjects who had no response options even available agrees with previous animal (Fleshner, Laudenslager, Simmons, & Maier, 1989) and human studies (Kamen, 1989), which have shown that inactivity associated with a submissive posture toward a dominant animal is associated with reduced immune responses. These findings suggest that future studies should evaluate how subjects' behavioral responses to stress, which do represent a form of active coping, influence immune activity.

The present data also suggest that responding may prevent the perception of uncontrollability, thus protecting one from the consequences of exposure to uncontrollable noise. Subjects without a behavioral response available to them may have experienced greater helplessness and therefore showed a corresponding reduction in NK activity. It is possible that if subjects in the Inescapable Noise/Response condition were exposed to the stress repeatedly, they would eventually have learned that none of their responses were effective, ultimately leading to reduced NK activity as well.

Differences between the current results and those obtained by Weisse et al. (1990) need to be explored. Although Weisse et al. (1990) found reduced immune responses in subjects exposed to controllable stress as compared to subjects exposed to uncontrollable stress, no significant differences in perceived control were found between these two groups. The two groups they compared are similar in their experience to subjects in EN and IN/R groups in our study—these groups showed no difference in NK activity poststress. The addition of a group that was exposed to uncontrollable stress with no response available indicates that perceptions of controllability, rather than actual lack of control, may be an important factor in the effects of uncontrollability. More work is needed to define the conditions under which uncontrollable stress does and does not have immunosuppressive effects.

Possible mechanisms for the effects found here need to be explored. As mentioned above, much work with animals has shown cortisol and catecholamine differences in response to uncontrollable stress and these are worthy of investigation in humans. However, it is not likely that neuroendocrine differences, if they existed, were caused by differences between conditions in behavior (i.e., button-pressing). Subjects in the IN/NR condition, who had no button to press, showed significant differences poststress from the experimental controls who also made no responses. Clearly, the biological mechanisms that may help explain the effects of uncontrollability on immune functioning remain elusive. Additionally, the current results showed no differences between conditions in lymphocyte sub-

set counts. Given that previous work using different stressors have shown differences (Manuck, Cohen, Rabin, Muldoon, & Bachen, 1991; Naliboff et al. 1991), clarifying the parameters of the stress as well as the parameters of immune functioning affected should be the focus of future work. Replication and refinement of the present findings is currently being conducted.

Another significant finding of this study is the apparent importance of control-relevant individual differences in moderating the immune response to uncontrollable stress. It appears that subjects who have a high desire for control, as well as those who are generally more optimistic, show the greatest reduction in immunoreactivity subsequent to uncontrollable stress. These findings suggest that those subjects who, by personal disposition, tend to expect positive and controllable outcomes are the most stressed when the actual outcome is contrary to their expectations. The fact that the moderating effects of these personality variables on NK activity last only a short time, however, is noteworthy. One hypothesis is that these traits, while making an individual susceptible to the acute consequences of uncontrollable stress, also confer the ability to rebound quickly once the stressor is over. We believe that this is the first experimental evidence for the moderating role of control-relevant personality traits in the human immunological response to uncontrollable stress. These results suggest that future studies should investigate more fully the influence that certain types of individual differences may have on immunomodulation after exposure to stress. Attention to identifying personality traits that may be especially relevant to the specific situation under consideration, however, is essential for this type of analysis (Mischel, 1977).

REFERENCES

Ader, R., & Cohen, N. (1985). CNS-immune system interactions: Conditioning phenomena. *Behav. Brain Sci.* **8**, 379–395.

Antoni, M. H., Schneiderman, N., Fletcher, M. A., Goldstein, D. A., Ironson, G., & Laperriere, A. (1990). Psychoneuroimmunology and HIV-1. *J. Consult. Clin. Psychol.* **58**(1), 38–49.

Burger, J. M., & Cooper, H. M. (1979). The desirability of control. *Motivation and Emotion* **3**(4), 381–393.

Cohen, S., Evans, G. W., Stokols, D., & Krantz, D. S. (1986). *Behavior, Health and Environmental Stress*. Plenum: New York.

Cohen, S., & Williamson, G. M. (1991). Stress and infectious disease in humans. *Psychol. Bull.* **109**(1), 5–24.

Dantzer, R. (1989). Neuroendocrine correlates of control and coping. In A. Steptoe (Ed.), *Stress, personal control, and health*. Wiley & Sons: Brussels.

Dorian, B. J., Garfinkel, P. E., Keystone, E. C., Gorczynski, R., Darby, P., & Garner, D. M. (1985). Occupational stress and immunity. *Psychosomatic Med.* **47**, 77.

Fleshner, M., Laudenslager, M. L., Simons, L., & Maier, S. (1989). Reduced serum antibodies associated with social defeat in rats. *Physiol. Behav.* **45**, 1183–1187.

Geiser, D. S. (1988). Psychosocial influences on human immunity. *Clin. Psych. Rev.* **9**(6), 689–715.

Gonzalez-Amaro, R., Alcocer-Varela, J., Martinez-Cordero, E., & Alarcon-Segovia, D. (1984). Natural killer cell-mediated activity in mixed connective tissue disease and its response to induction by interleukin 2. *J. Clin. Immunol.* **4**, 273–279.

Gorczynski, R. M. (1987). Analysis of lymphocytes in, and host environment of, mice showing conditioned immunosuppression to cyclophosphamide. *Brain Behav. Immun.* **1**, 21–35.

Hanson, J. D., Larson, M. E., & Snowdon, C. T. (1976). The effects of control over high intensity noise on plasma cortisol levels in rhesus monkeys. *Behav. Biol.* **16**, 333–340.

Irwin, M., Daniels, M., Smith, T. L., Bloom E. T., & Weiner, H. (1987). Life events, depressive symptoms, and immune function. *Am. J. Psych.* **144**(4), 437–441.

Irwin, M. R., & Hauger, R. L. (1988). Adaptation to chronic stress: Temporal pattern of immune and neuroendocrine correlates. *Neuropsychopharmacology* **1**(3), 239–242.

Jemmott, J. B., & Locke, S. E. (1984). Psychosocial factors, immunologic mediation, and human susceptibility to infectious disease: How much do we know? *Psychol. Bull.* **95**, 78–105.

Kamen, L. (1989). *Learned helplessness, cognitive dissonance, and cell-mediated immunity*. Unpublished doctoral dissertation, University of Pennsylvania.

Kamen, L., Rodin, J., Seligman, M. E. P., & Dwyer, J. (1991). Explanatory style and cell-mediated immunity in elderly men and women. *Health Psychol.* **10**(4), 229–235.

Kiecolt-Glaser, J., & Glaser, R. (1988). Methodological issues in behavioral immunology research with humans. *Brain Behav. Immun.* **2**, 67–78.

Kiecolt-Glaser, J. K., Fisher, L., Ogrocki, P., Stout, J. C., Speicher, C. E., & Glaser, R. (1987). Marital quality, marital disruption, and immune function. *Psychosomatic Med.* **49**, 13–34.

Kiecolt-Glaser, J. K., Glaser, R., Shuttleworth, E. C., Dyer, C. S., Ogrocki, P., & Speicher, C. E. (1987). Chronic stress and immunity in family caregivers of Alzheimer's disease victims. *Psychosomatic Med.* **49**, 523–535.

Kiecolt-Glaser, J. K., Speicher, C. E., Holliday, J. E., & Glaser, R. (1984). Stress and the transformation of lymphocytes by Epstein–Barr virus. *J. Behav. Med.* **7**, 1–12.

Laudenslager, M. L., Ryan, S., Drugan, P. C., Hyson, R. L., & Maier, S. F. (1983). Coping and immunosuppression: Inescapable but not escapable shock suppresses lymphocyte proliferation. *Science* **221**, 568–570.

Levy, S., Herberman, R., Simmons, A., Whiteside, T., Lee, J., McDonald, R., & Beadle, M. (1989). Persistently low natural killer cell activity: Immunological, hormonal and psychological correlates. *Natural Immun. Cell Growth Regul.* **8**, 173–186.

Lotzova, E., Savary, C. A., & Herberman, R. (1987). Impaired natural killer cell profile in leukemia patients. In E. Lotzova & R. Herberman (Eds.), *Immunology of natural killer cells*. CRC Press: Florida.

Lysle, D. T., Lyte, M., Fowler, H., & Rabin, B. S. (1987). Shock-induced modulation of lymphocyte reactivity: Suppression, habituation, and recovery. *Life Sci.* **41**, 1805–1814.

Maier, S. F., & Laudenslager, M. L. (1988). Inescapable shock, shock controllability, and mitogen stimulated lymphocyte proliferation. *Brain Behav. Immun.* **2**, 87–91.

Manuck, S. B., Cohen, S., Rabin, B. S., Muldoon, M. F., & Bachen, E. A. (1991). Individual differences in cellular immune response to stress. *Psychol. Sci.* **2**(2), 111–115.

Mischel, W. (1977). On the future of personality assessment. *Am. Psychol.* **32**(4), 246–254.

Naliboff, B. D., Benton, D., Solomon, G. F., Morley, J. E., Fahey, J. L., Bloom, E. T., Makinodan, T., & Gilmore, S. L. (1991). Immunological changes in young and old subjects during brief laboratory stress. *Psychosomatic Med.* **53**(2), 121–132.

Palmblad, J. (1981). Stress and immunological competence: Studies in man. In R. Ader (Ed.), *Psychoneuroimmunology*, pp. 229–257. Academic Press: New York.

Pross, H. F., Baines, M. G., Rubin, P., Shragge, P., and Patterson, M. S. (1981). Spontaneous human lymphocyte-mediated cytotoxicity against tumor target cells. IX. The quantification of natural killer cell activity. *J. Clin. Immunol.* **1**, 51–63.

Ratliff-Crain, J., Temoshok, L., Kiecolt-Glaser, J. K., & Tamarkin, L. (1989). Issues in psychoneuroimmunology research. *Health Psychol.* **8**(6), 747–752.

Rodin, J. (1986). Health, control and aging. In M. Baltes & P. Baltes (Eds.), *Aging and control*. LEA: Hillsdale, NJ.

Rosenbaum, M. (1980). A schedule for assessing self-control behavior: Preliminary findings. *Behav. Ther.* **11**, 109–121.

Schier, M. F., & Carver, C. S. (1985). Optimism, coping, and health: Assessment and implications of generalized outcome expectancies. *Health Psychol.* **4**, 219–247.

Seligman, M. E. P. (1975). *Helplessness: On depression, development, and death*. San Francisco: Freeman & Co.

Seligman, M. E. P., Abramson, L. Y., Semmel, A., & von Baeyer, C. (1979). Depressive attributional style. *J. Abnormal Psychol.* **88**, 242–247.

Shavit, Y., Lewis, J., Terman, G., Gale, R., & Leibeskind, J. (1984). Opiod peptides mediate the suppressive effect of stress on natural killer cell cytotoxicity. *Science* **223**, 188–190.

Sklar, L. S., & Anisman, H. (1979). Stress and coping factors influence tumor growth. *Science* **205**, 513–515.

Sklar, L., Bruto, V., & Anisman, H. (1981). Adaptation to the tumor-enhancing effects of stress. *Psychosomatic Med.* **43**, 331–342.

Vistainer, M. A., Volpicelli, J. R., & Seligman, M. E. P. (1982). Tumor rejection in rats after inescapable or escapable shock. *Science* **216**, 437–439.

Weiss, J. M. (1970). Somatic effects of predictable and unpredictable shock. *Psychosomatic Med.* **32**, 397–409.

Weiss, J. M., Sundar, S. K., & Becker, K. J. (1989). Stress-induced immunosuppression and immunoenhancement: Cellular immune changes and mechanisms. *Neuroimmune Networks: Physiol. Dis.* 193–206.

Weisse, C. S., Pato, C. N., McAllister, C. G., Littman, R., Breier, A., Paul, S. M., & Baum, A. (1990). Differential effects of controllable and uncontrollable acute stress on lymphocyte proliferation and leukocyte percentages in humans. *Brain Behav. Immun.* **4**, 339–351.

Welsh, R. (1981). Do natural killer cells play a role in virus infections? *Antiviral Res.* **1**, 5.

Whiteside, T., Bryant, J., Day, R., & Herberman, R. (1990). Natural killer (NK) cytotoxicity in diagnosis of immune dysfunction: Criteria for a reproducible assay. *J. Clin. Lab. Anal.* **4**, 102–114.

Whiteside, T., & Herberman, R. (1989). The role of natural killer cells in human disease. *Clin. Immunol. Immunopathol.* **53**, 1–23.

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