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Recent Japanese research, as well as pilot work preliminary to this current project, have pointed to a possible association between mood and lowered natural killer (NK) cell activity. In this previous work, a subgroup of individuals characterized by persistently low NK activity, and self-reported depression and fatigue, tended to report more serious illness on follow-up assessment. In this current study, we have accrued approximately, 104 normal individuals to this prospective project. Preliminary analyses have been carried out in order to identify the incidence of persistently low natural killer cell activity in this population of young adults, and to characterize the psychological profile associated with this pattern of NK activity. Subjects were tested serially, at baseline induction into the study, and at 2 week and 4 week follow-up. Baseline assessment included a complete physical examination and laboratory work-up in order to exclude anyone ill at the time of enrollment, and to have complete physical status information on all subjects at the time of accrual. NK activity, urinary catecholamine levels, and psychological status were also assessed at baseline, and at the specified follow-

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up intervals. Individuals with low NK activity were operationally defined as those having NK function either below the group mean, or below the group lowest quartile, at baseline and at two follow-up assessments. Results showed that in both univariate analyses, as well as in logistic regression models, age and the perception of environmental stressors or "hassles" predicted persistently low NK activity. Younger subjects, who perceived environmental events to which they were exposed as more serious in nature, were more likely to exhibit a persistently low NK profile over time than older individuals who perceived daily events as less important to them.

ANNUAL REPORT

Stress, Coping, and Infectious Illness: Persistently Low Natural Killer Cell Activity as a Host Risk Factor

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I. Introduction

The study of stress and coping responses linked with disease end-points in animal systems has revealed enhancement of disease risk under a variety of stress conditions (Visintainer, Volpicelli, and Seligman, 1982; Laudenslager, Ryan, Drugan, et al., 1983). A number of such studies have also examined endocrinological and immunological mechanisms potentially mediating behavior and disease end-points (Shavit, Lewis, Terman, et al., 1983; Sklar and Anisman, 1979; Greenberg, Dyck, and Sandler, 1985; Schneiderman, 1986). However, there are scant clinical data demonstrating a link between distress response and disease end-points mediated by regulatory mechanisms such as immune function.

A number of studies have demonstrated a correlation between stressful life events of various kinds and increased incidence of acute, infectious illnesses, such as upper respiratory infections and infectious mononucleosis, as well as outbreaks of herpes simplex (Ishigami, 1919; Hinkle and Plummer, 1952; McClelland, Alexander, and Marks, 1982; Kemeny, et al., 1986). Several investigations have also indicated an association between stressful events and lymphocyte alteration (Palmblad, Blomback, Egberg, et al., 1977; Bartrup, Luckhurst, Lazarus, et al., 1977; Crary, Borysenko, Sutherland, et al., 1983; Jemmott and Locke, 1984). However, with only a few exceptions (Kasl, et al., 1979, and Kemeny, et al., 1986), a major limitation of most human studies has been that they were not conducted in a prospective fashion, examining the association of life stress and coping ability with hormonal and immunological changes, with subjects then followed to assess the incidence of disease episodes. Since most studies have not made the final, longitudinal link with actual disease, the biological significance of stress-related immune impairment remains unclear.

Preliminary work by our research team suggests that it may be possible to identify a subgroup of vulnerable individuals as particular risk of infectious illness. For example, research carried out by Aoki, Herberman and colleagues (1985) points to a possible association between mood and lowered NK activity. They identified a subgroup within a patient sample characterized by low NK activity, remittent fever, and self-reported depression and fatigue. The depressive and fatigue-like symptoms were sufficiently prominent that these individuals had frequently been seen by psychiatrists rather than by other health care specialists. These investigators concluded that these individuals may be suffering from a new immunological disorder termed Low Natural Killer Cell Syndrome (LNKS), and reported some success in treating them with an immunopotentiator, letinen.

The Principal Investigator and Co-Principal Investigator on this proposed project (S. Levy and R. Herberman) conducted preliminary work in the

Biological Response Modifiers Program at the NCI with resultant data consistent with Aoki, et al.'s. That study examined the predictive value of daily stressors, personality, and coping factors, as well as repeated baseline measures of natural immunity (NK activity) and hormonal distress markers (excreted epinephrine and norepinephrine) relevant to episodes of infectious illness in a sample of healthy laboratory volunteers. Results from that prospective study revealed a subgroup at risk for disease, identified as having "low natural killer cell syndrome" (LNKS). Such individuals, operationally defined as having persistently low functional levels of NK activity across all three times of measurement (three baseline measures separated by two-week intervals), tended to report more serious illness over a three-month follow-up period. When comparing the psychological and demographic profile of the LNKS versus "normal" NK group, the LNKS individuals tended to be younger, report more daily distress, and had higher levels of excreted urinary catecholamine than individuals with normal NK activity.

Taken together, these studies suggest that inadequate coping (reflected in reports of depression and/or fatigue, coupled with report of high levels of daily hassles), psychological distress (reflected in higher levels of norepinephrine and epinephrine excretion) and immune function (specifically NK activity) may interact to increase risk for infectious illness.

The direction of association is unclear at this time. It is plausible that psychological distress produced by inadequate problem solving of life's hassles may compromise immune function and lead to the development of illness. On the other hand, low levels of natural immunity may produce cognitive and behavioral effects such as dysphoria and inability to cope. This project is designed to assess the relevant variables over a sufficient length of time to begin to understand these complex relationships.

II. Progress Report

During the last seven months since our first report, we have accrued an additional 44 normal healthy community volunteers, for a total of 104 accrued to this study. Accrual was ended on October 1, 1988, in order to be able to follow the last subject accrued for an additional twelve months. Due to a supplement to our original contract, we are now able to follow all subjects for a year in order to track illness episodes across four seasonal changes associated with differential exposure and infectious risk.

We have recently submitted a manuscript for review, reporting incidence of persistently low NK activity in our sample, and characterizing the psychological profile associated with this pattern of NK activity. This progress report will also detail those preliminary findings.

Demographics. The mean age of the sample accrued so far is 28.8, with a range of 18 to 44 years. Approximately 60% of the sample are female, and 90% are caucasian. Sixty percent of the sample are single, 30% married, and 11% divorced. Average years of education = 15.7 years, with a range of 10 to 27 years. Subjects were excluded on the following bases: Documented history of alcohol or drug abuse; history of psychiatric hospitalization; current use of

prescribed psychoactive agents; medically documented chronic disease (e.g., cardiovascular disease, diabetes, cancer, etc.); or current acute physical disease.

Psychological Measures. Mood and the perception of environmental stressors were assessed at baseline induction into the study, and at bi-weekly or monthly follow-up intervals as specified. Specifically, the Profile of Mood States (POMS) (McNair, Lorr, and Droppleman, 1971), and Kanner and colleagues' Hassles Scale (Kanner, Coyne, Schaefer, and Lazarus, 1981) were used in this study. The POMS, composed of six clinical subscales (tension/anxiety, depression/dejection, anger/hostility, vigor/activity, fatigue/inertia, and confusion/bewilderment), has the advantage of having been used extensively in studies of both physically and psychiatrically ill populations. In our previous research, we have used the POMS as a measure of current mood.

The Hassles Scale is a 117-item questionnaire in which respondents are instructed to indicate the occurrence of any items which have "hassled" them within a specified period of time (e.g., in the past week). Participants rated each hassle on a 3-point scale as having been "somewhat," "moderately," or "extremely" severe. From this information, two scores were created: 1) a frequency score, which is a simple count of the number of items checked; and 2) and intensity score, which is the mean severity reported by the participant for all items checked.

Laboratory Measures. Natural killer activity, lymphocyte subpopulations in the circulation, and urinary levels of epinephrine, norepinephrine, and dopamine were measured at baseline and follow-up periods. (Details of method supplied in the original application).

RESULTS

Table 1 shows Pearson correlations between major study variables. As can be seen, natural killer cell activity was positively correlated with chronological age, and norepinephrine excretion negatively correlated with the perception of frequent and severe hassles. Not surprisingly, catecholamine values were highly intercorrelated (e.g., epinephrine and norepinephrine values), as were subscales on the POMS (e.g., depression and fatigue). Both hassles scores were also significantly and positively correlated with POMS subscales and total scores on this measure.

Low NK function was operationally defined as having NK activity values below the mean or below the lowest quartile of the sample as a whole, calculated at the 25:1 effector:target cell ratio, at both baseline, and at two follow-up visits. We utilized the 25:1 ratio because this was the E:T ratio that Aoki and colleagues used in characterizing their series of patients with "low NK syndrome." Table 2 displays the proportion of the sample with low NK values as defined above. (Please note: Comparable data emerged when calculating proportions using NK activity at the 50:1 ratio. In fact, a perfect match was obtained for below the quartile stratification defined by the two E:T ratio distributions; only a minor and non-significant deviation obtained for the other stratification, with 9 "mismatches" for the whole sample.) As can be

seen, approximately one-third of the sample (36.8%) had NK activity values below the group mean on serial assessment. Approximately 14% of the sample had persistently low NK activity values, below the lowest quartile value, at three serial assessments.

Finally, both logistic and comparative probit regression analyses were carried out, using average values for predictor variables, in order to predict LNK versus other group membership. By using a linear logistic regression analysis (Cox, 1970), we were able to enter multiple variables into the equation in order to predict LNK activity. This method not only requires no distributional assumptions, but it can be used to identify risk factors that allow the prediction of events or states that are of particular interest; in this case, the probability of having persistently low NK activity or not based on the probability model developed.

Based on the significance of Pearson correlation coefficients, as well as theoretical interest, variables shown in Table 3 were entered into the regression analyses. As shown in Table 1, only the report of fatigue-like symptoms and the perception of seriousness of daily hassles were moderately correlated. Both epinephrine and norepinephrine were associated with LNK pattern in univariate analyses (norepinephrine more so by Pearson correlation and in the more conservative group comparison). These two neurotransmitters do tend to co-vary together ($r=.65$, $p<.001$) in this sample, but it is norepinephrine values that have been associated more frequently with depression (Schildkraut, 1978 and Anisman and Zacharko, 1982). Thus, our choice was to enter norepinephrine values into the model. In order to avoid problems with co-linearity, we elected to enter the severity of perceived daily hassles into the model, rather than mere frequency of hassles occurrence. Finally, because of theoretical interest in the experience and report of fatigue, we maintained this variable as a predictor, although it was only marginally significant in the more conservative univariate analyses. Average scores, reflecting more stable values for these variables, were entered into these regression analyses.

Table 3 shows the results from the logistic regression analysis. As can be seen, only age ($X = 1.9$, $p<.07$) and the perception of hassles severity ($X^2 = 2.1$, $p<.03$) remained in the model. This same model also resulted when we used the 50:1 E:T ratio distribution to stratify the two groups. A second analysis testing these same variables in a probit model confirmed the above findings, with age ($X^2 = 1.9$, $p<.05$) and hassles severity ($X^2 = 2.1$, $p<.03$) entering the model. Pearson Goodness of Fit Chi-Square = 64.6 (non-significant), indicating that the data adequately fit the model tested.

In addition, in order to test whether the number of NK cells in the periphery, reflected in proportion of cells identified by the Leu 19^+ marker, might be a more significant predictor of NK activity group membership--perhaps superceding other variables in the model--we repeated the logistic regression calculation, entering Leu 19^+ values into the equation. Table 4 shows the results of this analysis. As can be seen, Leu 19^+ values did not significantly contribute to prediction of LNK group membership. Thus, younger subjects, who perceived environmental events that occurred to them as more serious in nature, were more likely to exhibit a persistently low NK profile

over time than older individuals who perceived daily events as less important to them when they occurred.

DISCUSSION

It appears that persistently low NK activity is not all that uncommon in the population represented by this study. Consistent with our earlier NCI findings, approximately one-third of this population had NK activity consistently below the sample mean, and approximately 14 percent showed values persistently below the lowest quartile.

Several conclusions can be drawn from these data. First, there was a very striking age effect here, with younger subjects evidencing the LNK pattern significantly more than older subjects. This age effect was even more striking when one remembers that the age distribution for the whole study was not wide (18-45), and it is thus mainly the younger end of this rather narrow spectrum showing this NK activity pattern. In fact, when stratifying by mean age, persistent NK activity below the lowest quartile value was not observed in the older subgroup (age range 29-44 years).

One cannot ignore the fact that in both conservative and less stringent stratifications, peripheral blood from LNK subjects had a smaller average proportion of NK cells, marked by Leu 19^+ and Leu 11^+ antibody, than comparison samples. There were no significant differences in proportion of circulating cells at baseline entry into the study, and both percentage, and absolute numbers of NK cells were within normal range for all groups compared, including those with LNK below the lowest quartile in the sample. It is unlikely that these average differences in the size of the NK cell population fully explained the very wide functional difference in NK cells between the two groups (30% versus 47% activity at the 50:1 E:T ratio, $p<.001$; 15% versus 30% activity at the 25:1 E:T ratio, $p<.001$; 66 versus 146 lytic units, $p<.001$). Correlations between Leu 19^+, as well as Leu 11^+ values and NK activity tend to be rather modest ($r=.14$ and $r=.24$, respectively), and in logistic regression analyses predicting group membership, Leu 19^+ values did not significantly contribute to the predictive value of the logistic model tested. Thus, it is likely that LNK may be due to both a decrease in the cell population, as well as a decrease in the function of NK cells present.

Although the psychological findings are reminiscent of Aoki and colleagues' clinical description of "low NK syndrome," it should be borne in mind that our subjects are not directly comparable to Aoki and colleagues' sample, as ours was a normal, asymptomatic group, whereas their was a patient sample. However, if persistently low NK activity is the hallmark of a "syndrome" with potential health relevance, then characterizing a psychological profile that distinguishes the LNK group from others in the population might provide clues to the origin, or modulation of this host characteristic. And here, we have refined the measurement and quantified such a profile. In addition to younger chronological age, the perception of environmental stressors was found in both univariate analyses, and in multivariate predictor models, to be associated with the LNK profile. Since this is a replication of an earlier finding reported in an independent sample (Levy, 1986), we are rather confident that this association is not a study artifact, but is a valid

linkage between NK activity and the perception of the frequency and severity of environmental impingement. As other research has shown that the perception of daily "hassles" is an important predictor of subsequent illness (Weinberger, Hiner, Tierney, 1987), we believe that our findings may help explain such an association between environmental stress and health outcome.

Of major interest is that these findings emerged from a healthy sample of normal individuals who did not define themselves as incapacitated in any way. In fact, any signs of clinical illness made potential subjects ineligible for the study. The question remains whether we have identified a vulnerable sub-population at risk for illness, either over study follow-up, or over a life time. Whatever the source of significantly lower NK activity pattern (viral or otherwise), are such individuals particularly at risk related to environmental stressors, perhaps literally seeing their world as a more dangerous and threatening place, and hence subject to low mood? Is the host risk status, reflected in persistently low NK activity, or "low NK syndrome," exacerbated by perceived stress and negative mood factors, contributing to illness episodes? Again, future research will answer this latter question. At the least, this profile seems to be associated mostly with younger adults who are having difficulties in what are ordinarily difficult phases of life. Whether we have also discovered a constitutional vulnerability that has clinical significance remains to be seen.

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TABLE 1

Pearson Correlations Between Baseline Measures of Major Study Variables

	NK (25:1)	NK (12:1)	Lu	Age	Dopa	NE	Epi	H-F	H-S	Dep	Fat	Poms Total
NK (50:1)	.91	.8	.91	.37	.12	.14	-.06	-.19	-.17	.05	-.13	.08
NK (25:1)		.93	.95	.35	.06	.26	.12	-.26	-.24	.04	.00	.07
NK (12:1)			.93	.31	.10	.34	.20	-.26	-.25	.03	.00	.02
Lytic Units (LU)				.31	.01	.27	.11	-.19	-.19	.03	.01	.06
Age					.11	.10	.05	-.16	-.18	.04	.13	-.03
Dopamine (Dopa)						-.52	.30	.10	.10	-.11	-.12	-.10
Norepinephrine (NE)							.65	-.01	-.02	.10	.07	.15
Epinephrine (Epi)								.17	.18	.11	.17	.19
Hassles-Frequency (H-F)									.96	.41	.27	.43
Hassles-Severity (H-S)										.55	.35	.56
Depression (Dep)											.60	.89
Fatigue (Fat)												.75

PLEASE NOTE: Underlined correlations are significant at $p<.05$ or less.

TABLE 2

PROPORTION OF TOTAL SAMPLE

WITH LOW NK ACTIVITY

	%
% Below Mean NK activity Baseline + 2 follow-ups	36.8
% Below lowest quartile NK activity Baseline + 2 follow-ups	14.7

TABLE 3

Logistic Regression Predicting LNK Status

Variable	Beta	Std. Error	Beta/S. E.	P
Age	.08	.04	1.9	.07
Norepinephrine	-.04	.08	-.6	NS
Hassels - Severity	-.04	.02	-2.1	.01
Fatigue	.14	.10	1.5	NS

TABLE 4

Logistic Regression Predicting LNK Status

Variable	Beta	Std. Error	Beta/S. E.	P
Age	.08	.04	1.84	.07
Norepinephrine	-.01	.06	-.34	NS
Hassels -Severity	-.04	.02	-2.03	.01
Fatigue	.08	.10	.87	NS
Leu 19^+	.12	.10	1.1	NS