AD_____

GRANT NUMBER: DAMD17-94-J-4164

TITLE: Psychophysiological Reactivity and Immunological Sensitivity to Stress in Healthy Women at Familial Risk for Breast Cancer

PRINCIPAL INVESTIGATOR(S): Sandra G. Zakowski, Ph.D.
Doctor Dana H. Bovbjerg

CONTRACTING ORGANIZATION: Sloan-Kettering Institute for Cancer Research
New York, New York 10021

REPORT DATE: January 1996

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21701-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	January 1996	Annual (1 Jan 95 - 31 Dec 95)

4. TITLE AND SUBTITLE
Psychophysiological Reactivity and Immunological Sensitivity to Stress in Healthy Women at Familial Risk for Breast Cancer

5. FUNDING NUMBERS
DAMD17-94-J-4164

6. AUTHOR(S)
Sandra G. ZAkowski, Ph.D.
Doctor Dana H. Bovbjerg

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Sloan-Kettering Institute for Cancer Research
New York, New York 10021

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick
Frederick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

As of December 1995, 64 women with and without family histories of breast cancer have completed the first assessment of the proposed study. Of these, 38 have returned for the second experimental assessment. Subjects are exposed to 2 consecutive tasks designed to induce stress in a laboratory setting. Their psychophysiological and immune responses are assessed during and after stress exposure and compared to their own resting baseline for reactivity asesssments. Questionnaires measureing background information and chronic stress are also administered. Initial observations confirm that the experimental tasks are stressful and elicit marked immune changes which are in accordance with previously published data. Statistical analyses addressing the main hypotheses concerning contribution of family history of cancer to psychophysiological reactivity and immunological sensitivity to stress will be conducted upon completion of data collection as planned by the end of 1996.

14. SUBJECT TERMS
breast cancer

15. NUMBER OF PAGES
11

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

GENERAL INSTRUCTIONS FOR COMPLETING SF 298

The Report Documentation Page (RDP) is used in announcing and cataloging reports. It is important that this information be consistent with the rest of the report, particularly the cover and title page. Instructions for filling in each block of the form follow. It is important to ***stay within the lines*** to meet ***optical scanning requirements***.

Block 1. Agency Use Only *(Leave blank)*.

Block 2. Report Date. Full publication date including day, month, and year, if available (e.g. 1 Jan 88). Must cite at least the year.

Block 3. Type of Report and Dates Covered. State whether report is interim, final, etc. If applicable, enter inclusive report dates (e.g. 10 Jun 87 - 30 Jun 88).

Block 4. Title and Subtitle. A title is taken from the part of the report that provides the most meaningful and complete information. When a report is prepared in more than one volume, repeat the primary title, add volume number, and include subtitle for the specific volume. On classified documents enter the title classification in parentheses.

Block 5. Funding Numbers. To include contract and grant numbers; may include program element number(s), project number(s), task number(s), and work unit number(s). Use the following labels:

C	- Contract	**PR**	- Project
G	- Grant	**TA**	- Task
PE	- Program Element	**WU**	- Work Unit Accession No.

Block 6. Author(s). Name(s) of person(s) responsible for writing the report, performing the research, or credited with the content of the report. If editor or compiler, this should follow the name(s).

Block 7. Performing Organization Name(s) and Address(es). Self-explanatory.

Block 8. Performing Organization Report Number. Enter the unique alphanumeric report number(s) assigned by the organization performing the report.

Block 9. Sponsoring/Monitoring Agency Name(s) and Address(es). Self-explanatory.

Block 10. Sponsoring/Monitoring Agency Report Number. *(If known)*

Block 11. Supplementary Notes. Enter information not included elsewhere such as: Prepared in cooperation with...; Trans. of...; To be published in.... When a report is revised, include a statement whether the new report supersedes or supplements the older report.

Block 12a. Distribution/Availability Statement. Denotes public availability or limitations. Cite any availability to the public. Enter additional limitations or special markings in all capitals (e.g. NOFORN, REL, ITAR).

- DOD - See DoDD 5230.24, "Distribution Statements on Technical Documents."
- DOE - See authorities.
- NASA - See Handbook NHB 2200.2.
- NTIS - Leave blank.

Block 12b. Distribution Code.

- DOD - Leave blank.
- DOE - Enter DOE distribution categories from the Standard Distribution for Unclassified Scientific and Technical Reports.
- NASA - Leave blank.
- NTIS - Leave blank.

Block 13. Abstract. Include a brief *(Maximum 200 words)* factual summary of the most significant information contained in the report.

Block 14. Subject Terms. Keywords or phrases identifying major subjects in the report.

Block 15. Number of Pages. Enter the total number of pages.

Block 16. Price Code. Enter appropriate price code *(NTIS only)*.

Blocks 17. - 19. Security Classifications. Self-explanatory. Enter U.S. Security Classification in accordance with U.S. Security Regulations (i.e., UNCLASSIFIED). If form contains classified information, stamp classification on the top and bottom of the page.

Block 20. Limitation of Abstract. This block must be completed to assign a limitation to the abstract. Enter either UL (unlimited) or SAR (same as report). An entry in this block is necessary if the abstract is to be limited. If blank, the abstract is assumed to be unlimited.

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army.

NA Where copyrighted material is quoted, permission has been obtained to use such material.

NA Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

✓ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

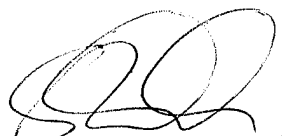
NA In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

✓ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

NA In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

NA In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

✓ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.



1/24/96

PI - Signature Date

Table of Contents

Introduction

Chronic and acute stress has been associated with alterations in immune measures including Natural Killer (NK) cell activity (e.g., Herbert & Cohen, 1993). Healthy individuals with family histories of cancers have been shown to have lowered NK cell cytotoxicity (e.g., Strayer et al., 1984, 1986; Hersey et al., 1979). As NK cells are thought to serve an important function in immune surveillance against neoplastic cells (Trinchieri, 1990) it is possible that deficits in NK cell activity in individuals at familial risk for cancer may contribute to their heightened risk of developing the disease. It therefore becomes important to determine the causes of this lowered NK cell activity. Aside from heritable deficits in NK cell activity it is also possible that the higher levels of distress that have been found in women with family histories of cancer (e.g., Kash et al., 1992) may be partly responsible for their immune deficits. The present study explores the possiblity that women with family histories of breast cancer may have higher psychophysiological reactivity and/or greater immunological sensitivity to stress than women without family histories of cancer. This is done using an experimental stressor paradigm that has been widely recognized in psychophysiological and psychoneuroimmunological research.

Body

Method

Subjects are exposed to two consecutive mental tasks that have been shown to affect psychophysiological reactivity (i.e., self-reported distress, cardiovascular changes, hormonal changes) as well as immune measures (i.e., NK cell activity) (e.g., Manuck et al., 1991; Stone et al., 1993; Zakowski et al., 1992). Self-reported distress, cardiovascular, hormonal and immunological measures are assessed before during and after stressor exposure at 15 to 30 minute intervals. Changes are assessed in response to the stressors and compared to resting baseline levels in order to determine the magnitude and duration of subjects stress reactivity and immunological sensitivity. These effects are compared to measures taken in response to a non-stressful control task. In addition, subjects complete questionnaires assessing family history of cancer, chronic distress, cancer-related symptoms of distress, etc. (see Measures). We hypothesize that: 1) Women with family histories of breast cancer show greater psychophysiological reactivity and immunological sensitivity than women without family histories of cancer. 2) Chronic stress, fear, and uncertainty associated with risk for cancer will partly account for subjects heightened psychophysiological reactivity and lower NK cell activity at baseline and in response to stress.

Measures

1. Psychobehavioral Study Measures. The standardized measures described briefly below were selected because of their possible relation to immune measures and because of their well established reliability and validity.

a. Measures of chronic stress. These questionnaires assess background levels of stress associated with daily life and specifically perceived risk of breast cancer. Their inclusion will permit assessment of group differences in distress and will enable us to examine the possible contribution of chronic stress to the psychophysiological and immunological responses to the laboratory tasks.

Life Experiences Survey (LES). The LES (Sarason et al., 1978) assesses the total number of life events and their impact, which has been reported to predict anxiety, depression, and psychological discomfort.

Brief Symptom Inventory (BSI). The BSI (Derogatis et al., 1982) with nine symptom dimensions and three global indices of distress has been used in previous studies of individuals with a family history of cancer.

The Perceived Stress Scale (PSS). The PSS (Cohen et al., 1983) assesses how unpredictable, uncontrollable, and overloading respondents find their lives, which may be related to immune function.

Impact of Event Scale (IES). The IES (Horowitz et al., 1979), assesses distress anchored to a specific stressor; in this case the threat of cancer.

Perceived Risk for Cancer. This face-valid questionnaire determines whether the women perceive themselves to be at increased risk for breast cancer.

b. Measures of stress mediators.

Courtauld Emotional Control Scale. The CECS assesses emotional control and expressivity, which has been suggested by some investigators to differ in individuals with cancer.

c. Other background measures. These questionnaires assess variables, such as demographic variables and health habits that may affect physiological and immune measures.

Demographic questionnaire. The purpose is to obtain basic demographic information such as age, race, socio-economic status, etc.

Daily Habits Questionnaire (DHQ). The DHQ is a face valid instrument developed by us to assess sleep, physical activity, eating patterns, cigarette smoking, alcohol consumption, use of licit and illicit drugs, and menstrual cycles, variables that may affect immune function.

2. Measures of acute stress in response to the laboratory manipulations Reactivity to the laboratory stressors will be assessed at three levels. Psychological distress will be assessed by self-report questionnaires, cardiovascular reactivity will be assessed by continuous monitoring of heart rate (HR) and blood pressure (BP), and biochemical

measures of stress will include changes in levels of stress hormones.

a. Self-report measures

Visual Analog Scales (VASs). VASs (Cella et al., 1986) will be used to provide measures of subjects': distress associated with the assessment visit, current levels of emotional distress, venipuncture distress, which may be related to immune function.

The Profile of Mood States (POMS). The POMS (McNair et al., 1971) assesses current levels of emotional distress.

b. Cardiovascular measures. Blood pressure and heart rate is monitored at set intervals using an automated monitoring device.

c. Endocrine measures

Catecholamines in plasma samples collected at each assessment will be assayed by the CRC Core Laboratory at CUMC under the direction of Dr. Imperato-McGinley using classic HPLC techniques.

Cortisol in plasma samples will be assayed by the CRC Core Laboratory at CUMC using commercial radioimmunoassay kits with high reliability.

3. Immune measures. The immune measure of primary interest is NK cell activity, because of the published data indicating deficits in individuals with a family history of cancer (e.g., Strayer et al., 1984). In addition, psychoimmune studies have repeatedly documented that NK cell activity is sensitive to emotional distress (Herbert & Cohen, 1993).

Quantification of leukocyte subpopulations. Complete blood counts (CBC) with differential is done on the day of assessment by a laboratory using laser light scatter and enzyme cytochemistry. Flow cytometric quantifications of well established lymphocyte subsets (e.g., CD3+, CD4+, CD8+, CD19+, CD4+CD45RA+, CD4+CD45-), with particular emphasis on enumerating natural cytotoxic effectors based on phenotype (e.g., CD2, CD3, CD16, CD56), is accomplished with two color immunofluorescence techniques.

Effector Cells and Culture Conditions. Mononuclear cells are isolated from heparinized blood samples by standard Ficoll-Hypaque (Pharmacia, Piscataway, NJ) gradient centrifugation routinely yielding cells with greater than 95% viability, which is used fresh for the assays described here.

Natural killer cell activity. Natural killer cell activity is assessed in classic chromium release assays using the natural killer (NK) cell sensitive K562 erythroleukemia line (Bonavida et al., 1983).

Blastogenic responses and production of cytokines. Following classic methodologies, isolated mononuclear cells are stimulated with: the classic T cell dependent mitogens.

Results

As of December 31, 1995 (Month 12 of the study) we have recruited a total of 64 women with and without family histories of cancer who have participated in the first session of the study. Of those participants, 38 have completed the second experimental session. We therefore expect that, in line with our proposed Statement of Work, we will have completed the full study with a total of 100 subjects by the end of 1996. In accordance with our Statement of Work (see Appendix K of the grant) we will begin data processing and analyses by Month 17 of the study period (i.e., May 1996) and therefore cannot present results from the present study. It is routine procedure for a short-term experimental study such as this one to analyze the data once its collection has been completed so as not to unintentionally influence the rigorous experimental procedures by experimenters expectations based on preliminary findings. Based on initial observations it can be concluded, however, that our experimental paradigm is successful in eliciting the expected stress and immune effects that have previously been shown in the literature independent of family history of cancer. That is, the mental tasks elicit reliable increases in self-reported distress, as measured by visual analog scales, increases in heart rate and blood pressure, as well as a biphasic response curve in NK cell activity with an initial increase followed by a subsequent decrease in activity as expected based on previous literature (e.g., Schedlowski et al., 1993). This confirms our expectations and previously published data on the effects of experimental tasks on stress and immunological measures and confirms the methodological soundness of our research design. The quantitative analysis of response differences between women with family histories of cancer and women without family histories of cancer addressing our main hypotheses (see above) will be conducted in accordance with our Statement of Work.

Conclusions

Preliminary observations confirm previous data showing that when subjects are exposed to stressful mental tasks in a controlled laboratory setting increases in psychological and cardiovascular indices of distress as well as changes in immune function are seen. Analyses addressing the major study hypotheses will be conducted upon completion of the data collection. The final results from this study will determine the role of stress in the reduced NK cell acitivity in women at familial risk for cancer and will provide potential mechanisms by which stress may be partly responsible for these immune deficits. To date no other studies have addressed these issues in populations at risk for cancer and the findings from this study will provide important information on how women at familial risk for cancer respond to stress both psychophysiologically and immunologically. The results will have important implications for designing stress-reducing interventions for women at familial risk for cancer to help them reduce the psychological impact of stressful events and as a consequence to attempt to attenuate the potentially deleterious effects of stress on the immune system in these women. The findings will also lead to further studies refining previous methodology in order to determine in greater detail the sources of distress in this population so as to be able to target more specific stressors for intervention purposes.

Finally, this study will contribute to a greater awareness of the importance of psychological issues in cancer risk for both researchers and clinicans.

References

Bonavida, B., Bradley, T.P., Grimm, E.A. Frequency determination of killer cells by a single-cell cytotoxic assay. 1983 Methods in Enzymology, 93:270-280.

Cella DF, Perry SW. Reliability and concurrent validity of three visual-analogue mood scales. Psychol Rep 1986; 59:827-833.

Cohen S, Karmarck T, Mermelstein R. A global measures of perceived stress. Journal of Health and Social Behavior 1983; 24:385-296

Derogatis LR, Spencer P. The Brief Symptom Inventory (BSI) Administration Scoring and Procedures Manual-I. Baltimore: copyrighted manuscript, 1982.

Herbert TB, Cohen S. Stress and immunity in humans: A meta-analytic review. Psychosomatic Medicine 1993; 55:364-379.

Hersey P, Edwards A, Honeyman M, McCarthy WH. Low natural killer-cell activity in familial melanoma patients and their relatives. Brit J Cancer 1979; 40:113-122.

Horowitz M, Wilner N, Alvarez W. Impact of Event Scale: A measure of subjective stress. Psychosom Med 1979; 41:209-218.

Kash KM, Holland JC, Halper MS, Miller DG. Psychological distress and surveillance behaviors of women with a family history of breast cancer. J Natl Cancer Inst 1992; 84:24-30.

Manuck SB, Cohen S, Rabin BS, Muldoon MF, Bachen EA. Individual differences in cellular immune response to stress. Psychological Science 1991; 2:111-115.

McNair DM, Lorr M, Droppleman LF. Manual: Profile of Mood States. San Diego:Education and Industrial Testing Service, 1971.

Sarason IG, Johnson JH, Siegel JH. Assessing the impact of life changes: Development of the life experiences survey. Journal of Consulting and Clinical Psychology 1978; 46:932-946.

Schedlowski M, Jacobs R, Sttratman G, Richter S, Haedicke A, Tewes U, Wagner T, Schmidt R: Changes of natural killer cells during acute psychological stress. Journal of Clinical Immunology 1993; 13:119-126.

Stone AA, Valdimarsdottir HB, Katkin ES, Burns J, Cox DS, Lee S, Fine J, Ingle D, Bovbjerg DH. Effects of mental stressors on mitogen-induced lymphocyte responses in the laboratory. Psychology and Health 1993; 8:269-284.

Strayer DR, Carter WA, Mayberry SD, Pequignot E, Brodsky I. Low natural cytotoxicity of peripheral blood mononuclear cells in individuals with high familial incidences of cancer. Cancer Research 1984; 44:370-374.

Strayer DR, Carter WA, Brodsky I. Familiar occurrence of breast cancer is associated with reduced natural killer cytotoxicity. Breast Cancer Research Treatment 1986; 7:187-192.

Trinchieri G. Biology of natural killer cells. Advances in Immunology 1990; 47:187-376.

Zakowski SG, McAllister CG, Deal M, Baum A: Stress, reactivity and immune function. Health Psychology 11:223-232, 1992