

**THE EFFECT OF MIRTHFUL LAUGHTER ON
STRESS AND NATURAL KILLER CELL
CYTOTOXICITY**

by

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Title of Dissertation: The Effect of Mirthful Laughter on Stress and Natural Killer Cell Cytotoxicity

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ABSTRACT

A survey of local cancer patients revealed that humor was a frequently used complementary therapy which helped them cope with stress. Introductory research has suggested that humor can decrease the perception of stress and perhaps improve the immune system's response to disease causing agents. However, there are a very limited number of rigorous intervention studies which document these effects. Using psychoneuroimmunology as a framework, the effect of mirthful laughter on stress and natural killer (NK) cell cytotoxicity was measured. Since the beneficial effect of laughter on NK activity has limited documentation in healthy males and no documentation in females, a clinical study would have been premature. Therefore, thirty-three healthy adult women were randomly assigned to either a control or humor group. Subjects in the humor group viewed a humorous video, whereas subjects in the control (distraction) group viewed a tour video. Instruments included the Life Experiences Survey, Stress-Arousal Check List, Situational Humor Response Questionnaire, Multidimensional Humor Scale, Humor Response Scale (HRS), and chromium release NK assay. Data revealed that stress scores decreased more in the humor group than in the distraction group ($U = 215.5$ $p = 0.004$); HRS scores correlated with post stress for persons in the humor group ($r = -0.655$ $p = 0.004$). Persons who laughed more had less stress following the video. Viewing a humorous video, in and of itself, did not significantly change NK activity ($t = 1.52$ $p = 0.138$), however, subjects who scored ≥ 25 on the HRS had significant increases in NK cytotoxicity over their baseline values ($t = 2.52$ $p = 0.037$), and as compared to the remaining participants ($t = 2.1$ $p = 0.04$). HRS scores significantly correlated with changes in NK cytotoxicity ($r = 0.744$ $p = 0.001$). Persons who laughed more had greater improvement in NK cytotoxicity. These findings indicate that laughter has the potential to reduce stress and temporarily improve NK activity in healthy women. As low NK activity has been linked to metastases and poorer prognosis, it is plausible that humor may be a useful complementary therapy in the care of persons with cancer.

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THE EFFECT OF MIRTHFUL LAUGHTER AND SENSE OF HUMOR ON STRESS AND NATURAL KILLER CELL CYTOTOXICITY

INTRODUCTION

This paper describes a dissertation project designed to investigate the effect of mirthful laughter on stress and natural killer (NK) cell cytotoxicity. In addition, the relationships among negative life events, sense of humor, humor response, stress, arousal, and baseline NK cell cytotoxicity were investigated.

Significance

Persons diagnosed with cancer often report greater stress than those diagnosed with other serious diseases. This phenomenon is not limited to persons in the United States and has been documented in both nursing and psychological literature (Halstead & Fernsler, 1992; Kumsaka & Dungan, 1993; Krause, 1993; Lierman, 1988). A diagnosis of cancer reportedly evoked feelings of uncertainty, fear, and horror in a group of Finnish patients (Krause, 1993). Grassman (1993) uses the descriptive word "victim" to illustrate how helpless those diagnosed with cancer feel. Feelings of loss of control add to the stress of cancer (Fletcher, 1992). Role function, relationships with others, as well as psychological and physiological functioning are all affected when patients are diagnosed and treated for cancer (Samarel & Fawcett, 1992).

For almost twenty years, it has been documented that increased stress levels can lead to changes in psychological and physiological functioning. In addition to changes in the usual "stress hormones" such as ACTH, cortisol, epinephrine, and norepinephrine, many other factors are influenced by exposure to stressors. For example, the production and release of prolactin, growth hormone, insulin, glucagon,

thyroid hormone, and gonadotrophin can be affected by physical and emotional stress (Curtis, 1979). Levels of neurotransmitters, neurohormones, cytokines, and various cells of the immune system can also be influenced by the stress associated with diseases such as cancer (Andersen, Kiecolt-Glaser & Glaser, 1994). The intricate interactions of neuropeptides, neurohormones, cytokines, and immune system components are just beginning to be explored in a field of research known as psychoneuroimmunology (PNI) (Caudell & Gallucci, 1995).

Unfortunately, clinical intervention research has lagged behind animal-based bench research in this arena. While most PNI researchers would agree that stressors can lead to decreased physiological and psychological functioning, there is no such agreement on which interventions can effectively modify the effects of stress on immune function. A number of complementary interventions have been developed to help improve quality of life and reduce the effect of stressors on psychological and physiological functioning. Complementary interventions offer a variety of ways for persons to cope with stress, in combination with standard medical care. However, the effects of various complementary interventions on stress and immune function are just beginning to be reported in the literature.

According to a study of cancer patients in the rural Midwest (Bennett 1995a), more than 87% were currently using at least one complementary intervention to cope with the stress of cancer. The majority of the patients were using prayer, which was not surprising given the conservative nature of many persons in the rural Midwest. Use of humor was the next most popular intervention, with 50% of the sample already using humor, and an additional 13% stating they would definitely try it. The use of humor to diminish stress, decrease pain, improve quality of life, and perhaps influence immune functioning has been discussed in several publications (Bellert, 1989; Erdman, 1991;

Gilligan, 1993; Groves, 1991; Henry & Moddy, 1985; Herth, 1984; Metcalf, 1987; Raber, 1987; Robinson, 1979; Ruxton & Hester, 1987; Ruxton, 1988; Samra, 1985; Scott, 1985; Shaw, 1984; Simon, 1988; Simon, 1989; Simon, 1990; Sullivan & Deane, 1988; Osterlund, 1983). This popularity is easy to understand. While complementary interventions such as relaxation therapy require large amounts of both staff and patient time to learn and practice, and hypnosis requires a minimum of a masters' degree to qualify for certification (Mott, 1994), mirthful laughter in response to a humorous stimulus is a natural occurrence and does not require any additional effort by the participant (Cogan, Cogan, Walts & McCue, 1987). Humorous stimuli are relatively easy to introduce into a person's life, because the use of humor does not require large amounts of time or money to implement, and produces few, if any, harmful side effects. This makes humor therapy attractive, and possibly even cost-effective.

Use of humor as a healing modality can be traced back to the Shriners' use of clowns in the 1930's to cheer up children hospitalized with infantile paralysis. The holistic or alternative medicine movement began in the 1950's. Humor was not initially considered a part of this movement, but was subsequently added as the movement widened to embrace more non-traditional modalities (Fry, 1994). In 1979, Cousins wrote *Anatomy of an Illness*, a widely popular book in which Cousins credited humor and laughter with his recovery from a serious collagen disease. Since then, many small and large humor projects have been initiated in an attempt to utilize the hypothetical benefits of humor (Fry, 1994). Unfortunately, despite the popularity of humor, there appears to be little documentation to scientifically support the use of humor as a complementary intervention. According to Fry, (1994) "as with most grass-roots movements, there is little laboratory, statistical, analytic, or otherwise scientific evidence to justify scientifically the huge degree of enthusiasm for uses of humor in this context" (p. 119). Since this

area of research is in the introductory phases, and relatively little is known about the effects of laughter on stress and immune function in healthy people, a clinical study of the effects of humor and laughter in cancer patients would have been premature. Therefore, this study investigated the effect of laughter and sense of humor on stress and immune function in a sample of healthy women.

Conceptual Framework

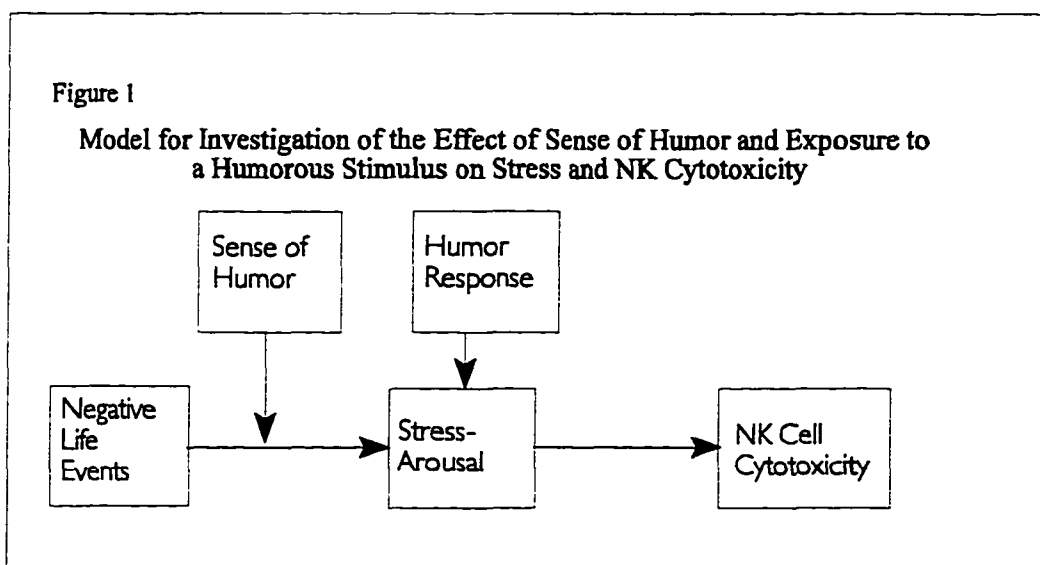
Interest in the influence of psychological factors on susceptibility to certain disease states goes back to the times of Galen, when it was noted that persons who developed cancer often had a "melancholic" personality (Kaplan & Reynolds, 1988). Since that time, numerous clinicians have shared anecdotal data concerning the development of cancer or other diseases in persons with certain psychological styles, or after a severe life event, such as bereavement (Locke & Kraus, 1982). Selye (1950) was one of the first to document the general effect of a stressor on the autonomic nervous system, neuroendocrine system, and lymphatic organs. According to his theory, environmental demands are viewed by the organism as stressful and produce a common stress response within the organism. This response leads to physiological arousal which, if unrelieved, can lead to structural and functional damage to the organism. Further studies established that activation of the stress response could also be triggered by acute emotions, physical exertion, cold, and pain (Cannon, 1953). Subsequently, Lazarus and Folkman (1984) broadened the definition of stress from Selye's concept of response to "environmental demands" to include psychological components such as appraisal and coping. According to Lazarus and Folkman (1984), stress is "a particular relationship between the person and the environment that is appraised by the person as taxing or exceeding his or her resources and endangering

his or her well-being" (p. 19). While Lazarus and Folkman's theory helped to explain the moderation of stressors by use of coping mechanisms, it did not attempt to explain the possible consequences of these coping mechanisms in terms of effects on immune functioning or health. A recent field of inquiry, psychoneuroimmunology (PNI), has attempted to bring all of these factors together.

Psychoneuroimmunology developed from a multifactorial model of illness that includes stress, coping, and resultant disease (Engel, 1962). This theory was further developed by Solomon (Solomon & Moos, 1964; Solomon, 1985; Solomon, 1987), to include the influence of stress on the immune system in the development of disease. Later, the term "psychoneuroimmunology" was coined by Ader and Cohen (1981), to describe the basic phenomena of this theory: interactions between the nervous system, the neuroendocrine system, and the immune system. These interactions may play a part in subsequent disease development/progression. According to PNI theory, the central nervous system (CNS), peripheral nervous system (PNS), endocrine system, and immune system are part of an intricate communication and feedback system. Therefore, any action that causes changes in one part of this system, such as the central nervous system, could potentially cause changes in the other parts of the PNI system, such as in the endocrine and/or immune systems. Use of PNI theory could explain how mental "stress", which causes changes in neurotransmitters in the CNS and changes in hormone levels in the endocrine system, could also induce changes in immune functioning, which ultimately can influence susceptibility to disease. Because PNI theory acknowledges the multifactorial nature of wellness and illness, it is particularly useful as a guide for nursing research and practice (Birney, 1991; Zeller, McCain & Swanson, 1996)

The following PNI propositions were tested in this dissertation:

- A complementary intervention (use of humor) can reduce stress and enhance or optimize immune function (Solomon 1987). For this study, a humorous video was used to stimulate mirthful laughter and to investigate the effect of humor response upon stress and NK cell cytotoxicity.
- Enduring coping style and personality factors (so-called "trait" characteristics) are able to modify the effects of negative life events on an individual's immune system (Solomon 1987). For this study, the relationships among negative life events, sense of humor, humor response, stress, arousal, and baseline NK cell cytotoxicity were investigated. (See figure 1 for model of study).



REVIEW OF LITERATURE

Immune Function and Cancer

Cancer cells stimulate an immune response that results in lymphocytic infiltration of the tumor site (Humphrey, Singla & Volence, 1980). This infiltration of immune cells appears to be a defense mechanism against the tumor. It has been demonstrated that there is an inverse correlation between numbers of infiltrating immune cells at the tumor site and several indicators of poor prognosis, such as extensive lymph node involvement and distant metastases (Shimakowara, Imamura, Yamanaka, Ishii & Kikuchi, 1982).

One of the immune cells that plays an important part in this response is the NK cell. NK cells are lymphocytes that are classified as non-B, non-T, or "null cells". NK cells can kill tumor cells *in vitro*, while leaving normal cells intact (Herberman & Ortaldo, 1981; Kiessling, Klein & Wigzell, 1975). The NK cell is a spontaneous killer that does not have to be activated by tumor antigens, nor is it limited by major histocompatibility complex requirements (Locke, et al., 1982).

In vitro studies have demonstrated that NK cells can kill a variety of cancerous human and animal cell lines including leukemia, carcinomas, sarcomas, and melanomas (Hanna & Fidler, 1980; Hanna & Barton, 1981; Hanna, 1986; Herberman, et al., 1981). Low levels of NK cell cytotoxicity have been correlated with tumor cell metastases in both humans and animals (Hanna, et al., 1981). In addition, there is some evidence to suggest that NK cell activity is important in the prevention of breast cancer micro-metastases (Barlozzari, Leonhardt, Wiltrout, Herberman & Reynolds, 1985; Ben-Eliyahu, Yirmiya, Liebeskind & Taylor, 1991; Brosschot, Smelt, de-Semt & Heijnen, 1991; Keast, 1981; Levy, Herberman, Maluish, Schlein & Lippman, 1985; Levy, Herberman, Lippman & D'Angelo, 1987; Steinhauer, Doyle, Reed & Kadish, 1982). Breast cancer

patients tend to have significantly reduced NK cytotoxicity compared with patients with benign breast disease or healthy female controls (White, Jones, Cooke, & Kirkham, 1982). Of additional note is the significant correlation between depressed NK cytotoxicity and lymph node involvement in patients with primary breast cancer (Levy, Herberman, Maluish, Schlein, & Lippman, 1985).

NK cell activity tends to be decreased in several types of cancer (Chakraborty, Chakraborty & Chattopadhyay, 1994; Gaspari, Fleisher & Kraemer, 1993; Oka, Yoshino, Hazama, Shimoda & Suzuki, 1993; Sedlmayr, Rabinowich, Elder, Ernstoff, Kirkwood, et al., 1991; Tan, Pang & Hong, 1993; Whiteside, Wang, Selker & Herberman, 1988), possibly due to serum inhibitory factors produced by cancer cells (Konjevic & Spuzic, 1992; Konjevic & Spuzic, 1993; Wanebo, Blackinton, Kouttab & Mehta, 1993) or cellular suppressive effects (Barlozzari, Leonhardt, Wiltrout, Herberman & Reynolds, 1985; Swisher, Kiertscher, Golub, Holmes & Roth, 1993). For example, pulmonary macrophages from persons with lung cancer have the ability to cause NK suppression (Swisher, et al., 1993). Perhaps because of their activity against cancerous cells, NK cell cytotoxicity has been linked to cancer stage (Carballido, et al., 1993; Garzetti, Ciavattini, Muzzioli, Goteri, Fabris, et al., 1994; Konjevic, et al., 1992; Konjevic, et al., 1993), cancer progression or remission, (Alonso, et al., 1994; Atzpodien, et al., 1993; Fujioka, et al., 1994; Maxwell, et al., 1993; Oka, et al., 1994), and cancer survival (Garzetti, et al., 1994; Yamasaki, Kan, Harada, Ichinose, Moriguchi, et al., 1993).

NK cell cytotoxicity is also important in the prevention of some types of viral illnesses, most notably herpes simplex virus type 1, Epstein Barr, and influenza viruses (Bonneau, Sheriadan, Feng & Glaser, 1991; Esterling, Antoni, Schneiderman & Carver, 1992; Kennedy, Kiecolt-Glaser & Glaser, 1988; Sheridan, Feng, Bonneau & Allen, 1991). This activity can be particularly significant for persons with cancer, as they tend

to have decreased immune function related to various cancer treatments, which leads to an increased susceptibility to certain viral illnesses.

While NK cells intrinsically have activity against tumor cells and viruses, they are more effective when activated by various lymphokines. Activated NK cells are known as lymphokine activated killer cells or LAK cells. Because NK cells infiltrate tumors (Kuppen, Basse, Goldfarb, Van-De-Velde, Fleuren, et al., 1994; Melder, Brownell, Shoup, Brownell & Jain, 1993) and can efficiently kill cancer cells following differentiation into LAK cells, activation of NK cells by interleukin-2 (IL-2) and other cytokines is being used in experimental immunotherapy for the treatment of drug resistant cancers, inoperable cancers, and leukemia (Alonso & Medenica, 1994; Anderson, Bach & Ochoa, 1988; Atzpodien, Kirchner, Korfer, Hadam, Schomburg, et al., 1993; Blaise, Viens, Oliver, Stoppa, Gabert, et al., 1991; Bubenik, Zeuthen, Bubenikova, Smiova & Jandlova, 1993; Carballido, Molto, Manazano, Oliver, Salmeron, et al., 1993; Cramer & Long, 1990; Feng, Zheng, Si, Lin, Chang, et al., 1993; Foreman, Rill, Coustan-Smith, Douglass & Brenner, 1993; Fujimoto, Omote, Mai, Natsuume & , 1992; Fujioka, Nomura, Hasegawa, Ishikura & Kubo, 1994; Gratama, Bruin, Lamers, Braakman, Stoter, et al., 1993; Klingemann, Deal, Reid & Eaves, 1993; Kochupillai, 1993; Lotzova & Savary, 1993; Maxwell, McDevitt, Reid, Sharpe, Feighery, et al., 1993; Oka, Hazama, Yoshion, Shimoda, Suzuki, et al., 1994; Rossi, Pericle, Rashleigh, Janiec & Djeu, 1994; Scudeletti, Filaci, Imro, Motta, Di-Gaetano, et al., 1993; Sekine, Shiraiwa, Yamazaki, Tobisu & Kakizoe, 1993; Whiteside, et al., 1988). Unfortunately, similar to many cancer treatments, immunoactive substances tend to produce stressful side effects.

The stress of cancer and the side effects of cancer treatment may lead to depression in immune function (Garzetti, Ciavattini, Goteri, Tranquilli, Muzzioli, et al., 1994; Levy, Herberman, Lippman & D'Angelo, 1987). According to PNI theory, it may be

possible to decrease the effect of these stressors on immune response using complementary therapies. In an attempt to decrease stress, improve quality of life, and perhaps enhance immune response, certain oncology patients and physicians have begun adding complementary therapies to their standard plan of care. This approach is utilized in an attempt to decrease the effects of stress and perhaps increase resistance to disease. It is important to note that complementary therapy involves incorporating various non-medical treatments into the total care of the patient. Complementary interventions are not alternative therapies intended to replace traditional medical care; instead, they are used as additional or adjunctive treatments to improve patient mental and physical well-being. However, as noted by Lerner and Kennedy (1992), "any therapy that can be used adjunctively can and has been used alternatively, and vice versa", so there tends to be some overlap in the use of terms (p. 32). Complementary interventions, such as imagery, relaxation therapy, exercise, nutrition, and use of humor are viewed as less invasive, and therefore less likely to produce negative side effects, but data concerning the effectiveness of such interventions in actually improving either immune functioning or well-being are just beginning to be reported in the literature.

According to a study of "unconventional" medicine in the United States (Eisenberg, Kessler, Foster, Norlock, Calkins, et al., 1993), 34% of 1,539 randomly selected adult survey respondents reported using unconventional therapy in the prior year. The most popular therapies used were exercise, prayer, relaxation techniques, and chiropractic use. Most respondents were using complementary therapies as an adjunct to standard medical care to treat chronic illness (36%), anxiety (28%), headaches (27%), or cancer (24%). Although the survey inquired about use of 16 common complementary therapies, it did not include use of humor.

Use of complementary therapies appears to be increasing in the United States and is frequently not reported by patients to their regular physicians. According to Eisenberg's extrapolation of the survey data, it is probable that 61 million Americans used at least one of the therapies studied. In addition, it was estimated that Americans spent about \$11.7 billion in out-of-pocket costs for these services. This is comparable to the out-of-pocket costs for hospital care in the U. S. and is 50% of the out-of-pocket costs spent for all physicians' services in the U.S. (Eisenberg, et al., 1993).

As mentioned previously, a recent survey of cancer patients in the rural Midwest (Bennett 1995a) revealed that many of these cancer patients were currently using complementary interventions, with 87.5% using at least one such intervention. Similar to the results of the Eisenberg (1993) study, prayer was the most commonly used intervention. Use of humor was the second most popular choice, with 50% of the sample already using humor, and an additional 13% stating they would definitely try it. There is also information to suggest that hospitals and nurses in the rural Midwestern area are interested in the use of humor as a complementary intervention. For example, at the 1995 Nurse's Day conference co-sponsored by hospitals in the East Central Illinois and West Central Indiana area, a humor therapist, Dr. Ann Weeks, was invited to conduct an all day seminar on the use of humor to reduce stress and improve health. According to PNI theory, it is possible that humor and/or sense of humor act to reduce the effects of negative life events on stress and immune function. Yet so far, there is relatively little data to document this effect. The following sections discuss the effects of stress and negative life events on immune function and well-being.

Stress and Immune Function

One of the first scientific studies to connect a psychological factor with a biological outcome was conducted by Ishigami in 1919. In a study of patients with

tuberculosis (TB), he found that phagocytosis of the TB bacilli was decreased in patients during episodes of "emotional excitement." He concluded that the stress of "contemporary life" led to decreases in immune functioning and increased susceptibility to TB (Ishigami, 1919).

Early experimental studies attempting to document a relationship between stress and disease frequently examined the result of physical stressors on disease development and progression utilizing animal models. It was observed that animals exposed to physical stressors developed lymphatic organ involution, reduction in lymphocyte numbers, and impaired resistance to infection following activation of the hypothalamic-pituitary-adrenal (HPA) axis (Selye, 1950; Selye, 1956). However, subsequent studies revealed that the reactions of the HPA axis are not the only ones involved in a stress response. It has been demonstrated that production and release of prolactin, growth hormone, insulin, glucagon, thyroid hormone, and gonadotrophin can be affected by both physical and emotional stressors (Curtis, 1979). Later work in this area focused on the effect of stress on various components of the immune system. Animals exposed to acute stressors have decreased antibody production in response to antigens, depressed B - and T - lymphocyte proliferation in response to mitogens, and depressed NK cell cytotoxicity (Monjan, 1981).

Further studies attempted to document the effect of physical stressors and/or negative emotional events on human immune function. It has been reported that stressors, including sleep deprivation and noise (Nieburg, Weiss, Navarrete, Strax, Teirstein, et al., 1979; Palmblad, Cantell, Strander, Froberg, Karlsson, et al., 1976; Palmblad, Petrini, Wasserman & Akerstedt, 1979), bereavement (Bartrop, Lazarus, Luckhurst, Kiloh & Penny, 1977; Irwin, Daniels & Weiner, 1987; Schleifer, Keller, Cameron, Thornton & Stein, 1983), divorce (Kiecolt-Glaser, Fisher, Ogrocki, Stout,

Speicher, et al., 1987), loneliness (Glaser, Kiecolt-Glaser, Speicher & Holliday, 1985; Kiecolt-Glaser, Ricker, Gerooge, Messick, Speicher, et al., 1984; O'Leary, 1990), academic stress (Kasl, Evans & Niederman, 1979; Jemmott, Borysenko, Borysenko, McClelland, Chapman, et al., 1984; Jemmott & Locke, 1984; Jemmott & Magloire, 1988; McClelland, Ross & Patel, 1985), and examination stress (Dorian, Keystone, Garfinkel & Brown, 1981; Glaser, Mehl, Gerald & Speicher, 1986; Glaser, Kennedy, Lafuse & Bonneau, 1990; Jemmott, et al., 1988; Kiecolt-Glaser, Garner, Speicher, Penn, Holliday, et al., 1984; Kiecolt-Glaser, Glaser, Strain, Stout, Tarr, et al., 1986; McClelland, Davidson & Saron, 1985) produce various levels of immune system depression in humans. Given the studies cited above, there appears to be sufficient evidence to support that physical stressors and cognitive stress can cause measurable changes in various immune system parameters. Therefore, it plausible that an intervention which reduces stress levels may also buffer the effect of stress on the immune system.

Stress and NK Activity

A number of stressors have been used to investigate the effect of stress on NK cytotoxicity in humans. In investigations of the effect of physical stressors, several studies report that painful physical stressors such as surgery suppress NK activity in both animals and humans (Ben-Eliyahu, Yirmiya, Liebeskind & Taylor, 1991; Bonneau, Sheridan, Feng & Glaser, 1991; Koltun, Bloomer, Tilberg, Seaton, Ilahi, et al., 1996; Page, Ben-Eliyahu & Liebeskind, 1994; Taylor & Gale, 1991; Weiss, Sundar, Becker & Cierpial, 1989). In a study of sleep deprivation it was reported that late-night partial sleep deprivation significantly reduced on NK cell activity in 23 healthy male volunteers (Irwin, Mascovich, Gillin, Willoughby, Pike, et al., 1994). Also, in a small study of six frail elderly males, strenuous exercise over a period of three months led to significantly decreased NK activity (Rincon, Solomon, Benton & Rubenstein, 1996).

Reports of the effect of mental stressors on NK activity are not as consistent. In a study of psychological stress and immune function in 56 gay men, it was reported that the stress of waiting for the results of an HIV test led to decreased NK activity in both seronegative and seropositive subjects. This immunosuppression disappeared in seronegative subjects after notification of their negative HIV status, indicating that the psychological stress of anticipating the diagnosis led to the decrease in NK activity (Antoni, LaPerriere, Schneiderman & Fletcher, 1991).

In contrast, in a study using the Stroop color-word test to investigate the effect of a mild experimental stressor on cortisol and NK activity, the results indicated that the stressor did not lead to significantly increased levels of cortisol nor significantly decreased levels of NK activity. The authors concluded that the Stroop test may not have been stressful enough to produce a physiological change in endocrine or immune parameters (Caudell & Gallucci, 1995). Another study investigated the effects of a variety of psychological variables on NK activity in 95 subjects. Subjects were assessed for daily hassles and self-reported symptoms using questionnaires. After controlling for gender, lifestyle, and endocrine parameters, there was no evidence to support the assumption that NK activity was related to the psychological variables measured. The study reported that changes in NK activity were only related to plasma epinephrine levels (Benschop, Jabaaij, Oostveen, Vingerhoets, Kirschbaum, et al., 1993). However, it is possible that some of the endocrine levels controlled for were actually part of the stress response, which would account for the lack of NK differences if the effects of endocrine parameters were removed.

In another study of stress and NK activity, cold pressor pain and mental arithmetic were used as stressors to determine the time it takes for stress to affect NK activity. In a sample of 31 men, NK activity was measured before, during, and after the

acute stressor exposure. Subjects in both the cold pressor group and the mental arithmetic group reported that the stressful experience caused distress. Increased heart rate and blood pressure were seen in the mental arithmetic group, and persons in this group also had increased NK activity during the task and 5 minutes after the task ended (Delahanty, Dougall, Schmitz, Hawken, Trakowski, et al., 1996). Persons in the cold pressor group did not demonstrate significant changes in NK activity. This study demonstrates that NK cells can react very quickly to stimuli. The increased NK activity seen in response to the brief mental stressor could have been related to epinephrine, as these subjects displayed other signs of sympathetic nervous system activation, such as increased heart rate and blood pressure. Epinephrine has been demonstrated to increase NK activity (Bachen, Manuck, Cohen, Muldoon, Raible, et al., 1995; Nomoto, Karasawa & Uehara, 1994), in addition to its classic effects on heart rate and blood pressure.

Similar results were reported in an earlier study of mental arithmetic stress in women. Younger women had increases in NK cell activity following a 12 minute mental arithmetic examination. However, older women did not show stress-related changes in NK activity. Again, this study demonstrates that brief psychological stress is associated with rapid changes in NK activity. The age related differences may have been due to differences in the way older women react to mental arithmetic, or may have been related to physiological changes associated with aging (Naliboff, Benton, Solomon & Morley, 1991).

These results are of interest, as while humor has been used as a stress reduction method, laughter has been noted to act as a mild mental stimulus, and is associated with increased heart rate and blood pressure (Fry, 1994). Therefore, it is possible that exposure to humor and/or laughter may act as a brief stressor or stimulus,

leading to increased NK activity as a result of mental stimulation, rather than acting as a buffer of the effects of stress on NK activity.

Given the above studies, it appears that physical stressors, particularly pain, are related to decreased NK activity, while brief mental stressors associated with increased heart rate and blood pressure may lead to increased NK activity. However, the effects of chronic physical and mental stress on NK activity and health are not as well documented. The section below outlines studies which link one type of chronic stressor, negative life events, with the development and progression of illness. The subsequent section describes current information regarding stress, NK cell activity, and physical health outcomes.

Negative Life Events and Development/Progression of Illness

The relationship between negative life events and the development/progression of illness has been most extensively documented in studies of cancer. Retrospective studies in humans have consistently documented that more previous stressful life events are reported by cancer patients than by persons of the same age with different diagnoses. Several retrospective studies have reported an association between acute loss, bereavement, and/or depression and the subsequent development of cancer (Bahnon, 1981; Kissen, 1966; Leshan, 1966; Locke & Kraus, 1982). Antecedent stressful life events also have been linked to increased TB and upper respiratory infections, (Holmes, Hawkins, Bowerman, Clarke & Joffe, 1957), cancer of the cervix (Goodkin, Antoni & Blaney, 1986), and breast cancer (Solomon & Amkraut, 1981). Further information on this subject can be found in the following reviews: Anisman, Irwin and Sklar, (1989); Anisman and Sklar, (1982); and Blaney, (1984).

There are some obvious problems with the use of retrospective analyses as noted by various reviewers. Subjects may not remember past events with complete

accuracy, or the trauma of being diagnosed with cancer may induce the subjects to view past events in a negative light (Cohen, 1985; Rabkin & Streuning, 1976). Prospective studies avoid this methodological flaw and provide data that may be more reliable.

Several prospective studies document the connection between stressful life events and the development of certain types of cancer (Anisman, Irwin & Sklar, 1989; Fox, 1978; Rogentine, van Kammen, Fox, Doherty, Rosenblatt, et al., 1979; Thomas, Duszynski & Shaffer, 1979). For example, in a study involving breast cancer, women undergoing biopsy who reported a greater number of stressful life events subsequently were diagnosed with malignant disease more frequently than women who reported fewer numbers of stressful life events (Derogatis, Abeloff & Melisaratos, 1979). In addition, breast cancer patients who experienced many severe, adverse life events during their illness had shorter remission times than those breast cancer patients who reported fewer or less severe adverse life events during their illness (Ramirez, Craig, Watson, Fentiman, North, et al., 1989).

While there are several studies documenting the relationships between negative life events and the development or progression of cancer as noted above, many have methodological problems. In addition, while it is frequently suggested that decreases in immune function may be the causative influence by which stress affects cancer development, most of these studies did not document immune function status. Thus, the mechanism linking the stressor with the subsequent development or progression of illness is lacking. Therefore, further studies in this area need to establish whether relationships exist among stressors, decreases in immune function, and the development or progression of illness. Studies designed to document this chain of events are reported in the next section.

Stress, NK Activity and Illness

A number of animal studies have been conducted using either breast tumor or viral models to determine the relationships among stress, decreased NK activity, and the development or progression of disease. In a study of stress, NK function, and the development of herpes simplex virus Type 1 (HSV-1) in mice, it was reported that restraint stress decreased NK cell activity and the number of HSV-1 specific cytotoxic T-cells, impairing the animals' immune response to the HSV-1 infection (Bonneau, Sheridan, Feng & Glaser, 1991).

A study by Taylor (1991) reported the effect of stress on lung metastases in male rats. Rats exposed to acute stress had a significant decrease in NK cytotoxicity and a twofold increase in surface lung metastases. The authors noted that the stress effect was time related. A decrease in NK activity and increase in tumor cell metastases was seen when the stressor was applied one hour before intravenous (IV) administration of tumor cells, but not if the stressor was applied 24 hours before administration of tumor cells (Taylor & Gale, 1991; Ben-Eliyahu, Yirmiya, Liebeskind & Taylor, 1991).

A similar study by this group investigated the stress of surgery on immune function and the subsequent development or progression of cancer. In a series of studies using an IV injection of tumor cells, it was noted that rats who had surgery demonstrated a decrease in NK cytotoxicity and a decrease in the number of circulating NK cells four hours post-operatively (Page, Ben-Eliyahu & Liebeskind, 1994). Rats that underwent surgery also had a twofold increase in numbers of lung metastases, compared with rats that had not undergone surgery. This effect appeared to be related to pain during surgery, as morphine administration decreased the number of observed metastases. Another study by this group used NK depleted animals and found that surgery increased tumor cell metastases, even when morphine was administered. The

authors suggest that NK cell activity and other factors play a role in the increase of tumor cell metastases seen following surgery, and that NK cells play a critical role in morphine's attenuating effects on this outcome. These results also reinforce the need to provide adequate pain relief for persons undergoing surgery (Page, et al., 1994).

The effect of housing environment and swim stress on immune function and cancer development was investigated in a unique animal model. Using male transgenic mice that develop cancer at 10-15 months of age, it was found that housing with aggressive non-sibling mice increased the incidence, number of tumors, and tumor burden, compared with sibling housed mice. Mice housed in social isolation had increased tumor burden compared with sibling housed mice. To investigate the effect of an additional stressor, a subset of mice from all three housing environments were exposed to swim stress. Swim stress significantly increased the incidence and size of tumors in mice housed with siblings, but did not significantly affect tumor burden in the non-sibling housed mice or the isolated mice. It should be noted that the effects of stress on tumor growth in this study were not correlated with change in NK functioning. NK cell activity, which is already reduced in these transgenic mice, was not significantly affected by housing environment or swim stress (Hilakivi-Clarke & Dickson, 1995).

The animal studies cited above document the effect of physical stressors on NK activity and the subsequent development of disease using tumor or virus induction models. Naturally, these methods cannot be used with human subjects, but at least one study has attempted to document the same effect in humans. In a longitudinal study of 106 adults healthy adults with persistently low NK cell cytotoxicity, the combined effect of increased stress and decreased immune function on health was documented. The investigators report that an increased risk for infectious disease was seen at the six-month follow-up point for those subjects who had both risk factors, a more stressful

environment, combined with persistently low NK activity (Levy, Herberman, Simons, Whiteside, Lee, et al., 1989). This study indicates that persons who already have lowered immune function, such as persons with cancer or HIV, may be at greater risk for the development of illnesses secondary to stress. It should be noted that this study was limited to persons with low NK functioning, so the effect of a stressful environment on rate of infection in persons with normal NK activity was not determined.

Given the previously cited studies, there is considerable evidence that physiological and mental stressors, such as negative life events, can influence the neuroendocrine and immune systems. These changes have been documented for various neurohormones and specific immune system cells. See Kiecolt-Glaser and Glaser (1995), Kiecolt-Glaser, Cacioppo, Malarkey and Glaser (1992), O'Leary (1990), and Schulz (1992), for further review of stress and human immune functioning.

Researchers have frequently used measures of negative life events to indicate stress. This methodology is supported by studies which report that exposure to negative life events is correlated with measures of psychological distress, such as stress, anxiety, or depression (Anderson & Arnoult, 1989; Conger, Lorenz, Elder, Simons & Ge, 1993; Irwin, Patterson, Smith & Caldwell, 1990; Johnson & Sarason, 1978; Labott & Martin, 1987; Nezu, Nezu & Blissett, 1988; Toves, Schill & Ramanaiah, 1981). However, stress is a cognitive/affective response, not an event (Cohen, 1987). Exposure to a certain life event does not necessarily trigger the same cognitive/affective response in all people. How a person views a given event, how they cope with the event, and the emotional response triggered must also be taken into consideration (Cohen, 1987). For instance, Locke et al., (1984), found no relationship between life change stress (LCS) and NK cell activity (NKCA). However, subjects who reported both high levels of LCS and psychological symptoms (viewed as being "poor copers") had significantly lower NKCA

than those subjects who had high levels of LCS and low levels of psychological symptoms (good copers). Also, in a study of bereavement and depressed NK functioning, (Irwin, Smith & Gillin, 1987), it was found that changes in NK activity were not directly related to bereavement, but related to changes in depressive symptoms. Therefore, persons who responded to bereavement with depression were more likely to have subsequent immune suppression. This research demonstrates that the way a person responds to stress, which may be related to personality style and coping mechanisms, is as important as the number of stressful life events to which a person is exposed. The following section discusses how one coping mechanism, sense of humor, may act to diminish the effects of stress.

Sense of Humor and Stress

The use of humor as a coping mechanism to reduce the influence of stressful events (Giddan, 1987; Prerost, 1987), and to improve the ability to relax (Prerost, 1993) has been documented in studies of college students. In addition, sense of humor has been connected to higher levels of self-esteem. In studies using the Coping Humor Scale (CHS) and/or the Situational Humor Response Questionnaire (SHRQ), higher sense of humor scores were associated with lower levels of loneliness, depression, and stress, as well as higher levels of self-esteem (Kuiper & Martin, 1993; Kuiper, Martin & Olinger, 1993; Martin, Kuiper, Olinger & Dance, 1993; Overholser, 1992). Sense of humor was also related to higher quality of life scores in a study of undergraduate students (Kuiper, Martin & Dance, 1992).

Studies of nurses have revealed that humor is frequently used as a coping mechanism to decrease the effects of stress. Warner (1991) noted that student nurses use humor to cope with the stress of psychiatric nursing clinical (Warner, 1991). This was determined by examining the students' written narratives of various clinical

situations and their responses. A second study examined how nurses responded to a crisis in connection with the hurricane Hugo disaster. Nurses working in the devastated area reported that talking with each other, humor, religion, and altruism helped them to cope with the stress of helping victims of the disaster (Weinrich, Hardin & Johnson, 1990). A third study using qualitative methodology noted that emergency room personnel engage in various forms of humor to help cope with the stress of emergency situations (Rosenberg, 1991).

Since people frequently report using humor to cope with life events, or stress in general, the effectiveness of humor in reducing stress and mood disturbance related to stress has also been examined. Several studies document that sense of humor is related to improved mood in the face of increased life stressors. In a study by Martin and Lefcourt (1983) fifty-six college students completed self-report questionnaires on life events and sense of humor (SHRQ, CHS, and four other measures) as well as the Profile of Mood States (POMS). The students with higher scores on five out of the six humor measures had decreased pathological mood, even though they reported more stressful life events than students who scored low on the humor measures (Martin & Lefcourt, 1983). In a second study, reported in the same article, sixty-two college students completed the Life Experiences Survey, the SHRQ, and the POMS. The subjects were also asked to make up a 3-minute comedy routine about various objects, such as an old shoe. This last technique was devised to elicit the ability to create humor. The results indicated that persons who were able to create humor on demand had a lower association between stressful life events and depressed mood. A significant correlation ($p < .05$) was found between the production of humor and scores on the SHRQ.

However, Porterfield (1987) reported being unable to replicate Martin and Lefcourt's (1983) results. A sample of 220 college students completed the college students' life events schedule, the SHRQ, the CHS, and the Center for Disease Studies Depression Scale (CES-D). Physical illness (Inventory of Physical Symptoms) was also included as an outcome measure. Depression scores were positively related to the number of negative life events ($p = .000$) while being negatively related to scores on the SHRQ ($p = .002$) and CHS ($p = .009$). These data suggest that increased negative life event scores are related to increased levels of depression, while increased sense of humor scores are related to reduced levels of depression. However, when sense of humor and life events were combined, they became a non-significant predictor of depression. There was also a significant relationship between negative life events and physical symptoms ($p = .000$) but not between sense of humor and physical symptoms. These data suggest that while sense of humor was related to decreased depression scores, the relationship between negative life events and well-being may not be influenced by sense of humor.

A third study examined the effect of sense of humor on depression and anxiety (Nezu, Nezu & Blissett, 1988). A sample of 87 college students took the Life Event Scale, the State-Trait Anxiety Indicator, and the Beck Depression Inventory (BDI). Sense of humor was measured using the CHS and the SHRQ. Sense of humor (measured by either scale) and life events combined to be a significant predictor of depression scores on the BDI (CHS $p < .002$, SHRQ $p < .001$). These results are similar to those obtained by Martin and Lefcourt (1983) and support the proposition that sense of humor can act to reduce the effect of negative life events on depression scores. However, sense of humor was not directly related to anxiety, nor did it act to reduce the effect of life events on anxiety scores.

In a more recent study of 100 college students (Kuiper & Martin, 1993), the effect of sense of humor on self-esteem, perceived stress, negative self-content, depressive self-concept, dysfunctional attitudes, and depression (CES-D) was examined. Sense of humor was measured on three different scales; the SHRQ, CHS, and Sense of Humor Questionnaire. Sense of humor was negatively correlated with perceived stress ($p < .001$), negative self-content ($p < .025$), depressive self-concept ($p < .01$), and dysfunctional attitudes ($p < .01$). Sense of humor was positively correlated with increased self esteem scores ($p < .01$). However, when evaluating depression using the CES-D, only one humor scale was significantly correlated with depression. Scores on the Sense of Humor Questionnaire metamessage subscale were negatively correlated with CES-D depression scores ($p < .05$). As life events were not included in this study, the relationship between life events and depression and the possible influence of sense of humor on this relationship was not examined. Kuiper concluded that it appears sense of humor has a weak effect on depression, but appears to have a larger and more significant effect on long term depressive personality factors such as negative self-content (Kuiper, et al., 1993).

Finally, in a combined report of four studies of 104 female executives, the influence of perfectionism, humor, and optimism on the relationship between daily hassles and measures of well-being was examined. It was reported that both sense of humor and optimism interacted with daily hassles to decrease burnout and increase self-esteem, while perfectionism interacted with daily hassles to decrease self-esteem and increase burnout. Women who were high in perfectionism also had increased reports of physical symptoms compared with women who scored high on measures of sense of humor and optimism (Fry, 1995).

In summary, most studies of sense of humor have supported the notion that sense of humor is correlated with increased self-esteem, decreased negative self-content, and decreased dysfunctional personality attributes. It also appears that sense of humor can act to reduce the effects of daily hassles on self-esteem, burn out, and physical illness. The effect of sense of humor on depression is not as clear, with some studies finding a weak direct relationship (Kuiper, et al., 1993; Porterfield, 1987), and others finding that humor acts to decrease the effect of life-events on depression, rather than having a direct effect on depression (Martin, et al., 1983; Nezu, et al., 1988). Given the results of these studies, it is plausible to propose that sense of humor can reduce the effect of negative life events on self-reported stress and depression.

Laughter and Stress

The use of laughter to reduce symptoms such as pain and distress is an exciting topic for nursing clinical investigation. Several nursing reports advocate the use of humor to reduce stress, relieve pain, or as an adjuvant therapy to improve overall quality of life. In one of the few comparison studies available, laughter and relaxation therapy were used to determine which was more effective at reducing both psychological and physiological measures of stress (White & Camarena, 1989; White & Winzelberg, 1992). The results indicated that relaxation training was more effective than laughter at reducing the physiological measures of stress in their sample of college age students. Nonetheless, both relaxation training and laughter significantly reduced psychological measures of stress, when compared with the control group.

In a study of the effect of laughter on stress in persons undergoing dental surgery, it was found that those persons who laughed and joked more frequently before the dental procedure reported less stressful subjective experiences following the procedure

(Trice & Price, 1986). The investigators indicated that laughter may have reduced both stress and pain perception in persons undergoing dental procedures.

Another study examined the effect of laughter on both mood and pain perception in a sample of elderly persons living in a long-term care facility. The experimental group viewed humorous videos, while the control group watched non-humorous videos. The group exposed to the humorous videos subsequently reported decreased pain and improved mood, compared with those who were exposed to the non-humorous videos (Adams & McGuire, 1986). These findings concerning the effect of laughter on pain perception were supported by a later study that used college students to examine the effect of laughter on pain tolerance. The results indicated that subjects who were exposed to a humorous video had increased pain tolerance to TENS unit stimulation compared with the control group, who watched a non-humorous video (Hudak, Dale, Hudak & DeGood, 1991).

The effect of humor on psychological functioning was examined in a sample of hospitalized schizophrenics. The experimental group watched more than 70 humorous movies during a three-month period. The control group watched a variety of different movies over the same length of time. Exposure to the humorous movies led to significant improvements on the Brief Psychiatric Rating Scale (BPRS) and increased positive staff-patient interaction (Gelkopf, Kreitler & Sigal, 1993). A later study by this same group found that while laughter had led to improvements in the previous study, sense of humor, measured by the coping humor scale (CHS), did not correlate with measures of hostility, aggression or anger (Gelkopf & Sigal, 1995). Given the results of these two studies, the positive effect of laughter, but not sense of humor, on mood in schizophrenic patients is supported.

It is plausible, however, that use of humor may not be effective at reducing certain types of stressors. A recent study examined the effectiveness of humor, play, and gaming on reducing work load stress in nurses (DesCamp & Thomas, 1993). While play activities successfully reduced stress, use of staff humor and unit oriented games did not significantly affect stress levels in nursing staff (DesCamp, et al., 1993). It should be noted that this study did not use a control group, nor did it document laughter. It was assumed that the nurses who engaged in staff humor were indeed laughing. It is possible that insufficient laughter was produced to significantly influence work load stress.

In summary, laughter appears to improve mood, reduce psychological measures of stress, and reduce perception of pain. The mechanisms that underlie this effect may involve release of psychological tension, which could act as a buffer of stressful events on affect. However, it is also possible that there are physiological mechanisms involved as well as psychological ones. The next section outlines studies that examine the physiological changes that accompany laughter.

Physiological Effects of Laughter

If humor has a role in healing, one would expect some measurable physical effects to be related to either a sense of humor or mirthful laughter. A number of studies have documented the various effects of laughter on the body. Laughter results in increased heart rate, skin temperature, blood pressure, pulmonary ventilation, and skeletal muscle activity, as well as changes in brain activity (Fry, 1994). The various physiological effects of laughter have been examined in several research studies. It should be noted that most of these early studies used all male samples, the physiological effects of laughter upon female subjects are mostly undocumented. Recognizing this limitation, the physiological effects of laughter are outlined below.

One very early study examined the effect of laughter upon muscle tone. It was demonstrated that periods of intense laughter led to temporarily decreased skeletal muscle tone. During laughter, various muscle groups are activated, while the period immediately after the laugh leads to general muscle relaxation. This relaxation can last up to 45 minutes (Paskind, 1932).

In later studies of the effects of laughter on muscle tone, it was reported that the physical act of laughter affects a variety of skeletal muscles. Smiles activate facial, neck, and scalp muscles. Chuckling and laughter acts on muscles in the thorax, shoulders, abdomen, and diaphragm. So called "belly-laughter" can act on all of the above while causing spontaneous movement of the arms, legs, and pelvic muscles. This muscle activity can be similar to that seen in aerobic exercise. After laughter, a period of relaxation follows, which allows the muscles involved to return to their normal metabolic state (Fry, 1986; Fry, 1992; Fry, 1994).

In addition to changes in muscle tone, laughter also stimulates the respiratory system. Laughter affects respiratory patterns by increasing both rate and depth of respirations. Laughter causes greater expirations than inspirations and greater respiratory excursion (Fry, 1977). This relatively greater expiratory effect leads to blowing off excess carbon dioxide and water vapor which builds up in stale residual air (Fry, 1994). Circulation and oxygen transport are also temporarily increased during laughter (Fry, 1994). However, an earlier study by the same author noted that laughter did not significantly improve oxygen saturation levels (Fry, 1971). It should be noted that the early oxygen saturation study was conducted using a healthy population whose oxygen saturation rate would be expected to be near or at 100%. It is possible that there was no room for improvement in this group. Determining the effect of laughter on

oxygen saturation rates in a more compromised population, such as persons with chronic lung disease, would be useful to nurses and respiratory therapists.

A later study by Fry and Savin (1988), demonstrated that hard laughter leads to increased heart-rate, respiratory rate, and oxygen consumption. Laughter affects the cardiac system by temporarily increasing heart rate, circulation, and blood pressure, followed by a recovery state in which blood pressure and heart rate drop below resting levels (Fry & Savin, 1988). This recovery stage or relaxation effect is important in what Fry calls the "sparing mechanism" (Fry 1994, p. 115) . While laughter can cause considerable increases in heart rate and blood pressure, similar to that seen in aerobic exercise, few persons have ever had a heart attack during laughter, demonstrating that laughter has a sparing effect, probably due to the recovery period seen following acute periods of laughter (Fry, 1994).

Other studies examined the effect of mirth and laughter upon the sympathetic nervous system. Mirth is an emotional response to a humorous stimulus, and frequently results in mirthful laughter, which is a behavioral response to a humorous stimulus. According to theories of emotion (Duffy, 1962; Mandler, 1962), all intense emotions, regardless of their content, lead to activation of the sympathetic nervous system. This was demonstrated to some extent by a study in which three groups of subjects (all male) were exposed to either a humorous movie, a sad movie, or a neutral movie (Averill, 1969). Physiological responses were determined by heart rate, blood pressure, respiratory rate, skin temperature, and galvanic skin response (GSR). Both the humorous stimulus and the sad stimulus produced significant increases in GSR, indicating activation of the sympathetic nervous system. However, while exposure to a sad stimulus caused significant increases in blood pressure over the control group, the blood pressure of those exposed to humor remained stable, even though the GSR

reading of both groups would indicate activation of the sympathetic nervous system (Averill, 1969). The results of this study suggest that a humorous stimulus may buffer some of the effects of sympathetic nervous system activation upon blood pressure.

A more recent study of the effect of laughter on the sympathetic nervous system response was designed to determine if use of humor could reduce anxiety and heart rate during a stressful waiting period. Fifty-three college students were told that they would receive a shock after a brief waiting period. Subjects in the experimental group listened to a humorous tape while waiting to receive the shock, the placebo control group listened to a non humorous tape during this waiting period, and the non-intervention control group did not listen to a tape. Subjects in the humor group reported decreased anxiety during the anticipatory period ($p < .05$); those with the highest level of sense of humor (measured by SHRQ) had the lowest reported anxiety ($p < .01$). In contrast, the heart rate of the three groups were not significantly different, in all groups an increased heart rate was noted during the waiting period ($p < .001$). The authors concluded that the effect of humor may be primarily cognitive, rather than having a physical stress reduction capability, because heart rate was not significantly different among the groups (Yovetich, Dale & Hudak, 1990). However, as Yovetich pointed out, laughter tends to increase heart and respiratory rate in itself, and, therefore, heart-rate may not have been the best measure of reduced anxiety for this experiment. It would have been interesting to determine the effect of this intervention on GSR and blood pressure, as these measures of anxiety may be less influenced by the physical effects of laughter, and thus have given more distinct results.

Research examining the effect of laughter upon stress hormones has generated conflicting results. One early study looked at the effect of viewing four different films on urinary excretion of epinephrine and norepinephrine (Levi, 1965). The four films were

chosen to elicit different emotions. The first was a natural-scenery film, which was expected to be very bland and not to elicit any strong emotions. A second film was funny and was expected to produce mirthful laughter. A third film was a war movie, devised to be tragic and agitating. The fourth film was chosen to elicit anxiety, being a gruesome ghost story. All 20 female subjects were shown the four films on four consecutive days. The subjects all viewed the same film each day, starting with the neutral movie on day one, and ending with the anxiety provoking film on day four. The subjects served as their own control group, with urinary hormone levels measured during a 90 minute control period before each film, during each film, and finally during a 90 minute post film session. Urinary epinephrine levels decreased significantly during the natural-scenery film ($p < .01$). Urinary epinephrine levels increased significantly during the war film ($p < .05$). Urinary epinephrine and norepinephrine both increased significantly during the humorous film ($p < .05$). Urinary epinephrine increased most during the anxiety provoking film ($p < .05$); norepinephrine levels also increased significantly during this film (Levi, 1965). The conclusion of the authors was that there is a correlation between increased urinary excretion of epinephrine and strong emotional arousal, independent of the type of emotion being elicited. However, the methodology involving the subjects all viewing the films in the same order is a limitation of this study. The results obtained could be due to the order of film viewing, as the first film led to the lowest levels of hormone excretion, while the last film led to the highest levels.

A more recent study also examined the effect of a humorous film on a wider variety of stress hormones, using direct testing of hormone levels in serum. To determine the effect of laughter upon stress hormone levels, Berk, et al., (1989a) exposed experimental subjects to a humorous video, while the control group was kept in a quiet room for the same amount of time. Endocrine testing was performed before,

during, and after exposure to the experimental or control condition. The results demonstrated that serum cortisol ($p = 0.011$), growth hormone ($p = 0.0005$), and plasma dopac, a metabolite of dopamine ($p = 0.025$), decreased with laughter. There was no significant change in serum prolactin, beta-endorphins, plasma epinephrine, or plasma norepinephrine in either the experimental or the control group. The control group demonstrated no significant changes in any of the endocrine measures used. The authors concluded that as cortisol is immunosuppressive, laughter may act as an immunoenhancer by decreasing levels of this particular stress hormone (Berk, et al., 1988; Berk, et al., 1989a). This study, while controlled, used a rather small sample ($N=10$) and included male subjects only.

According to some authors in humor therapy, use of humor is believed to improve immune function by blocking production of stress hormones, such as cortisol and by increasing the release of immunoenhancers, such as beta-endorphin (MacHovec, 1991; Martinez, 1989; Metcalf, 1987; Sullivan & Deane, 1988). However, data to support this aspect of humor therapy is scant. In fact, according to a published interview with Steven Locke, a psychoimmunologist at Harvard, "if there's anything newsworthy about this subject, it's how little research has been done, considering how widely accepted the ideas are" (Long, 1987, p. 28). The next section outlines what is currently known about sense of humor, laughter, and immune system functioning.

Effects of Humor on the Neuroendoimmune System

According to PNI theory and several recent studies, it appears that humor may influence health through moderation of stress chemicals and/or immunoenhancement (Berk, Tan, Eby, Carmons & Vorce, 1984; Berk, Tan, Nehlsen - Cannarella, Napier, Lewis, et al., 1988; Berk, Tan, Fry, Napier, Lee, et al., 1989; Berk, Tan & Fry, 1993; Lefcourt, Davidson-Katz & Kueneman, 1990; Martin & Dobbins, 1988; Morreall, 1991).

Laughter can decrease levels of glucocorticoids (Berk, et al., 1989), and physiologic concentrations of glucocorticoids have been demonstrated to reduce NK cell cytotoxicity (Berk, et al., 1984). In addition, previous humor studies have reported that subjects who are exposed to a humorous video have a significant increase in IgA ($p = 0.015$), interferon ($p = 0.024$), the number of activated T cells ($p=0.007$), and number of NK cells ($p=0.013$) (Berk, et al., 1993; Berk & Tan, 1995).

Several studies have demonstrated that exposure to a humorous stimulus can result in immunoenhancement, determined by increased levels of salivary immunoglobulin A (SIgA) (Dillon, Minchoff & Baker, 1985; Labott, Ahleman, Wolever & Martin, 1990; Lambert & Lambert, 1995; Lefcourt, et al., 1990). Secretory IgA is found in body secretions such as tears, saliva, colostrum, sweat, and mucus. It acts to prevent local infections in areas such as the respiratory or GI tract. The following section reviews these studies concerning the effect of exposure to a humorous stimulus and sense of humor on SIgA.

In a randomized crossover study, 10 college students viewed two videos, a humorous video and an instructional video (used as a control) (Dillon, et al., 1985). The effects of exposure to a humorous video and sense of humor, measured by the coping humor scale (CHS), on SIgA levels were examined. The findings indicated that exposure to a humorous video (*Richard Pryor Live*) significantly increased SIgA levels ($p < .025$). In addition, scores on the CHS were positively correlated ($p < .05$) with SIgA levels **before** viewing the videos, demonstrating that subjects with greater sense of humor also had increased salivary IgA overall. Interestingly, scores on the CHS were actually **negatively** correlated with increases in SIgA ($p < .10$) following the humorous video. It was suggested by Dillon that as the subjects with higher humor scores had

higher baseline SIgA before the film (.02), they may not have increased as much from the intervention, due to a possible ceiling effect.

To determine if sense of humor acted to reduce the effect of stress on immune functioning, Martin, et al., (1988), used a sample of 40 college students. Stress was measured by Daily Hassles Scale (DHS), while sense of humor was measured by the SHRQ, CHS, and the Sense of Humor Questionnaire (SHQ). Immune function, determined by levels of SIgA, was measured at two different times during the study, about one and a half months apart. Only scores on the SHRQ and SIgA at time two were positively correlated ($p < .05$); all the other humor scores and SIgA correlations were not significant, and six out of the eight correlations were negative (non-significant) correlations. These results would appear to indicate that sense of humor has no direct effect on salivary immune functioning, or at most, a very modest effect. However, data from this study indicated that sense of humor may act to reduce the effect of stress on SIgA. Adding sense of humor scores to the stress scores in a multiple regression statement produced a significant increase in amount of variance in SIgA which could be predicted from the equation ($p < .05$). The relationship between daily hassles and SIgA varied as a function of sense of humor. These results suggest that sense of humor, rather than having a direct effect on SIgA, may act to buffer the effect of stress on salivary IgA.

One recent publication reports the results of three separate studies of humor and immune response in college students (Lefcourt, et al., 1990). This report documents that in all three studies ($n = 45$, $n = 34$, $N = 62$) subjects' salivary IgA levels increased significantly after a brief humorous stimulus ($p < .005$). These results clearly support the immunoenhancing actions of an exposure to a brief humorous stimulus on SIgA. The influence of sense of humor on SIgA levels was more complicated. Sense of

humor was measured using the SHRQ and the CHS. Contrary to Dillon's findings, in study number one, scores on the humor scales were unrelated to baseline SIgA, while scores on the CHS were positively related to increases in SIgA following the stimulus ($p < .02$). In study number two, no significant correlation was found between sense of humor and pre or post SIgA levels. And in study number three, scores on the SHRQ were only marginally related to increases in post stimulus SIgA ($p < .10$). Therefore, it is questionable if sense of humor (as measured by the CHS or SHRQ) influences levels of SIgA. It should be noted, that while a similar methodology was used in these three studies, a different humor stimulus was used. In studies number one and three, a humorous audio tape, *The 2,000 -Year Old Man* was used. In study number two, subjects viewed a humorous video, *Bill Cosby Himself*. Lefcourt (1990) suggested that the video was found humorous by all subjects, which could override the effect of sense of humor. The audio tape was not rated as funny by the subjects. It may be that only subjects with a greater sense of humor benefited from the audio tape, and therefore led to the correlation between sense of humor and SIgA in studies number one and three.

Finally, to determine the influence of sense of humor on a variety of immune function measures, Dobbin, Harth, McCain and Martin (1990; 1991) examined the effect of three psychological variables; type A, locus of control, and sense of humor (measured by CHS) on immune functioning following examination stress (Dobbin, 1990; Dobbin, Harth, McCain & Martin, 1991). The results indicated that exam stress produced significant immune suppression as measured by decreases in T-cell response to stimulation by concanavalin-A ($p < .01$) and pokeweed mitogen ($p < .01$). Exam stress also led to decreased plasma levels of IL-1 beta ($p < .01$) and interferon gamma ($p < .05$). Exam stress did not lead to significant changes in salivary or plasma cortisol levels ($p < .91$). However, this study found no relationship between any of the measured

psychological variables and immune function following examination stress. The author concluded that the role of psychological traits in the physiological reaction to stress should be questioned (Dobbin, 1990). While this conclusion appears to be broad, considering the bulk of literature to support the role of other psychological factors as stress modifiers, it does suggest that sense of humor (as measured by CHS) may not act to reduce the effect of exam stress on the immune functions measured in this study.

In review, it appears that exposure to a brief humorous stimulus significantly increases one measure of immune function, salivary IgA. The effect of sense of humor on SIgA and its possible interaction with a humorous stimulus is not as clear. One study reported that sense of humor was negatively correlated with increases in SIgA following a humorous stimulus (Dillon, et al., 1985), two studies found that subjects with greater sense of humor scores had larger increases in SIgA following a humorous stimulus (Lefcourt, et al., 1990), and lastly one study reported that sense of humor was not significantly related to SIgA levels before or following a humorous stimulus (Lefcourt, et al., 1990). It should be noted that the use of salivary IgA as a measure of immune function has been challenged by some authors (Mouton, Fillion, Tawadros & Tessier, 1989; Stone, Cox, Valdimarsdottir & Neale, 1987), due to the effect of variations in individual saliva flow rate and the use of stimulated and non-stimulated samples in some studies. In addition, the clinical significance of increased levels of salivary IgA in cancer patients is questionable. Therefore, the effect of humor and sense of humor on immune function needs further documentation using more valid and reliable measures of immune function.

According to a conference report by Cohen (1987), there is no general agreement among PNI researchers concerning which immune tests should be used in

PNI research. Listed below are three points of view that Cohen recorded from the conference.

1. Apply virtually every test available, hoping that one test will detect a reproducible change. However, while this method was favored by the psychologists, the immunologists were concerned that chance occurrences of significant changes might result. Even with statistically significant changes, there may not be any clinical relevance.
2. Focus the immune testing on the parameter that is believed to be related to an expected outcome, such as NK cell functioning in persons with cancer, or T cell profiles in persons with AIDS. "This was referred to by M. Reite as the 'theory-driven' approach" (p. 34). However, because so little is known about the influence of small changes in specific immune system parameters on infectious or malignant disease, it is often very difficult to determine if an immune change will in fact lead to a clinically significant outcome.
3. The third view was to let the outcome drive the testing. "First establish that a particular sort of stressful event or personality type does, in fact, incur a higher incidence of a disease that could be related to immune system compromise. Once this is firmly established, a search can be instituted for immune-related intervening variables" (p.34). However, these type of studies tend to be long, expensive, and difficult to conduct.

NK Cell Cytotoxicity as an Immune Outcome Measure

For this study, the theory-driven method was used. As the use of humor was one of the most popular complementary interventions reported by a sample of rural cancer patients in the Midwest (Bennett 1995a), an immune outcome measure connected to cancer development and progression was the logical choice. As noted earlier, NK cells

have demonstrated ability to kill tumor cells and various viruses. This ability may have clinical applicability in the treatment of cancer.

Many studies examining the effects of stress and coping on the immune system are now using NK cell cytotoxicity as an indicator of immune system functioning (Antoni, LaPerriere, Schneiderman & Fletcher, 1991; Brosschot, et al., 1991; Irwin, Daniels, Bloom & Weiner, 1986; Irwin, Patterson, Smith & Caldwell, 1990; Naliboff, Benton, Solomon & Morley, 1991; Zamai, Papa, Marinozzi & Caroli, 1992). According to Schulz and Schulz (1992), NK cell assays give some of the clearest and most replicable results in this type of research. Therefore, NK cell cytotoxicity was chosen as the immune outcome measure for this study.

Previous Studies of the Effect of Exposure to a Humorous Stimulus and Laughter on NK Cytotoxicity

Empirical evidence concerning the effect of humor on NK cytotoxicity is scant and conflicting. Only two studies have examined the influence of a humorous stimulus on NK cytotoxicity. The first study used 22 breast cancer patients to determine the effect of viewing both a humorous video and a distressing video on NK activity and number of NK cells (Wise, 1989). Subjects met in groups of three on two consecutive evenings to view each of the two 20 minute videos, in a randomized crossover design. Blood samples were taken from an indwelling catheter before and immediately after each video, at 30 minutes following the video, and at 23 hours following the first video (just before viewing the second video). Sense of humor was also included in this study, measured using the Coping Humor Scale (CHS). Scores on the CHS were positively correlated with baseline numbers of NK cells, which would indicate that subjects with higher coping humor scores also had more NK cells. However, contrary to what was expected, no significant changes were noted in NK numbers or cytotoxicity at the end of

either video or during the recovery period. This unpublished dissertation also included another unexpected finding; NK cytotoxicity was significantly **decreased** 23 hours after viewing the humorous video, and was significantly **increased** 23 hours after viewing the distressing film. Wise suggested that this could be a rebound effect, from a change in NK cytotoxicity (in the expected direction) sometime in the preceding 22 hours, or could be related to anticipation of the film immediately to follow. The 23-hour post-stimulus test point was immediately prior to viewing the second video, and served as the pre-stimulus data point for the second film. Unfortunately, data was not collected 23 hours after the second film. Therefore, it is possible that the results seen 23 hours after the first video were related to anticipation of the upcoming video, rather than a delayed effect of the video seen the previous evening. This is a confounding factor that needs to be avoided in future humor research. In addition, as NK levels are known to be reduced in patients with cancer, using cancer patients as the subjects in this investigational work may have led to an additional confounding factor. Lastly, as this study involved running multiple NK assays over a period of several weeks, some data on day to day NK assay variability would have added to the strength of this report.

In a controlled experimental study of ten subjects, NK cell cytotoxicity was significantly increased following a humorous video (Berk, Tan, Napier & Evy, 1989). The experimental group viewed a 60 minute humorous video. The control group sat quietly in the same room for the same length of time a few days later. Samples for NK cytotoxicity were drawn at baseline and immediately after the movie or the quiet time. Blood samples were also drawn every ten minutes during the study for other physiological measures, such as beta endorphins, cortisol, and ACTH (Berk, et al., 1989). The experimental group displayed significantly increased NK cell cytotoxicity from baseline to recovery ($p < .008$), while the control group did not. A limitation of this

study is that the experimental and control samples for NK cytotoxicity were run on different days. The samples for the endocrine measures of the experimental group were frozen, then run in the same assay with samples from the control group. However, because NK cytotoxicity decreases with cell freezing, the NK samples were tested as fresh samples on each of the two days of the study. Therefore, the control pre/post NK samples and the experimental pre/post NK samples were not run together in one assay (personal communication, S. Tan, Loma Linda Medical Center, January 19, 1996). This methodology is somewhat problematic, as it subjects the experimental versus control data to interassay variability. Fortunately, the pre/post samples of either group viewed alone would not be affected by interassay variability, so the findings concerning change in the experimental group could be considered valid using a strictly pre-post methodology. However, current recommendations are for the experimental and control samples to be tested in the same assay to prevent interassay variability between the control and the experimental results (Moye, Richardson, Post-White & Justice, 1995). Berk's (1989) methodology did not include sense of humor, documentation of humor response, or psychological measurement of stress. It also involved a small sample, all male subject group, and was published in abstract form only.

Summary of Review of Literature

While use of humor is a popular complementary intervention with cancer patients in the rural Midwest, the effectiveness of this intervention in improving immune function is somewhat tentative. Several studies have demonstrated an increase in salivary IgA levels following exposure to a humorous video (Dillon, et al., 1985; Labott, et al., 1990; Lambert, et al., 1995; Lefcourt, et al., 1990). Sense of humor does not appear to have a direct effect upon SIgA levels, but may have an effect on numbers of NK cells. While sense of humor may also act to reduce the effect of stress on SIgA levels, reliability of

the SIgA test itself is questionable and the clinical significance of increased SIgA levels is not well documented. NK cell cytotoxicity is a more reliable measure of immune functioning and has demonstrated clinical significance in persons with cancer. Unfortunately, there are only two studies in the literature that document the effect of exposure to a humorous stimulus on NK cytotoxicity. The first study to use NK cytotoxicity (Wise 1989) as an outcome measure had confounding factors, small sample size, and no control group. The second study (Berk et al., 1989a) used a small sample of male subjects, did not include sense of humor, and ran the experimental and control NK samples in separate assays. The two studies have yielded conflicting results. An additional problem with most of the studies of humor and immune function cited is the lack of humor response measurement. While mirthful laughter is a common response to a humorous stimulus, subject responses may vary. Documentation of humor response is needed to determine the effect of mirthful laughter on immune function. Therefore, additional research is necessary to determine the influence of sense of humor and mirthful laughter upon NK cytotoxicity.

METHODOLOGY

The purpose of this research project was to determine the effect of mirthful laughter and sense of humor on stress and NK cell cytotoxicity in a sample of healthy adult females.

Research Aims

1. To determine the effect of a brief period of mirthful laughter on NK cell cytotoxicity.
2. To determine the effect of a brief period of mirthful laughter on self-reported stress and arousal.
3. To determine the relationships among negative life events, sense of humor, humor response, stress, arousal, and baseline NK cell cytotoxicity.

Participant Recruitment and Screening

Participant Inclusion Criteria

As the effect of humor on NK cell cytotoxicity in healthy individuals has not yet been established, using a compromised patient sample in this exploratory study would have been premature. Therefore, healthy, adult, non-pregnant, female volunteers were recruited from a university setting and from within a rural Midwestern community by use of posters, newspaper advertisements, and word of mouth. The use of the all female sample was based on a number of considerations. First, in a recent survey of local cancer patients, females expressed more interest in the use of humor to reduce stress than did males, so future clinical use of a humorous stimulus would more likely include female subjects (Bennett 1995a). Second, the only published research to demonstrate a possible effect of laughter upon NK cytotoxicity used an all male sample (Berk, et al., 1989), thus the effect of laughter upon NK cytotoxicity in females has not yet been

demonstrated. Lastly, males tend to have higher NK cytotoxicity than females, so a mixed gender sample could introduce more variance than is desirable in this type of research (Benschop, Jabaaij, Oostveen, Vingerhoets, Kirschbaum, et al., 1993; Evans, et al., 1992; Irwin, et al., 1992). Therefore, the sample for this project was limited to healthy females. Additional subject selection criteria are addressed in the following section.

Participant Exclusion Criteria

Because several acute and chronic disease processes, surgery (Lukomska, Waldemar, Engeset & Kolstad, 1983; McCulloch & MacIntyre, 1993; Nakamura, Morikawa, Koike, Sakai, Yamaguchi, et al., 1994), and even pregnancy (Adam, 1982) have been demonstrated to alter immune function, these factors were controlled by excluding subjects who reported being pregnant, a diagnosis of chronic illness (such as rheumatoid arthritis, lupus, cancer, or other disease processes that involve the immune system), or having had an infectious illness or minor surgery (not requiring general anesthesia) in the prior two weeks. In addition, subjects who reported having major surgery (requiring general anesthesia) in the prior six months were also excluded.

Lifestyle factors such as sleep, exercise, and general nutrition can also affect immune functioning (Kusaka & Morimoto, 1992). In general, sleep deprivation leads to immune suppression (Irwin, Mascovich, Gillin, Willoughby, Pike, et al., 1994; Moldofsky, Lue, Eisen, Keystone & Gorczynski, 1986; Palmblad, et al., 1979). Therefore, persons who reported having had less sleep than usual in the preceding three nights were excluded. Changes in sleep cycles, such as those seen in shift workers, can also influence immune functioning by altering circadian rhythms (Anders, 1982; Fukuda, Ichikawa, Takaya, Ogawa & Masumoto, 1994; Gatti, Delponte & Cavallo, 1987; Gatti, Sartori, Cavallo, Del Ponte, Carignola, et al., 1987; Maestroni, Conti & Pierpaoli, 1986;

Maestroni, Conti & Pierpaoli, 1987; Masera, Carignola, Staurenghi, Sartori, Lazzero, et al., 1994). Thus, persons working on the 11:00 P.M. - 7:00 A.M. shift, or those who had been rotated to this shift within three days of data collection, were excluded from this study.

It has been demonstrated that moderate regular exercise tends to enhance immune functioning, whereas suppressed immune function has been noted after extensive exercise (Temoshok, 1987; Antoni, Schneiderman, Fletcher, Goldstein, Ironson, et al., 1990; Esterling, et al., 1992; Filteau, Menzies, Kaido & O'Grady, 1992; LaPerriere, Antoni, Schneiderman & Ironson, 1990). Thus, persons who reported exercising more than seven hours each week were excluded from the study.

Overt nutritional deficits can lead to immunosuppression (Filteau, et al., 1992; Kiremidjian, Roy, Wishe, Cohen & Stotzky, 1994; Saxena, Esteban & Adler, 1980) and there is some indication that nutritional supplements can influence immune functioning (Berger, German, Chiang, Ansari, Keen, et al., 1993; Filteau, et al., 1992; Kiremidjian, et al., 1994). Persons who reported weight changes of more than five pounds in the prior month were excluded, along with persons who reported taking herbal remedies or nutritional supplements in excess of a regular daily multivitamin and/or calcium supplement.

Other factors to consider were the use of medications, alcohol intake, and smoking. Alcohol use, street drugs, and many medications have been demonstrated to alter immune functioning (Dax, Adler, Nagel, Lange & Jaffe, 1991; Grunberg & Baum, 1985; Jaffe, 1980; Klimas, Blaney, Morgan, Chitwood, Milles, et al., 1992; Nakachi & Imai, 1992; Saxena, et al., 1980; Windle, Mondul, Whitney, Cummings & Stadler, 1993). Therefore, persons who reported drinking more than the equivalent of ten ounces of alcohol a week or use of tobacco products, street drugs, or immunosuppressant

medications/treatments (steroids, chemotherapy, radiation treatments) were excluded from this study.

There are also some data to indicate that pain and anxiety related to venipuncture can alter NK functioning in persons with acute needle phobia (Girgis, Shea & Husband, 1988). Therefore, persons who reported previous symptoms of fear or fainting when having their blood drawn, and those who stated that they are afraid to have their blood drawn, were excluded from this study.

Lastly, several studies have documented a connection between psychological depression and immune system suppression. Persons with depression have reduced NK cytotoxicity (Caldwell, Irwin & Lohr, 1991; Irwin & Livnat, 1987; Irwin, et al., 1987; Irwin, Smith & Gillin, 1992; Kronfol, Nair, Goodson & Goel, 1989; Nerozzi, Santoni, Bersani & Magnani, 1989; Urch, Muller, Aschauer & Resch, 1988), and depression is associated with decreased proliferative responses to pokeweed mitogen and concanavalin A (Schleifer, Keller & Stein, 1987). There is also indication that suppression of NK cell numbers and NK cell cytotoxicity are related to severity of depression. These changes appear to be gender related, with women affected more than men (Evans, Folds, Petitto & Golden, 1992). A meta-analysis of the depression and immune function literature indicated that clinical depression is associated with alterations in cellular immunity, lower response to mitogens, reduced NK cell activity, and alterations in numbers of several white blood cell populations. There is also evidence of a linear relationship between intensity of depressive symptoms and indicators of cellular immunity (Herbert & Cohen, 1993). Therefore, persons being treated for depression and/or taking antidepressant medications were excluded from this study. In addition, as persons in the general population may be depressed, without having a diagnosis of depression (Deardorff, et al., 1985; Evans, et al., 1992; Hammen,

1980; Irwin, et al., 1987), the Center for Epidemiologic Studies Depressed Mood Scale (CES-D) was used to screen for depressive symptoms in potential subjects. Persons with a CES-D score over 16 were not included in subject selection. See table 1 for a summary of inclusion and exclusion criteria for this study.

Table 1. Human Subjects Inclusion and Exclusion Factors	
Inclusion Factors	Exclusion Factors
1. Female	1. Acute illness or minor surgery in the preceding two weeks
2. Over the age of 18	2. Chronic illness
3. Non-Pregnant	3. Major surgery in the preceding six months
	4. Use of tobacco products or street drugs
	5. Use of alcohol over 10 ounces per week
	6. Taking immunosuppressant or antidepressant medications
	7. Weight loss or gain of more than 5 pounds in the preceding month
	8. Use of nutritional supplements other than one daily multivitamin and/or a calcium supplement
	9. Sleeping less than usual in the preceding three nights before the study
	10. Working the 7-11 shift in the past three days
	11. Exercising more than seven hours per week
	12. Fear of having blood drawn for lab testing
	13. CES-D score over 16 at pre-screening time

Participant Screening Procedures

Over seventy women indicated interest in the research project by contacting the researcher. These potential participants then were screened by telephone interview two to three weeks in advance of the study, to determine if they met the research criteria. The Center for Epidemiologic Studies Depressed Mood Scale (CES-D) and an investigator designed tool were used during the telephone interview to assist in

screening subjects for this project (See Appendix A). Persons who scored over 16 on the CES-D during pre-screening were excluded from the study (Deardorff & Funabiki, 1985; Evans, et al., 1992; Hammen, 1980; Irwin, et al., 1987). Five potential subjects were rejected during the pre-screening period due to CES-D scores over 16. Anyone who scored over 16 on the CES-D was informed that the screening test indicated that she had symptoms of depression, and she was referred to her personal physician and the community mental health center for assistance. Twenty other potential subjects were rejected during the pre-screening for a variety of reasons, including scheduling problems, pregnancy, needle phobia, chronic illness, and use of herbal remedies. After the screening process, forty subjects were selected to participate in data collection, based on subject availability. These volunteers were then scheduled for one of the four data collection days. A reminder letter was mailed to the participants one week before their scheduled date for participation. If a subject called in to cancel their participation, another date was arranged, or another subject meeting the criteria was selected. On the day of data collection, additional screening was performed to determine if the subjects still met all the inclusion and exclusion criteria. The CES-D was repeated at this time. As some studies have found that depression screening tools tend to produce false positives, particularly in younger persons, a cut off score of over 18 on the CES-D was used to reject data from participants who met the earlier screening criteria (Hammen, 1980; Kendall, Hollon, Beck. A., Hammen & Ingram, 1987). In the end, thirty-four participants met the inclusion and exclusion criteria on the day of data collection. Data from one of these subjects were subsequently excluded as a statistical outlier, as her baseline NK activity was beyond three standard deviations of the mean NK activity for persons in this study.

The number of subjects scheduled for participation in data collection was based primarily on available funding considerations. The NK assay costs approximately \$25.00 per sample, and subject payments were \$30.00 each. The number of subjects participating in this study was greater than the number of subjects in either of the previous two humor and NK studies and sufficiently large to allow the use of parametric statistics. Funding for this study was provided by Lambda Sigma chapter of Sigma Theta Tau International and Indiana State University (ISU) Research Committee. Lab space and equipment were provided by Indiana University - Terre Haute Center for Medical Education.

Human Subjects Requirements

Institutional Review Board (IRB) approval was obtained before obtaining funding for this project. Written informed consent was obtained from the subjects prior to data collection (See Appendix B). Subject confidentiality was maintained by coding subject data with subject identification numbers and by reporting group results only. Data collected for this study are maintained in a computer database in a locked office. The computer is password protected. Hard copies of subject records are maintained in a locked file cabinet in a locked office. The subjects were exposed to no more risk than that involved in a routine medical lab exam. Universal precautions as described by the Centers for Disease Control were utilized at all times (U.S. Department of Health and Human Services, 1987). Results of the study, including the subject's individual test results and explanation of the results as average, below average, or above average were made available to the subjects if they so desired.

Random Assignment

Subjects were randomly assigned to either the distraction control group or the humor experimental group using random numbers. The random numbers were provided by Susan Shott Ph.D., using computerized random number generation and subject code numbers. The daily assignment sheets with random numbers were mailed directly to a research assistant, who was responsible for coding all subject materials and directing subjects to their assigned groups. All psychological instruments and blood samples were labeled with the subject's code number. The primary researcher was blind to subject group assignment while conducting the NK assays. However, as the pre-test samples arrived at the lab about one hour before the post-test samples, the primary researcher was not blind to pre vs. post condition of samples.

Use of Videos as a Stimulus

Humor Videos

Several studies have been conducted which use some type of humorous stimulus as an intervention (Adams & McGuire, 1986; Berk, et al., 1988; Berk, et al., 1989; Berk, et al., 1989; Dillon, et al., 1985; Hudak, Dale, Hudak & DeGood, 1991; Labott, et al., 1990; Lefcourt, et al., 1990; Wise, 1989). Most of the studies have used humorous videos, but audio tapes have also been used (Lefcourt, et al., 1990). The following movies have been successfully used in PNI humor research: *Bill Cosby, Himself* (Hudak, et al., 1991; Labott, et al., 1990; Lefcourt, et al., 1990), *Gallager- Over Your Head* (Berk, et al., 1988; Berk, et al., 1989a; Berk, et al., 1989b), and *Richard Pryor Live* (Dillon, et al., 1985). Many people suggested various comical movies for use in this research project. However, when choosing a humorous stimulus, there are several elements to be considered. What one person views as funny, another may view as silly, or even

offensive. This is particularly true if the subjects are from different age groups or cultures. Older persons tend to find old movies funny, like Abbot and Costello (Adams, et al., 1986), while younger people may find these films outdated and boring. Persons with a conservative background may find comedians such as Richard Prior offensive. And the use of an audio tape may not stimulate as much laughter in persons who are visually oriented (Lefcourt, et al., 1990). In the pilot study (Bennett, 1994), the video - *Bill Cosby, Himself* was successfully used as the humorous stimulus. This video, while somewhat outdated, has been successfully used in prior humor research. It tends to be found funny by many subjects, and does not contain offensive language. For this study, both experimental and control subjects viewed videos in groups of four to five. Three humorous videos were available for use in this project: *Bill Cosby, Himself*, Tim Allen, *Men are Pigs*, and *Robin Williams: Live at the Met*. The subjects were informed of the videos available and asked for their preferences during the telephone screening process. Subjects were informed that the Tim Allen video contained language that could be offensive, and the Robin Williams video contained language that could be offensive, along with sexually explicit material. This method followed the assumption that the subjects would pick the movie style that they find most humorous, and thus maximize the effect of the humor stimulus. The majority of subjects chose the Bill Cosby video, which may have been viewed as a "safe" alternative by the subjects. Persons who expressed a preference for either Tim Allen or Robin Williams were placed on a waiting list, to attempt to form a group for these movies. These persons were eventually dropped from the study, as it was not possible to find enough other subjects wanting to view these videos on the same day. The persons choosing either Tim Allen or Robin Williams tended to be younger, and from the university setting, while many of those choosing Bill Cosby were older and from the community setting. In future humor

research with this population, a continued search will be made for comedy that does not include use of offensive language or sexually explicit material.

To facilitate a humor response, subjects were asked to sit in a comfortable lounge environment while watching the video. After the video and all study instruments were completed, refreshments were available for everyone attending. This methodology was suggested by Dr. Lee Berk's humor research team at Loma Linda Medical Center as helpful in recruiting participants for their humor and immune function research.

Distraction Control Video

Another methodological problem was the choice of the distraction or control stimulus. The distraction control group was to view some type of video, to support that any immune changes in the experimental group were not related to sitting down and viewing a video tape, but rather that the response invoked by the video was the important factor. An optimal neutral stimulus should not evoke humor, sadness, anger, or frustration, as the effects of various emotions could alter immune functioning (Futterman, Kemeny, Sharpiro & Fahey, 1994; Labott, et al., 1990). In effect, the control tape should alter emotions only minimally. In previous research, various types of videos had been used as a control stimulus. A video on planting flowers, *Annals and Hanging Baskets* was effectively used by Hudak, et al. (1991) in their study of the effect of humor on pain tolerance. An educational tape, *The Thin Edge: Anxiety*, was effectively used as a control stimulus in a sample of psychology students by Dillon and Baker in their 1985 study of humor and salivary IgA. Other humor investigators have avoided the use of a neutral video by substituting an hour of class (Lefcourt, et al., 1990) and a quiet time protocol (Berk, et al., 1989b) in place of a neutral video. Because it is sometimes difficult to judge what one person will find funny, sad, or relaxing, the quiet time protocol was chosen by Berk (1989b). Of course, this did not account for the effects of viewing a

video, but did allow pre and post-testing of the control group, with the same intervening time interval as in the experimental group. For this project, the control subjects were asked to view two non-humorous videos as a means of distraction during the waiting period. The videos, *A Video Tour of Scotland* and *A Video Tour of the Caribbean Islands*, were chosen to be non-humorous, non-violent, non-anxiety provoking distraction. Distraction control subjects sat in a lounge environment while watching the two non-humorous videos. Following the video and instrument completion, the control subjects met and had refreshments with members of the humor video group and members of the research team.

Instruments

Humor Response Scale

To determine if mirthful laughter affects the immune system, it was important to document this response from the subjects. It was probable that some subjects would not be able to relax and respond normally to a humorous stimulus in a controlled research setting. In addition, some persons may naturally respond to a humorous stimulus with smiles, but not overt laughter. It was necessary to account for this possibility. In the pilot study, subjects were observed during the video for signs of overt laughter. In addition, subjects were asked to self rate their laughter on a 5-point scale following the video (Labott, 1990). Data from persons who did not laugh aloud were to be analyzed separately to look for significant differences, but all subjects laughed aloud during at least part of the film. For this project, a 4-point movie rating scale was added to assess how funny the subjects found the movie to be. Scores from the self-report laughter item and the self-report movie rating item were then combined into a movie response score. In addition, an observer joined the humor group to observe and rate

each subject on mirthful laughter, while being as inconspicuous as possible. The Humor Response Scale (HRS -developed for this dissertation, See Appendix C) was used by the observer to rate each subject's humor response. The observer was an experienced nurse researcher who had helped develop the HRS and was trained in the use of this scale by the primary researcher. Each subject was observed in rotation for two minute time intervals at each checkpoint. The checkpoints started five minutes after the beginning of the film and proceeded at 10 minute intervals throughout the video. The subjects were rated at each checkpoint on their average response during the two minute time frame. The rating was conducted using a scale from 1 -7, with one being no humor response and seven being nearly continuous laughter plus spontaneous body movements. These ratings were then summed for a total humor response score. To avoid spurious differences due to inter-rater variability, the same observer rated all subjects in the humor group.

Measuring Sense of Humor

The concept of humor is very complex and, therefore, remains difficult to measure. Qualitative style interviews and production of humor have problems due to the subjective nature of the test and the time it takes to administer, while self-report scales suffer from social desirability contamination. Everyone wants to believe they have an above average sense of humor. This has led to a variety of humor testing modalities being used in humor research, such as interview schedules, having the subjects come up with impromptu comedy routines, and several self-report scales. Each method has its benefits and drawbacks. Some studies have tried to equate sense of humor with measures of cheerfulness and optimism (Friedman, Trucker, Tomlinson-Keasey & Schwartz, 1993), while others have gone as far as to use the number of hours of

televised sit-com's viewed to determine the effect of humor exposure on the general health of the United States population (Silberman, 1987).

Most published studies have used self-report instruments with established reliability, such as the SHRQ (Martin & Lefcourt, 1984), the CHS, (Martin & Lefcourt, 1983) and the Sense of Humor Questionnaire (SHQ) (Svebak, 1974). Many studies examining sense of humor use one or more of these self-report scales, and may also include a qualitative creative humor test as well (Rosenberg, 1991). These studies have been able to support a connection between humor and health. Sense of humor was significantly correlated ($p < .01$) with perceived physical health (Cornell Index) in a sample of 51 college students (Carroll, 1990; Carroll & Shmidt, 1992). In addition, sense of humor (SHRQ) and using humor as a coping mechanism (CHS) were positively correlated with measures of morale and perceived health in a sample of noninstitutionalized older adults (Simon, 1990). However, a retrospective study of 159 college students reported that coping humor (CHS) does not reduce the influence of stressful life events upon physical health (Anderson & Arnoult, 1989). According to the Anderson study, stressful life events and personal control beliefs alone predicted more than 20% of the variance in the reported symptoms of illness for the previous 10 weeks. Scores on the CHS were not significantly related to reported past physical health. It should be noted that the physical health tool used in Anderson and Arnoult's (1989) study to determine students' health over the previous 10 weeks was developed for the project, with no report of reliability nor validity.

Sense of Humor Tools

For this project, sense of humor was measured using Martin and Lefcourt's Situational Humor Response Questionnaire (Martin & Lefcourt, 1984) and the Multidimensional Sense of Humor Scale (MDHS) (Thorson & Powell, 1993). Both of the

humor measures have established reliability and validity using a college student sample. The SHRQ is a 21-item scale that elicits how often the subject would laugh or smile in response to many positive and negative situations. Cronbach's alpha for the SHRQ is reported to be .70 to .85 (Martin, et al., 1988). In addition, significant correlations between SHRQ, observations of spontaneous laughter, and peer ratings of sense of humor have been reported (Martin & Dobbin, 1988). The SHRQ had more variability than the other humor scales used in the pilot study and appears to be less affected by the social desirability factor. The SHRQ is available through Martin and Lefcourt (1984).

The MDHS is a relatively new measure of sense of humor. It is designed to measure four factors of humor: 1. humor production and social uses of humor; 2. humor appreciation; 3. coping or adaptive humor; and 4. attitudes toward humor. It has been tested in a college age population, is reportedly age and gender neutral, and has an established Cronbach alpha of .92 (Thorson & Powell, 1993). In the pilot study, the scores on the MDHS were most closely correlated with changes in NK values following the intervention and more closely correlated with change in stress scores following the intervention; therefore, this test appeared to be more sensitive to the psychological changes occurring during exposure to a humorous stimulus, despite having less variability in scores (Bennett 1994). The MDHS is available through Thorson and Powell (1993).

The use of two sense of humor scales was recommended for this study as the scales measure two different aspects of sense of humor. The SHRQ determines how a person will respond to a variety of mostly ambiguous situations, while the MDHS is designed to measure humor production, appreciation, coping, and attitudes. In addition, it should be noted that humor scales are still in development, so the use of more than

one scale may give the best results. Finally, the use of more than one data collection tool allows for triangulation of a difficult to measure variable.

Measurement of Negative Life Events and Stress

According to a conference report by Cohen (1987), some basic principles should be considered when conducting PNI research using stress and life events.

Stress is impossible to define, but it can be generally agreed that stress is not what happens, but how the subject reacts to what happens.

Therefore, inventories of life change that do not take into account the subject's perception of the event (negative or positive) are of limited use (Cohen, 1987, p. 34).

Because of this limitation, stress and negative life events were determined using instruments that have been successful in previous PNI research. Both of the instruments had established reliability and validity. The Stress-Arousal Check-List (SACL), which was tested in the pilot study, was retained to measure self-reported stress and arousal levels. In addition, the Life Experiences Survey (LES) (Sarason, Johnson & Siegel, 1978), a life events tool which takes into account the number of stressors and how the individual perceives the stressor, was added to help determine the relationships among negative life events, sense of humor and stress. The instruments are described in more detail in the following sections.

Negative Life Events

The Life Experience Survey (LES) is a 57-item self-report instrument designed to measure life changes over the previous 6 -12 months and the subjects' perception of these changes. The LES asks the subjects to rate the perceived impact of each event at the time that they experienced that particular event. Ratings are on a seven-point likert-like scale, with scores from -3 (extremely negative) to +3 (extremely positive). The scale

is designed to give two types of scores, a negative stress score and a positive stress score. The negative stress score has been correlated with scores on the Beck Depression Inventory ($p < .05$) (Beck, Ward & Mendelson, 1961), and this correlation was demonstrated to be modified by locus of control ($p < .02$) (Sarason, et al., 1978). In addition, the impact of LES negative life event scores on negative mood has been demonstrated to be modified by sense of humor (measured by the CHS, SHRQ, and SHQ-MS) (Kuiper, et al., 1992; Martin, et al., 1983). However, coping humor (CHS) did not act as a modifier of LES scores on depression (Beck Depression Inventory) and mood (Multiple Affect Adjective Check List) in a study by Anderson and Arnoult (1989).

Test-retest correlations for the negative stress scores range from .56 - .88. The positive scale is not as reliable with scores ranging from .19 to .53. The authors stated that this could be due to actual changes in life events during the 6-week period between the two testing times, as positive life events tend to occur more often than negative life events (Sarason, et al., 1978). As the positive scores have not significantly correlated with other measures, such as depression or locus of control, they may be less useful in this type of research. Therefore, positive life events scores were not included in the data analysis for this research project.

The LES has two parts, a general scale for the overall population and an additional 10 questions designed for use with students, to determine the effects of stressful academic events. The addition of the later ten questions makes it particularly useful in research with students. For this study, persons who were students were instructed to fill out this additional section, while persons who were not students omitted this section. As life events that are not experienced by subjects do not affect the overall scoring of this tool, the additional items for students were summed together with the general scale

items to create one LES score for each subject. The instrument is available through Sarason et al. (1978).

Self-Reported Stress- Arousal

While there is not general agreement among psychologists as to the best way to measure stress, the Stress-Arousal Check List (SACL) (Mackay, Cox, Burrows & Lazzarini, 1978) has been successfully used in several studies of stress and coping (Cox & Kerr, 1990; Hollingworth, Matthews & Hartnett, 1988; McCormick, Taylor, Rivolier & Cazes, 1985; Watts, Cox & Robson, 1983). The SACL was designed to measure current (state) perceived stress and arousal. The SACL has two subscales, one to determine self-reported stress, and one to determine self-reported arousal. It has been tested in several populations. Validity of this instrument has been confirmed by factor analysis and scores on the SACL have been demonstrated to increase when subjects are placed in a stressful situation (Duckro, Korytnyk & Vanderberg, 1989; McCormick, Walkey & Taylor, 1985). It also has been demonstrated to correlate with physiological measures of stress and arousal (Norton, Howard & Brown, 1991). Internal reliability scores range from .81-.86 (Fischer, Hansen & Zemore, 1988). The SACL, as originally formatted, used a complex series of plus, minus, and question mark symbols, which had to be converted to numbers during scoring. To ease test taking and scoring, these symbols were converted to a standard Likert scale format for use in the pilot study. No changes were made in the questions on the tool. The test-retest reliability coefficient for the modified SACL used in the pilot study was .977, which is excellent. Scores on the SACL significantly decreased following the video and were also correlated with changes in NK cytotoxicity following the intervention (Bennett, 1994). Therefore, this tool appears to be capturing at least part of the psychological changes occurring following exposure to a humorous stimulus and was retained for use in the dissertation. In addition,

including a measure of stress-arousal helped to determine the relationships among negative life events, sense of humor, humor response, stress, arousal, and baseline NK cell cytotoxicity. The SACL is available through Mackay (1978).

Depression

Given that underlying levels of depression may be found in any community sample, and depression can suppress NK functioning, the Center for Epidemiologic Studies Depressed Mood Scale (CES-D) was added to screen potential subjects for depressive symptoms (Deardorff & Funabiki, 1985; Evans, et al., 1992; Hammen, 1980; Irwin, et al., 1987). The CES-D is a 20-item likert scale designed to screen for depressive symptoms in the general public. The CES-D has been found reliable, with an internal consistency alpha of .85 for the general population, and has correlated significantly with a number of other depression mood scales. The CES-D produces one score with a possible range of 0-60, with higher scores indicating greater depressive symptoms. It is generally accepted that scores above 16 are indicative of greater risk for depression (Radloff, 1977). Potential subjects with CES-D scores above 16 were excluded from the study and referred to either the Indiana State University counseling center (if students) or to a local community mental health center. As it was noted that some subjects could become depressed in the period between the pre-screening and actual data collection, the CES-D was also given on the day of data collection. On data analysis, it was noted that CES-D screening detected six participants who scored above 18 on the day of data collection. Data from these participants were not included in further data analysis. The CES-D is available through Radloff (1977).

Considerations for NK Research in Humans

Chromium Release NK Assay

The method used for measuring NK cell cytotoxicity was a modification of the standard four hour chromium release assay previously described by Ortaldo, Bonnard, and Herberman (1977). See Appendix C for detailed description of assay procedure. In brief, K562 target cells were labeled with 250 μCi of $\text{Na}^{51}\text{CrO}_4$ for 60 minutes in a 37°C water bath. Target cells were shaken gently every 15 minutes during incubation to assist with even uptake of chromium. Effector cell suspensions were adjusted to 5×10^6 mononuclear cells per milliliter and serially diluted for use in the assay. Triplicate aliquots (0.1 ml) of a fixed number of cells from the target cell suspension were added to effector cell suspensions at 40:1, 20:1, 10:1, and 5:1 effector-target ratios in 96-well U-bottomed microtiter plates. Spontaneous release was determined in wells containing only target cells in RPMI 1640 with 10% FCS. Maximum counts were determined using unlysed, labeled targets. Plates were incubated for four hours in a 5% carbon dioxide incubator at 37°C. Plates were centrifuged at 150g for 5 minutes, and supernatant was harvested using Skatron harvesting filters. Target cells release radioactivity in direct proportion to numbers of target cells lysed. Radioactivity was measured in a Beckman gamma counter in counts per two minutes. Numbers from the gamma counter were entered into a spreadsheet to calculate percent lysis for each of the effector-target cell ratios. Percent lysis numbers were calculated for each of the four effector-target ratios. Percent lysis data were entered into the Expfit (version 3) computer program, which calculated lytic units for each sample. Lytic units (LUs) are defined as the number of effector cells required to kill a certain percentage of target cells (Pross, Baines, Rubin, Shragge, & Patterson, 1981). Increased lytic unit values indicate greater cytotoxic

capabilities. Expressing the NK assay data in LUs provides a measure of NK activity that is not dependent on a particular effector-target ratio, making it easier to compare results across studies which use different effector-target ratios. See appendix C for more details.

Sample Collection Procedures

There are many things to consider when collecting specimens for NK cell research. A minimum of 10 cc of blood is recommended for each assay. The blood must be well-mixed in a heparinized vacutainer tube or syringe. If the sample is drawn too fast or through too small a needle, the red cells can be lysed, which may alter the results of the test. There are also some data to indicate that pain and anxiety related to venipuncture can alter NK functioning in persons with acute needle phobia (Girgis, Shea & Husband, 1988). Therefore, persons with acute needle phobia were excluded. The problem with pain related to sample collection procedures is more complex. To circumvent the influence of venipuncture related pain on immunity, 22 gauge butterflies with heparin locks were used in the pilot study (Bennett 1994), but problems with clotting, saline contamination of the sample, and subject complaints of pain from the indwelling needles led to a search for a better technique. Regular indwelling intracaths were tried with a few volunteers in a small pilot at ISU, but the subjects complained that the intracath start hurt much worse than the vacutainer stick, and the presence of the intracath was annoying during the intervention. In addition, starting the intracath and keeping it patent was technically more difficult than the use of vacutainer sticks. Lastly the use of an analgesic cream (EMLA) was considered. EMLA cream does decrease the sensation of venipuncture, but unfortunately requires a prescription, and must be applied one hour in advance and covered with an occlusive bandage, which then must be removed before the venipuncture. Also, while there are some data concerning the effects of EMLA

cream on standard laboratory values, there are no data concerning the effect of EMLA cream on NK cytotoxicity (Amdisen & Vuld, 1991). As all other NK studies have used venipuncture without the benefit of analgesia, the use of EMLA cream could introduce an unnecessary confounding variable. Therefore, 21 gauge vacutainer needles, in two separate sticks, were used to collect the blood samples for this study.

Timing of Sample Collection and Assay Procedures

There is some evidence that NK cell cytotoxicity decreases throughout the day, showing diurnal variations (Fukuda, et al., 1994; Gatti, et al., 1987; Masera, et al., 1994). Ideally, all blood specimens should be collected on the same day at the same time (Kiecolt-Glaser et al., 1988), and tested immediately (Whiteside, Bryan, Day & Herberman, 1990 & Whiteside, et al., 1989). Fresh samples are preferred to frozen samples, as the freezing and thawing process introduces increased variance in NK cell cytotoxicity (Sabbe, De Bode & Van Rood, 1983; Whiteside, Bryan, Day & Herberman, 1990; Whiteside, et al., 1989). In addition, as several studies report that changes in NK activity appear rapidly in response to various stimuli, drawing the post-test blood sample immediately following the videos was necessary (Bachen, et al., 1995; Delahanty, et al., 1996; Herbert, Cohen, Marsland, Bachen, Rabin, et al., 1994; Nomoto, et al., 1994). Because the humor intervention would take approximately two hours, a series of studies was conducted to determine what effect holding the pre-intervention samples for two hours would have on NK cytotoxicity. The effect of various two-hour holding conditions on NK cytotoxicity was determined. Holding the whole blood samples at room temperature, on ice, and at 37 degrees C for two hours significantly altered the NK cytotoxicity, while holding separated lymphocytes at room temperature did not significantly alter NK cytotoxicity (Bennett, 1994). Therefore, blood was drawn immediately before and after the videos, and lymphocyte separation took place

immediately after each set of samples was drawn. These methods helped avoid any NK changes related to whole blood holding time. In addition, all trial runs took place at the same time of day to help control for the effects of diurnal variation. The use of a control group also helped to account for any possible diurnal effects during the two hours between pre and post-testing.

As a limited number of samples could be run at the same time in the Terre Haute Center for Medical Education Lab, either the study had to be very limited in sample size, or the data had to be collected on separate days. The use of separate lab days was somewhat problematic, as target cell changes could lead to interassay variations. Unfortunately, there tends to be a substantial amount of day to day variation in results obtained using the standard Cr ⁵¹ release assay (Bloom, Akiyama, Korn, Kusunoki & Makinodan, 1988; Whiteside, Bryan, Day & Herberman, 1990). This variability may be due to actual changes in the donor's NK cytotoxicity, or may be due to changes in target cell susceptibility (Nielson, Kimose, Linnet, Moller & Bukh, 1989). The inherent variability in tissue cell cultures such as the K562 target cell population (Lozzio & Lozzio, 1979) makes it difficult to compare results obtained from different labs, or from one lab on different days (Whiteside, Bryan, Day & Herberman, 1990).

Methods to Control for Day to Day Variability

In order to help control for any day to day interassay variability, the following approaches were used. The data collection days were as close together as possible, with all data collection taking place in less than a two-week time frame. Samples from both the control and experimental subjects were assayed together on each data collection day. In addition, a study of NK interassay variability was undertaken by the researcher before data collection for this project. Reliability testing of frozen donor cells

from five subjects over a period of five weeks demonstrated a mean test-retest reliability of 0.777 for the standard ^{51}Cr release NK assay (Bennett, 1995).

A new modification of the standard assay was tested also during this reliability study, in side by side assays using the same frozen donor cells as those tested with the standard assay. The modified assay used target cells grown up in one culture, then frozen in lots for use during the entire study (See appendix C for modified assay procedure). One lot of the prepared target cells were thawed 72 hours before each assay, then split 24 hours before use in the assay. The modified procedure yielded a mean test-retest reliability of 0.904, over the five-week period (Bennett, 1995). As the modified procedure demonstrated less interassay variability, this assay method was used for the dissertation data collection.

Use of Daily Control Samples

To document any day to day variability, it has been recommended that labs run frozen control samples from donors with established NK cytotoxicity, along with fresh control samples from donors with established NK cytotoxicity in each assay (Whiteside, et al., 1989; Whiteside, Bryan, Day & Herberman, 1990). If the fresh samples are within their normal range, but the frozen samples are out of normal range, the assay is considered valid, because of the problems that can occur with the freezing and thawing process. If the frozen samples are within normal range and the fresh control samples are out of line, the assay is considered valid, because donors may have day to day changes in NK function that could account for this result. If both fresh and frozen control samples have results that fall outside their normal range (mean \pm 2 SD), the assay results should be rejected (Whiteside, Bryan, Day & Herberman, 1990; Whiteside, et al., 1989).

For this project, 100 cc of blood were drawn from two volunteer control subjects, a high responder - male (control #1) and a low responder - female (control # 2). Mean responses in lytic units (LU) were established for each subject, based on the results of at least ten previous assays using frozen cells. The mean response for control # 1 was 87.4 LU (SD = 33.3), while the mean response for control # 2 was 20 LU (SD = 12.8). The control subjects' lymphocytes were frozen in lots to be used as the frozen control samples for this entire project. One week before the start of data collection, one aliquot of each frozen control was thawed and tested. Control # 1 tested at 86.6 lytic units and control # 2 tested at 18.1 lytic units. These results were within 2 lytic units of the control donors previously established mean baseline values, demonstrating the validity of the assay and the stability of these subjects' NK cell cytotoxicity after freezing. Each day of the assay one lot of lymphocytes from both frozen donors was used as frozen controls.

In addition to the daily frozen controls, fresh samples from two volunteers with previously established baseline NK values were used each day of the project as fresh controls (Control # 3 mean LU = 18 SD = 8.1 and control # 4 mean LU = 17 SD = 5.5, based on at least ten assays). Also, to help control for possible changes in target cell susceptibility to lysis, the assay results were to be rejected if the spontaneous release was greater than 10% of the maximum release, or if target cell viability (after labeling) was less than 80%. These control procedures help to decrease interassay variability due to target cell changes (Kiecolt-Glaser & Glaser, 1988; Sabbe, et al., 1983; Whiteside, et al., 1989).

Supplies and Equipment

Everything possible was done to avoid any introduction of variance in the NK assay. It has been demonstrated that changes in lab supplies and equipment can account for more variance than changes in subjects (Sabbe, et al., 1983), and, therefore,

certain precautions were taken. Sufficient medium, fetal bovine serum, plasticware, etc. were purchased at the start of the study, so that it was not necessary to order more medium or supplies during the study. Changing medium or serum can lead to significant changes in lab values. In addition, sufficient K562 for the entire study was grown in one batch, then frozen to standardize all cells at the same place in the growth cycle. Using cells from more than one culture of K562 in subsequent assays can lead to significant differences in NK cytotoxicity (Kiecolt-Glaser & Glaser, 1988; Sabbe, et al., 1983; Whiteside, et al., 1989).

Summary of NK Assay Control Measures

As separate lab days were used to collect data, the following measures were taken to decrease interassay variability:

1. All assays were conducted in triplicate. Each triplicate was examined to determine measures of central tendency and check for outliers. The data were accepted or rejected based on criteria developed by Stone, et al. (1991) to increase the reliability and validity of immune function assays.
2. The different experimental phases were conducted as close in time to each other as possible. This decreased the time between assays.
3. Each day's experimental and control samples were run in the same assay. This helped to prevent differences due to interassay variability between the experimental and control subjects.
4. The modified NK assay was used, which is more reliable over a five-week period than the standard assay.
5. To help detect any interassay variations, donor cells from a high and low responder were frozen in lots to be used as frozen controls samples for each NK assay. Two fresh control samples were also run in each NK assay. If the day's assay results

showed that the frozen and fresh samples were beyond the accepted range for these control samples, the assay results would be rejected as invalid. For this project, all of the control samples tested within their accepted range in all assays.

6. The K562 cells were labeled and viability was ascertained before data collection procedures each day. If the K562 viability was less than 80% or the spontaneous release was greater than 10% of the maximum release, another batch of K562 cells were to be used. This helped to detect problems with interassay variability due to target cell changes, without wasting research data. One tested batch of K562 cells was kept in continuous culture in case the frozen target cells were found to have too low a viability or too high a spontaneous release. These cells were to act as a back-up, to prevent losing a day's worth of data collection due to possible target cell problems. While keeping target cells in culture is the standard method in most labs, use of these back-up cells would probably have introduced greater interassay variance than use of the standardized frozen target cells. Fortunately during this project, the spontaneous release remained below 10% of the maximum release and all targets had a viability greater than 80% on all days of the study, so the back-up target cells did not have to be utilized.
7. Adequate plasticware, medium, serum, chromium, and other supplies and equipment used in the NK assay were purchased in advance, and tested in advance, to avoid introducing variability related to changing plasticware or serum during the research project.
8. To assist with comparison of this work with other NK studies that may use different effector-target ratios, all NK assay results were reported in lytic units (LU) (Bryant, Day, Whiteside & Herberman, 1992; Pross, et al, 1981) as recommended by Whiteside et al. (1989).

Summary of Methodology

This study used a quasi experimental, pre-post test design to examine the effect of a brief period of mirthful laughter on stress and NK cell cytotoxicity. In addition, the relationships among negative life events, sense of humor, stress, arousal, humor response, and baseline NK cell cytotoxicity were investigated. Healthy women were recruited from the rural Midwest using a variety of methods. The participants were screened for inclusion and exclusion criteria during a telephone interview two to three weeks before data collection. Forty women were selected to participate in data collection, on one of four different days. Written informed consent was obtained from all subjects. The participants were randomly assigned to either the humor intervention or the distraction control group. All data were coded with subject identification numbers. Equal numbers of control and experimental subjects were scheduled for each research day. Subjects completed a demographic tool, the LES, to test for exposure to negative life events; the SACL, to test levels of stress and arousal, the SHRQ and MDHS, to test for sense of humor, and the CES-D, to test for the presence of depression on the day of the study. Following psychological testing, 10 cc of blood were drawn from each subject to test for baseline NK cell cytotoxicity. Lymphocyte separation was performed at this time by the primary researcher. The research assistant coded all subject materials, directed subjects to either the humor intervention or the distraction control groups and started the videos. The primary researcher remained blind to subject assignment during the data collection period. The subjects were blind as to which group they were in until the videos started, at which time they were able to tell from the video content if they were in the distraction group or the humor group. The members of the humor group watched *Bill Cosby Himself*, and were observed and rated on their humor response.

The members of the distraction group watched two short video tour movies. Following the videos, members of both groups completed the SACL again, then had blood samples taken for the post NK test. The entire experience took about three hours of the participants' time. The post-test blood samples were coded and taken to the lab immediately for testing. Extensive control procedures were used to document that the NK assay results were valid and reliable over all four days of testing. The results from each of the study days were then combined for data analysis.

RESULTS

Forty subjects participated in the data collection process. Six of these subjects were subsequently noted to have CES-D scores above 18 on the day of data collection, thus data from these subjects were not included in the analysis. One other subject's data were excluded, as her baseline NK activity tested three standard deviations above the mean NK activity. Therefore, data from a total of 33 subjects (17 from the humor group, 16 from the control group) were used for the final data analysis.

Data Analysis

Triplicate data from the NK assay were examined to determine measures of central tendency and check for outliers. The data were accepted or rejected based on criteria developed by Stone, et al. (1991). NK cell activity was converted into standardized lytic units (LU) using the method described in previous research (Poss, Baines, Shragge & Patterson, 1981). A total score was obtained for each subject on each of the following instruments; LES, SHRQ, MDHS, and CES-D. Pre and post-test stress and arousal scores were obtained using the SACL. Humor Response Scale scores were obtained for subjects in the humor group. Subjects in the control group were not observed for humor response, as no humor response was expected from the distraction subjects. Data from each of the instruments and the pre and post-NK levels were entered into a computer data base for data analysis using SPSS for Windows and WINKS 4.1, Professional Edition. The data were examined for normality using histograms. Data found to be approximately normally distributed were analyzed using parametric statistics. Non-normally distributed data were analyzed using non-parametric methods. A sample size of thirty-two insured an 85% chance of detecting a population Pearson correlation

coefficient of 0.5. The 0.05 significance level was used as the level to determine statistical significance.

Sample Demographics

Demographic data were obtained for all participants. All of the subjects were healthy adult Caucasian women. Range and mean demographic data from the subjects are displayed in Table 2. The subjects assigned to the distraction group weighed more on average (mean weight 180 lbs) than those assigned to the humor group (mean weight 153 lbs, $t = 2.43$, $p = 0.02$). However, as subject weight did not significantly correlate with any of the outcome variables, this was not considered to be a factor in this study.

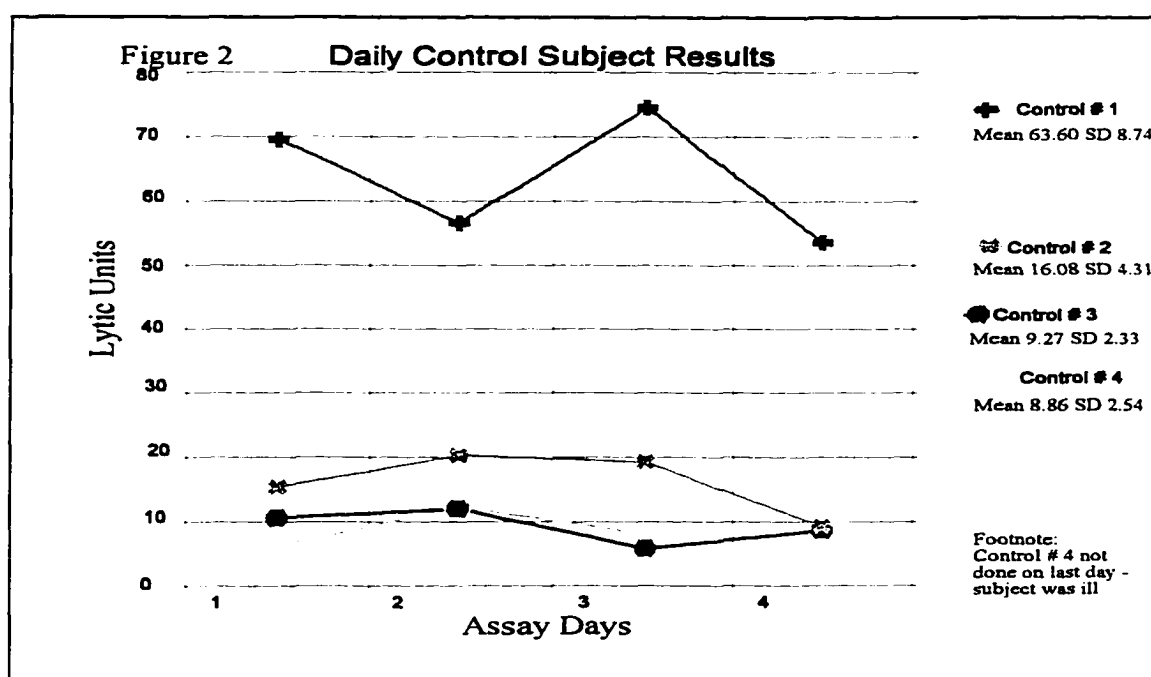
Table 2.		Subject Demographic Information		
Variable	Distraction (N=16) Experimental (N=17)	Mean	Range	Standard Deviation
Age (years)	Distraction.....	41	25-65	12.6
	Experimental.....	41	19-69	14.2
Weight* (lbs)	Distraction.....	180	126-230	32.4
	Experimental.....	153	104-216	31.2
Number of Children	Distraction.....	1	0-5	1.3
	Experimental.....	0.82	0-4	1.4
Total # of hours spent at work or school per week	Distraction.....	30	0-60	20.3
	Experimental.....	39	0-60	18.1

* Significant
Between-Group
Difference
 $p \leq .05$

NK Assay Control Data

On three of the four research days, two frozen controls and two fresh controls were run with the subject data. On the last research day, two frozen controls and one fresh control were run, as control # 4 was sick that day. All of the control samples tested

within two standard deviations of their previously established means on each of the four days and most tested well within one standard deviation. These data suggest that the NK assay results were valid on each day of the research project (Whiteside, Bryan, Day & Herberman, 1990). In addition, the control sample data indicated that there was very little day to day variability in the assay results (see figure 2). The mean standard deviation for the frozen samples was 6.25 lytic units (range 4.9 -10.1 LU), and the mean standard deviation for the fresh samples was 2.43 lytic units (range 2.7-5.5 LU). Interassay correlations for the daily controls ranged from 0.979-.999, with a mean correlation coefficient of 0.991, indicating highly reliable NK assay results over the four days of data collection.



Baseline Data

Despite randomization, data analysis revealed significant differences between the subjects assigned to the distraction group versus the humor group on some baseline

measurements (see Table 3). The subjects assigned to the distraction group were significantly more aroused, on average, at baseline than those assigned to the humor group ($t = 2.73$ $p = 0.01$), while the subjects assigned to the humor group were significantly more stressed, on average, at baseline than those assigned to the distraction group ($t = -3.3$ $p = 0.002$). Finally, the subjects assigned to the humor group had significantly higher baseline NK activity, on average, than those assigned to the distraction group ($t = -2.08$ $p = 0.045$). It should be noted that at the time the baseline measures were being conducted, the subjects were unaware of their group assignment. Because of the significant between-group differences in baseline arousal, stress, and NK cytotoxicity, change scores were used in the analysis of these measures to determine between-group differences following the videos. Pre and post-test data for each of the research variables are given in Table 3. Analysis of data as related to each of the research aims is discussed in the following section.

Variable	Distraction Group (N=16)			Humor Group (N=17)		
	Mean	Range	Standard Deviation	Mean	Range	Standard Deviation
Life Events Scale	8.2	0 - 30	8.5	9.4	0 - 36	9.9
Depression Multidimensional Humor Scale	5.4	0 - 15	5.4	6.1	1 - 15	4.5
Situational Humor Response	112	93 - 143	12.5	113	89 - 145	12.3
Pre-Arousal *	50	37 - 63	8.1	48	39 - 70	8.2
Post-Arousal	43	24 - 50	7.0	36	22 - 45	6.2
Pre-Stress *	31.3	14 - 49	11.2	34	20 - 45	7.8
Post-Stress	21	17 - 32	5.1	28	18 - 41	7.7
Pre-NK Activity *	19.8	17 - 28	3.1	21	17 - 30	4.1
Post-NK Activity	24	5 - 65	19.0	42	5 - 83	30.0
* Significant	32	12 - 73	17.2	38 LU	3.8 - 100	28.1
	Between	Group	Differences			

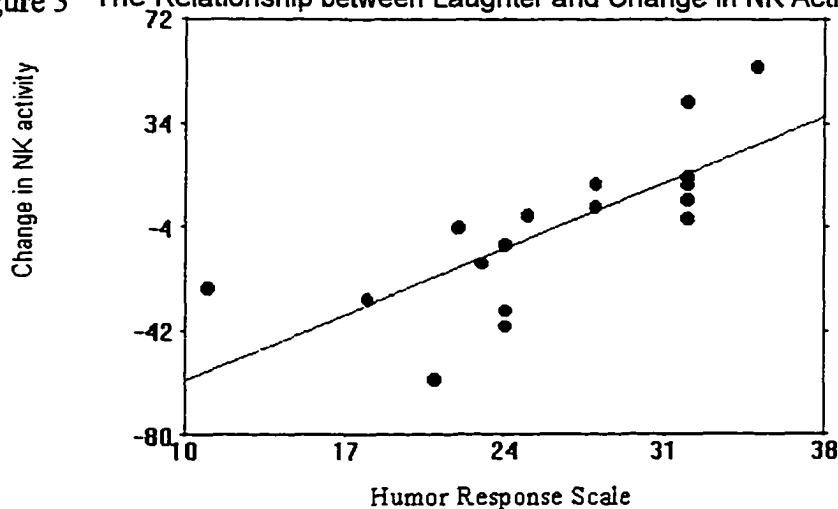
Results for Research Aims

The Effect of a Brief Period of Mirthful Laughter on NK Cell Cytotoxicity

The Student's t test was used to determine if there were any overall between-group differences in NK change following the videos. According to the t test, there was no statistically significant difference in NK change between subjects who viewed the humor video, compared with subjects who viewed the distraction video ($t = 1.52$ $p = 0.138$). Therefore, simply viewing a video or diurnal changes did not lead to statistically different post NK activity.

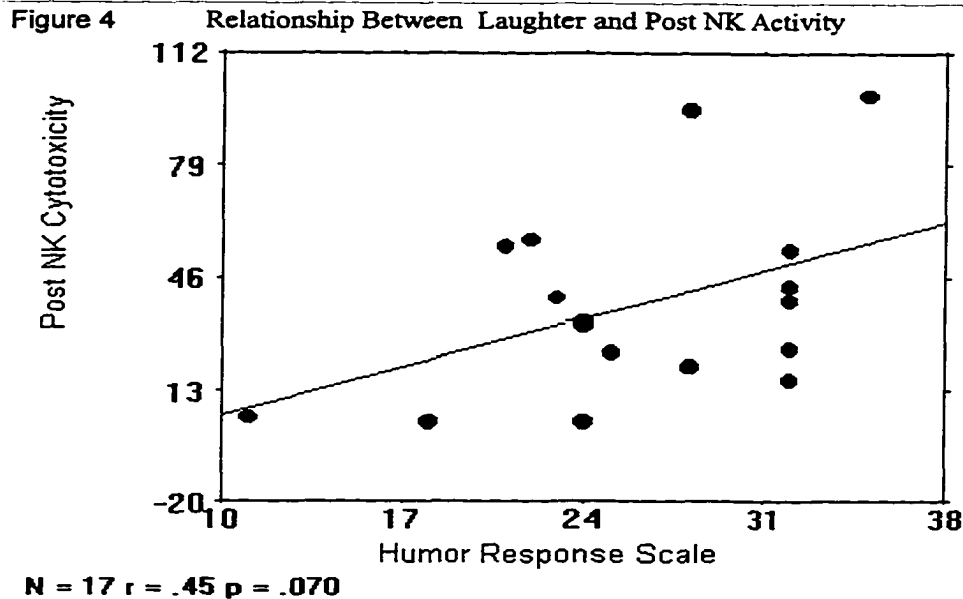
However, when mirthful laughter was taken into consideration, using the Humor Response Scale (HRS), a different picture emerged. Scores on the HRS significantly correlated with change in NK cytotoxicity for subjects in the humor group ($r = 0.774$ $p = 0.001$ see figure 3).

Figure 3 The Relationship between Laughter and Change in NK Activity



$r = .774$ $p = .001$

Scores on the HRS also tended to correlate with post NK cytotoxicity for persons in the humor group ($r = .45$ $p = 0.07$ see figure 4).



Further examination revealed that subjects ($n = 9$) who scored a 25 or above on the HRS, which indicated mirthful laughter responses, had significantly increased post-NK activity, compared with their baseline NK activity (mean increase of 15.77 lytic units, $t = 2.52$ $p = 0.037$). In addition, subjects that scored a 25 or above on the HRS had significantly greater positive change in NK activity compared with other subjects in the humor group ($t = 4.85$ $p = 0.002$), and compared with all other participants in this study ($t = 2.1$ $p = 0.04$). The cut-off score of 25 was selected based on scatter plot data, and based on the instrument itself. Subjects were scored six times during the video. In order to score a 25 or above, they would have to receive at least one rating of five or above. Ratings of one through four indicated no humor response to a maximum response of rare laughter or chuckle, while ratings of five through seven included the responses of intermittent laughter to nearly continuous laughter with belly laugh.

It should be noted that persons who watched the humorous video and did not display mirthful laughter (N=8) had significantly decreased NK cytotoxicity at post testing ($t = 4.4$ $p = 0.003$). These data reinforce that it is not simply exposure to a humorous stimulus that improved immune function, but the response that is elicited by the video. Viewing a supposedly humorous video, and not finding it funny, while other people around you are laughing, perhaps triggers an affectively negative experience. This may have accounted for the decreased NK cytotoxicity noted in these subjects.

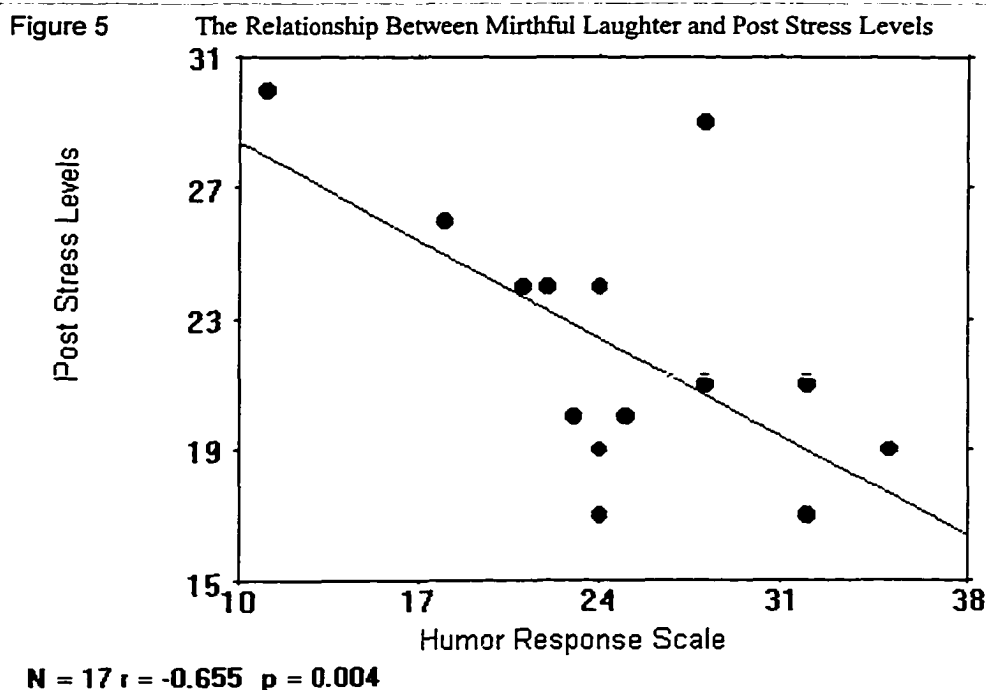
Scores on the sense of humor instruments were not significantly correlated with observed humor response during the video, (SHRQ $r = 0.115$ $p = 0.66$; MDHS $r = 0.276$ $p = 0.284$). Sense of humor, as measured by the MDHS ($r = 0.37$ $p = 0.14$), and the SHRQ ($r = 0.216$ $p = 0.40$), did not correlate with change in NK activity. In addition to sense of humor and observed humor response, subjects were asked to self-rate their laughter on a 5-point scale, and to rate how funny they found the movie on a 4-point scale. These two items were then combined into a movie response score. Movie response scores did not significantly correlate with observed humor response ($r = .261$ $p = .331$), sense of humor (SHRQ $r = .186$ $p = .474$, MDHS $r = .100$ $p = .701$) or with change in NK activity ($r = .043$ $p = .869$). However, two subjects in the humor group did not complete these items, and many of the other subjects from the humor group had to be reminded to complete this section during the refreshment period. Better placement of these items in the test packet and reminding the subjects to complete this section immediately after the video may have improved the quality of the data obtained by these self-report items.

The Effect of Mirthful Laughter on Stress-Arousal

The data for change in stress scores following the videos did not meet the criteria for normality of distribution, therefore, a non-parametric method was used to determine

between-group differences for change in stress. While stress scores decreased somewhat for persons in both groups, according to the Mann-Whitney U test, stress scores decreased significantly more for persons in the humor group, compared with persons in the distraction group ($U = 215.5$ $p = 0.004$). This change in stress scores appeared to be independent of the change in NK activity seen in persons in the humor group (Spearman's $\rho = 0.21$ $p = 0.262$). In addition, the change in self-reported stress did not appear to be related to sense of humor, as scores on the sense of humor scales did not correlate with the change in stress for those viewing the humorous video (SHRQ Spearman's $\rho = 0.17$ $p = 0.42$, MDHS Spearman's $\rho = 0.075$ $p = 0.61$). However, scores on the HRS were negatively correlated with post-stress levels. Persons who laughed more reported lower post stress levels ($r = -0.655$ $p = 0.004$, see figure 5).

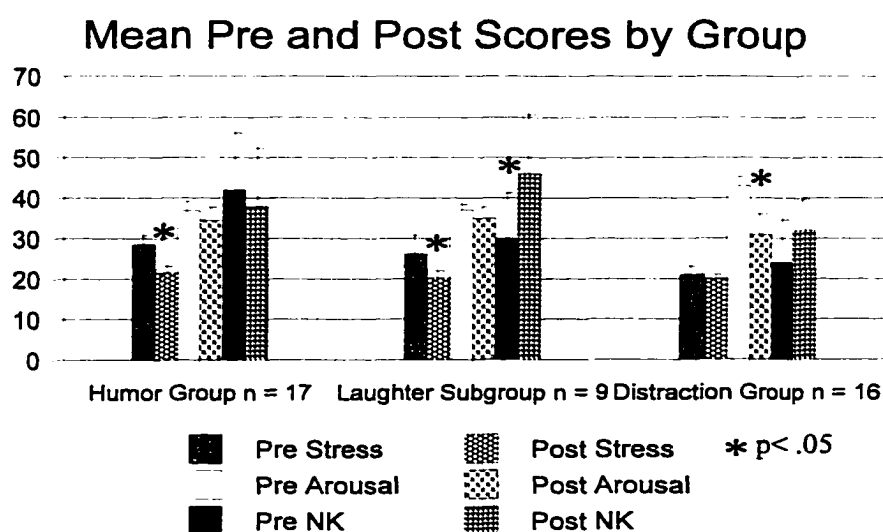
Interestingly, change in stress scores did tend to negatively correlate with change



in NK activity for members of the distraction group (Spearman's $\rho = -0.29$ $p = 0.081$). Decreased stress levels were related to positive change in NK activity, even though the distraction group as a whole did not have significantly decreased stress following the videos. It is possible that for some people, taking the afternoon off work and watching a tour video was sufficient to decrease their stress levels and increase their NK activity, but these changes were not statistically significant.

The data for change in self-reported arousal, measured using the arousal items on the SACL, were normally distributed. Student's t test indicated that arousal decreased significantly for persons in the distraction group, compared with those in the humor group ($t = -2.82$ $p = 0.008$). Subjects in the distraction group reported greater agreement with feelings of sleepiness and drowsiness following the video tours. This decrease in arousal was not correlated with change in NK activity ($r = -0.054$ $p = 0.84$) for members of the distraction group. See figure 6 for pre vs. post-test values by group.

Figure 6



Relationships Among Life Events, Sense of Humor, Laughter, Stress, Arousal, and NK Cytotoxicity

Standard multiple regression diagnostics were used to check for nonlinearity and outliers (Shott, 1990). If necessary, data transformation were conducted to correct non-normality and to model nonlinear relationships. Correlations among the variables tested are displayed in a correlation matrix (see table 4). The significant correlations are discussed in the following sections.

Table 4. Baseline Correlations Among Variables (N = 33)		Correlation (Significance)					
	LES	CES-D	MDHS	SHRQ	Pre-Arousal	Pre-Stress	Pre-NK
Negative Life Events (LES)		.422 (.016)	.165 (.368)	.07 (.702)	-.016 (.929)	.212 (.245)	-.029 (.877)
Depression (CES-D)			-.194 (.288)	-.27 (.135)	-.547 (.001)	.484 (.004)	.257 (.148)
Multidimensional Humor Scale (MDHS)				.594 (.0005)	.278 (.123)	-.181 (.322)	-.177 (.333)
Situational Humor Response Questionnaire (SHRQ)					.231 (.204)	-.264 (.145)	-.267 (.140)
Pre-Arousal						-.203 (.265)	-.388 (.028)
Pre-Stress							.235 (.196)

Positive Baseline Correlations

Negative life events scores from the LES correlated with scores on the CES-D ($r = 0.422$ $p = 0.016$). Persons with higher negative life events scores had more symptoms of depression. This relationship was present, even though persons with CES-D scores more than 16 were not scheduled to be participants, and persons scoring more than 18 on the day of data collection were not included in data analysis. Scores from the CES-D

also correlated with baseline stress scores from the SACL ($r = .484$ $p = .004$). Persons with increased symptoms of depression also had higher stress levels. Scores on the two humor scales, the SHRQ and the MDHS, correlated with each other ($r = .594$ $p < .0005$). Sense of humor scores did not significantly correlate with any of the other baseline variables.

Negative Baseline Correlations

Scores on the CES-D negatively correlated with arousal scores from the SACL ($r = -.547$ $p = .001$), with persons having higher CES-D scores also reporting decreased arousal levels. Arousal scores also negatively correlated with baseline NK cytotoxicity ($r = -.388$ $p = .028$), with persons reporting decreased arousal symptoms also having higher NK cytotoxicity. As this was the only variable that significantly correlated with NK cytotoxicity at the baseline measurement, multiple regression to predict baseline NK activity was not done.

Post Intervention Correlations

A number of post intervention correlations were performed, to determine what factors were related to change in stress and immune function for persons in both the humor group and the distraction group. These correlations and trends are discussed in the following sections.

Pre-NK and Change in NK following the Videos

Overall, a negative correlation was found between pre-NK levels and NK change ($r = -.465$ $p = 0.007$) for subjects in both groups. Those persons who had higher levels of NK activity at the start of the study had less positive NK change than persons who started with lower levels of NK activity. This could be due to a possible ceiling effect, similar to that noted in SIgA levels by Dillon et al. (1985); persons who start out with

relatively high NK levels may not show much improvement. It could also be related to regression towards the mean, with persons having lower pre-NK scores having increased post-NK levels, and persons having higher pre-NK scores ending with decreased post-NK scores. This finding is noteworthy because, despite random assignment, persons in the humor group started with higher levels of pre-NK activity than persons assigned to the distraction group. This would lead us to assume that persons in the humor group should, therefore, have had less positive NK change, compared with persons in the distraction group. Yet following the videos, persons in the humor group did not have significantly different NK change than persons in the distraction group. Moreover, persons who displayed mirthful laughter actually had higher levels of positive NK change than persons in the humor group who did not laugh ($t = 4.85$ $p = 0.0002$) and all others in the study ($t = 2.1$ $p = .04$). Multiple regression analysis suggested that HRS scores (mirthful laughter) may have acted to reduce the effect of pre-NK levels on NK change. Pre-NK levels alone accounted for 29% of the variance in NK change scores for persons in the humor group ($r = -.54$ $p = 0.025$). HRS scores alone accounted for 55% of the variance in NK change scores for persons in the humor group ($r = .74$ $p = 0.001$). Pre-NK plus HRS scores accounted for 67% of the variance in NK change scores for persons in the humor group (Pre-NK $p = 0.04$, HRS $p = 0.001$, $R^2 = 0.669$ total $p < 0.005$). These data suggest that mirthful laughter can act to improve NK activity, even for persons who already have higher levels of NK activity.

Stress Levels and Change in NK Activity

The only other variables that correlated with change in NK scores for members of both groups were pre and post-stress levels. Both pre-stress ($r = -.37$ $p = .034$) and post-stress ($r = -0.43$ $p = 0.012$) levels correlated significantly with NK change, with persons having higher stress levels also demonstrating lower NK change following the

videos. These data indicate higher stress levels are correlated with less positive change in one measure of immune function, NK activity.

Summary of Results

Overall, while there was not a significant difference in change in NK activity between persons in the two groups, scores on the Humor Response Scale significantly correlated with change in NK cytotoxicity for subjects in the humor group ($r = 0.774$ $p = 0.001$) and scores on the HRS tended to correlate with post NK cytotoxicity for persons in the humor group ($r = .45$ $p = 0.07$). Subjects who averaged a 25 or above on the HRS ($N=9$), which indicated laughter responses, had significant increases in NK cytotoxicity over their baseline values (mean increase of 15.77 lytic units, $t = 2.52$ $p = 0.037$). In addition, participants who displayed mirthful laughter had significantly greater increases in NK activity when compared with the rest of the participants in this study ($t = 2.1$ $p = 0.04$). These data indicate that while viewing a humorous video, by itself, was not related to improved NK activity, mirthful laughter, measured by the HRS, positively correlated with change in NK activity and post NK activity for persons in the humor group. Those subjects who scored a 25 or over on the HRS had significantly improved NK activity following the humorous video.

With respect to stress score changes, there was a significant difference between members of the humor group, and members of the distraction group ($U = 215.5$ $p = 0.004$). For subjects in the humor group, post stress levels were significantly decreased from their baseline values (paired $t = 4.48$ $p = 0.001$). In addition, scores on the HRS significantly correlated with post stress levels for persons in the humor group ($r = -.656$ $p = .004$). Pre ($r = -.37$ $p = .034$) and post ($r = -0.43$ $p =$

0.012) stress scores significantly correlated with change in NK activity following the intervention for persons in both groups.

Examination of the baseline variables revealed significant positive correlations between negative life events and depression, depression and stress, and scores on the two humor scales. Significant negative baseline correlations were found between depression and arousal, and arousal and NK activity. The following section includes discussion of the research findings in relation to PNI theory.

DISCUSSION

This study tested two PNI theory based propositions: 1. A complementary intervention (use of humor) can reduce stress and enhance or optimize immune function (Solomon 1987). To investigate the effect of humor response upon stress and NK cell cytotoxicity, a humorous video was used to stimulate mirthful laughter.

2. Enduring coping style and personality factors (so-called "trait" characteristics) are able to modify the effects of negative life events on an individual's immune system (Solomon 1987). To evaluate this proposition, the relationships among negative life events, sense of humor, humor response, stress, arousal, and baseline NK cell cytotoxicity were investigated. The sections below discuss the results of this study, as they relate to these two PNI propositions.

Test of PNI Proposition 1: The Effect of Exposure to a Humorous Stimulus and Mirthful Laughter on Stress and Immune Function

Exposure to Humor and Stress

In this study, stress scores decreased to a greater degree for persons who viewed the humorous video as compared with persons in the distraction group. For persons in the humor group, stress levels were significantly lower following the video, compared with their baseline values. Reduction in stress did not appear to be related to a subject's sense of humor, as measured using either the SHRQ or the MDHS. These data indicate that exposure to a humorous stimulus significantly decreases stress, regardless of an individual's sense of humor, as measured by current sense of humor instruments. However, the above results should be viewed with some caution. Unfortunately, despite randomization, persons in the humor group reported higher levels of pre-intervention stress than persons in the distraction group, and overall, persons with higher pre-

intervention stress scores had greater decreases in stress following the videos ($r = -0.875$ $p = 0.00$). This could have influenced the change in stress findings for the humor group. Therefore, more research with an increased sample size is needed to determine if exposure to a humorous video is sufficient to produce a significant change in stress.

Laughter and Stress

Mirthful laughter, measured on the humor response scale, was significantly correlated with stress scores following the humorous video. As mirthful laughter was not significantly correlated with pre-intervention stress, or change in stress scores, this finding was not affected by the between-group differences in pre-intervention stress.

The reduction of stress following mirthful laughter found in this study is supported by previous findings. In a study of dental patients and stress, it was noted that patients who joked and laughed before dental surgery reported less psychological stress (Trice & Price, 1986). In another study, subjects who were exposed to a brief period of mirthful laughter had significantly reduced anxiety, compared with their pre-test scores (White & Winzelberg, 1992). Finally, a study of the effects of mirthful laughter on physiological measures of stress found that cortisol decreased more rapidly in persons who laughed, compared with persons in the control group (Berk, Tan, Napier & Evy, 1989). Therefore, taken with the results from this study, there appears to be evidence to support the notion that a reduction of psychological and physiological measures of stress occurs in response to a brief period of mirthful laughter.

Exposure to a Humorous Video, Mirthful Laughter, and Immune Function

As a whole, NK cytotoxicity change scores were not significantly different for participants who were exposed to a humorous video, when compared with persons exposed to two distraction videos. Therefore, exposure to a humorous video alone did not significantly affect NK cell cytotoxicity. However, persons who displayed mirthful

laughter in response to the humorous video, as measured on the HRS, had significantly greater positive change in NK cytotoxicity than the other participants in this study. They also had significantly increased NK cytotoxicity following the video, compared with their baseline values. Finally, scores on the HRS, indicating mirthful laughter, tended to correlate with post NK activity for all persons in the humor group. These data indicate that a subject's behavioral/physiological response was the operative factor in affecting NK change, not merely exposure to a humorous video.

A negative correlation was found between pre-NK levels and NK change for all subjects in this study, possibly due to the previously described ceiling effect (Dillon et al. 1985) or regression towards the mean. Persons who started out with relatively high NK levels tended to have decreased NK activity following the videos. As noted before, despite random assignment, persons in the humor group started with higher levels of pre-NK activity than persons assigned to the distraction group. If the effects of pre-NK levels on NK change were the major factor in this study, persons in the humor group should have had less positive NK change overall, compared with persons in the distraction group. Yet following the videos, persons in the humor group who displayed mirthful laughter had higher levels of positive NK change than all other persons in the study. As noted in the results section, multiple regression analysis suggested that mirthful laughter may have acted to reduce the effect of pre-NK levels on NK change. Pre-NK levels alone accounted for only 29% of the variance in NK change scores for persons in the humor group, while HRS scores accounted for 55% of the variance in NK change scores. Therefore, the effect of mirthful laughter was greater than the effect of pre-NK levels on NK change for persons in the humor group. Pre-NK plus HRS scores accounted for 67% of the variance in NK change scores for persons in the humor group (Pre-NK $p = 0.04$, HRS $p = 0.001$, $R^2 = 0.669$ total $p < 0.005$). These data suggest that

mirthful laughter can act to improve NK activity, even for persons who already have higher levels of NK activity.

These findings of improved NK activity following a brief period of laughter in healthy women add further support to similar findings by Berk et al. (1989) in a study of ten male subjects. Men who were exposed to a humorous video had significant increases in NK cytotoxicity, compared with those who engaged in a quiet time activity. Taken together with the results of this study, there is evidence to support that a brief period of mirthful laughter can lead to significantly increased NK activity in the time period immediately after the laughter, in both male and female subjects.

Sense of Humor, Stress, Laughter, and Immune Function

There were no correlations between scores on the two humor scales, the Situational Humor Response Questionnaire and the Multidimensional Humor Scale, with observed laughter, change in stress, or change in immune function. In previous studies of sense of humor, mixed results have been reported. One study found that sense of humor was negatively correlated with increases in SIgA following a humorous stimulus (Dillon, et al., 1985). Two studies reported that subjects with greater sense of humor scores had larger increases in SIgA following a humorous stimulus (Lefcourt, et al., 1990). A fourth study reported that sense of humor was not significantly related to SIgA levels before or following a humorous stimulus (Lefcourt, et al., 1990). In addition, a study by Dobbin (1990), which investigated subjects under stress, noted that there was no relationship between sense of humor and immune function following exam stress. Finally, in a study by White and Winzelberg (1992), scores on the SHRQ did not correlate with stress reduction following a brief period of mirthful laughter.

Taken with the findings from this study, it appears that sense of humor, as measured by currently available instruments, may not correlate with changes in immune

function or stress following exposure to a humorous stimulus. This could be related to a number of factors. The construct of "sense of humor" is difficult to define and operationalize. It is possible that current instruments do not adequately capture a subject's sense of humor. It is also possible that sense of humor, as operationalized by current instruments, is not related to a subject's potential to produce laughter in response to a humorous video in an experimental setting. The SHRQ was originally designed to determine how a person would respond to various situations, many of which most people would not necessarily view as humorous. Scores on the SHRQ have been noted to correlate with observed laughter during subject interviews (Martin, 1984). However, it may not indicate which subjects will laugh more during a humorous video, which is deliberately designed to appeal to a broad audience. It could be that if the stimulus is strong enough, even subjects with lower sense of humor will respond with laughter. According to Lefcourt (1990) if the video is found humorous by all subjects, it could override the effect of sense of humor. It is also possible that the invasive measures necessary to conduct this experiment interfered with some subject's ability to laugh, thus overshadowing the effect of sense of humor in these subjects.

Stress and Immune Function

Lower levels of both pre-intervention stress and post-intervention stress correlated with more positive change in NK activity, no matter which group the subjects were in. It is possible that subjects who were very stressed could not respond in a positive way to either the distraction or humorous video. However, it should be noted that pre-intervention stress did not significantly correlate with Humor Response Scores for persons in the humor group, so it cannot be assumed that higher stress levels led to decreased mirthful laughter in these subjects. It is possible that subjects with higher stress levels had higher levels of cortisol and other stress hormones, and several

studies have documented the negative relationship between cortisol levels and NK cell cytotoxicity (Caudell & Gallucci, 1995; Gatti, Delponete & Cavallo, 1987; Gatti, Sartori, Cavallo, Del Ponte, Carignola, et al., 1987; Gatti, Masera, Pallavicini, Sartori, Staurengi, et al., 1993; Ironson, Field, Scafidi, Hashimoto, Kumar, et al., 1996; Koltun, Bloomer, Tilberg, Seaton, Ilahi, et al., 1996). Therefore, levels of stress hormones may have affected the subjects' physiological response following the videos, but as cortisol was not measured in this study, this mechanism is speculative.

Members of the humor group had significantly decreased stress levels following the video, and those who displayed mirthful laughter also had significantly higher post-intervention NK activity. However, it cannot be assumed that change in stress was related to the improved NK activity for these subjects, as change in stress scores did not significantly correlate with change in NK activity for subjects in the humor group. Therefore, when using a humorous video as an intervention, one cannot assume that change in stress scores following the video indicates that the subjects would also have improved immune function. In this study, only those subjects that scored above a 24 on the HRS had significantly improved NK cell activity, and humor response scores were the strongest predictor of NK change for persons in the humor group. It appears that mirthful laughter was the determining factor in NK change for persons in the humor group.

Possible Mechanisms for Changes in NK Activity Following Mirthful Laughter

Cortisol is frequently used as a physiological measure of stress, and high levels of cortisol tend to decrease NK cytotoxicity. In Berk's (1988; 1989) study of the physiological effects of laughter, it was reported that a brief period of mirthful laughter led to decreased cortisol and increased NK cytotoxicity. Unfortunately, cortisol was not measured in this study, so this possible mechanism remains speculative. In this study,

mirthful laughter was related to decreased self-reported stress. It is possible that those who had significant decreases in stress also had decreases in cortisol, which could have accounted for some increase in NK cytotoxicity. However, if this is the underlying mechanism, it could be expected that change in stress would correlate with change in NK activity. As noted in the previous section, these two measures did not correlate significantly in this study, which places some doubt on the role of cortisol as the underlying mechanism.

Another possible mechanism is that the increase in NK cytotoxicity was related to decreased pain perception. Prior studies have demonstrated that laughter can reduce pain perception (Adams & McGuire, 1986; Hudak, Dale, Hudak & DeGood, 1991; Trice & Price, 1986), and pain has been demonstrated to decrease NK cytotoxicity (Ben-Eliyahu, Yirmiya, Liebeskind & Taylor, 1991; Bonneau, Sheridan, Feng & Glaser, 1991; Koltun, Bloomer, Tilberg, Seaton, Ilahi, et al., 1996; Page, Ben-Eliyahu & Liebeskind, 1994; Taylor & Gale, 1991; Weiss, Sundar, Becker & Cierpial, 1989). There are also some data to indicate that the pain from venipuncture may be sufficient to cause decreased NK activity, particularly in persons who are very anxious about the procedure (Girgis, Shea & Husband, 1988; Ward, Mefford, Parker, Chesney, Taylor, et al., 1983). It is possible that those persons who laughed had decreased anxiety and pain during the post-intervention blood drawn, which led to increased NK activity, relative to their baseline values and the values of subjects who had not been laughing.

There are also some data which suggest that laughter can cause changes in lymphocyte movement, leading to increased numbers of NK cells in the periphery. In a study of 10 males, it was noted that exposure to a 60 minute pre-selected humor video led to increases in T-cell and NK cell numbers (Berk, Tan & Fry, 1993). It should be noted that this mechanism does not necessarily increase the cytotoxicity per NK cell.

With increased numbers of NK cells in the periphery, NK cells make up a higher percentage of lymphocytes in the assay, thus producing overall increased cytotoxicity values. However, Wise (1989) examined both NK activity and NK cell numbers and found no significant differences in either of these measures following a humorous video. As NK cell numbers were not determined in this study, further investigation of this phenomena is indicated.

Another mechanism which could lead to changes in NK cells numbers is related to the effects of sympathetic nervous system stimulation. Laughter stimulates the sympathetic nervous system, resulting in increased heart rate, blood pressure, respiration, and galvanic skin response (Fry, 1977; Fry, 1986; Fry, 1992; Fry, 1994). Sympathetic nervous system stimulation can also lead to the release of epinephrine, which has been demonstrated to result in increase NK cytotoxicity (Bachen, et al., 1995; Nomoto, et al., 1994). There are also data to suggest that acute increases in epinephrine can lead to changes in NK cell movement, resulting in increased numbers of NK cells in the periphery. Increased peripheral NK cells can lead to increased overall cytotoxicity values, but not necessarily increased activity per cell, as noted above (Bachen, Manuck, Cohen, Muldoon, Raible, et al., 1995; Benschop, Rodriguez-Feuerhahn & Schedlowski, 1996). In contrast, chronically elevated levels of epinephrine have been demonstrated to correlate with decreased numbers of NK cells in the periphery, which could lead to decreased cytotoxicity levels (Knudsen, Kjaersgaard, Jensen & Christensen, 1994).

Unfortunately, the data currently available concerning laughter and epinephrine levels are not clear. An early study of emotions and sympathetic nervous system activation reported that both urinary epinephrine and norepinephrine levels increased significantly during a humorous film ($p < .05$) (Levi, 1965). Yet a later study that included

a series of serum epinephrine levels reported that epinephrine levels did not change significantly in the laughter group, compared with the control group (Berk, Tan, Napier & Evy, 1989). Unfortunately, epinephrine levels were not measured in this study, so this mechanism is again, rather speculative.

Of course, it is likely that there is more than one mechanism involved in the immune response to laughter. According to Fry "we do not laugh merely with our lungs, or chest muscles, or diaphragm, or as the result of a stimulation of our cardiovascular activity. I believe that we laugh with our whole physical being. I expect that this total participation will eventually be recognized" (Fry 1994, p. 114).

Test of PNI Proposition 2: Relationships Among Negative Life Events, Sense of Humor, Humor Response, Stress, Arousal, and Baseline NK Cell Cytotoxicity

To test the second PNI proposition, the relationships among negative life events, sense of humor, humor response, stress, arousal, and baseline NK cell cytotoxicity were investigated. Results of these correlations are discussed in the following sections.

Negative Life Events and Depression

As in other studies, increased negative life events did correlate with increased symptoms of depression (Billings, Cronkite & Moos, 1983; Conger, Lorenz, Elder, Simons & Ge, 1993; Irwin, Daniels, Bloom & Weiner, 1986; Irwin, Patterson, Smith, & Caldwell, 1990; Roehl & Okun, 1984; Safranek & Schill, 1982), but not with self-reported stress, arousal, or baseline NK cytotoxicity. It is possible that this was partly related to the particular attributes of the instruments used in this study. The LES scale measures exposure to negative life events over the prior year; the CES-D is designed to detect any symptoms of depression experienced over the prior week; and the SACL is designed to

measure feelings of stress and arousal present only at the immediate time. This may be why reports of previous negative life events correlated with past or underlying symptoms of depression, but not with feelings of stress or arousal at the time of participation. The lack of correlation of previous negative life events with baseline NK cytotoxicity may have also been due to the immediate nature of NK activity. NK activity tends to respond to stimuli very quickly (Bounds, Betzing, Stewart & Holcombe, 1994; Delahanty, Dougall, Schmitz, Hawken, Trakowski, et al., 1996; Futterman, Kemeny, Sharpiro & Fahey, 1994; Herbert, Cohen, Marsland, Bachen, Rabin, et al., 1994; Hoffman, 1995), and therefore, may not reflect previous exposure to negative life events, particularly if the events have little or no effect on the subjects' current emotional status. Therefore, the connection between increased negative life events and depressive symptoms was supported by this study, but not the connection between past negative life events and current stress or NK cytotoxicity.

Depression, Stress, and Arousal

A statistically significant baseline correlation was found between symptoms of depression and self-reported stress. This was expected, as persons who are experiencing negative emotions at the time of testing, may also report experiencing other negative emotions over the past week, and vice versa (Anisman & Zacharko, 1982; Anisman, Irwin & Sklar, 1989). In addition, there was a significant negative correlation between depression and arousal. Persons reporting more symptoms of depression over the past week also had more agreement with symptoms of decreased arousal, such as feeling sleepy, relaxed, tired, or lethargic. As insomnia, hypersomnia, and feelings of lethargy are symptomatic of depression, it was expected that depression would correlate with decreased arousal. Therefore, the connection between depression and stress-arousal was supported in this study.

Arousal and Baseline NK Cytotoxicity

A negative baseline correlation was noted between arousal and NK cytotoxicity, with those persons reporting decreased arousal levels during pre-testing also having increased NK cytotoxicity at pre-testing. This finding was possibly due to deactivation of the sympathetic nervous system in those persons who could report feeling sleepy or relaxed at this baseline point. The self-report instruments were completed immediately before the subjects had their pre-test venipuncture, a procedure that tends to arouse some minor feelings of fear or anxiety in most people. Persons who reported decreased arousal at this particular point in time were probably less anxious about the procedure, and this may account for the correlation between arousal scores and NK activity at the pre-test time. This correlation was not noted following the videos, so it cannot be generalized that decreased arousal scores on the SACL will correlate with NK activity under all conditions.

Sense of Humor

Scores on the two sense of humor tools did not significantly correlate with anything at baseline, except each other. This demonstrates that the tools are measuring similar attributes in the subjects, but the attributes measured did not appear to be significantly related to baseline measures of stress, arousal, depression, or NK activity. Therefore, data from this study do not support the assumption that individuals with a better sense of humor, as measured by either the SHRQ or the MDHS, have better baseline immune function, as measured by NK cell cytotoxicity. Previous studies that have examined the effect of sense of humor on baseline immune function measured by salivary IgA have had mixed results. Sense of humor, measured by the Coping Humor Scale (CHS) (Martin & Lefcourt, 1983), correlated with baseline SIgA in a study by Dillon et al. (1985). Martin and Dobbin's (1988) study of sense of humor and hassles reported that

sense of humor, measured by the SHRQ, correlated with SIgA at time one in their study, but not at time two. They caution that the lack of correlation at time two casts doubt on the robustness of the correlation between sense of humor and baseline SIgA. Lastly, in a series of three studies, Lefcourt et al. (1990) reports that sense of humor, measured by both the CHS and the SHRQ, did not correlate with baseline SIgA in any of the three studies. Therefore, taken with the results from this study, it seems unlikely that sense of humor, as measured by the SHRQ or MDHS, correlates with baseline immune function. However, as this current study is the only report that examined the relationship between sense of humor and baseline NK activity, more research should be conducted to validate this finding.

Strengths, Limitations, and Recommendations

Strong points in this study include use of a control group, random assignment to groups, use of control samples to document NK assay reliability during each day of the study, and the primary researcher remaining blind to subject assignment.

This study was limited by a relatively small sample size and some unfortunate between-group differences at baseline, which existed despite use of random assignment. A larger sample may have increased the power of some of the correlations found in this study. As noted earlier, the sample size was limited by available funding. This study was also limited by lack of minority representation, as none of the volunteers who responded to the university or community notices were minorities.

As this study was limited to healthy women, the results should not be generalized to other populations, such as those with acute or chronic illness. More research is needed to determine if laughter would have the same effect on a person who has an acute or chronic illness. In addition, the results of this study only apply to the time frame

immediately following a brief period of laughter. More research is needed to determine the effects of a longer period of laughter, how long the effects last, and whether the effects are cumulative.

As noted in the results section, the self-report laughter item and the self-report movie rating item were not completed by some subjects, and were completed during the refreshment period by several other subjects. This limited the quality and quantity of data obtained by these items. Including these items on a separate page and reminding the subjects to complete this section immediately following the video would improve the data obtained from these self-report questions. In addition, it would have been helpful to have more information on how the subjects in both groups perceived the videos. Anecdotal comments from subjects in the distraction group suggests that while some persons found the distraction videos boring, others found them relaxing, enjoyable, and even funny. Use of video recording or other means to document subject responses to both the control and experimental videos would be helpful in future research.

The use of video tours as a neutral control stimulus was somewhat problematic. As noted above, it is difficult to know what some people find funny or relaxing. Based on observed laughter, some subjects apparently found Bill Cosby more funny than others. In the distraction group, while subject responses were not directly observed, some subjects reported afterwards that they really enjoyed the tourism videos, others found them boring, and most subjects stated the videos made them sleepy. These informal statements were supported by a significant decrease in arousal scores following the control videos. White and Winzelberg (1992) had similar problems with their study of laughter and stress. They used a national geographic video as a neutral stimulus, and found that even subjects in the control group had decreased stress following the control

video. It appears that almost nothing can really be a completely neutral stimulus in this type of research.

While the CES-D is commonly used as a screening tool in community populations, it may have led to false positives in this study. As this had been a problem in pre-screening, and the literature indicates that a cut-off of 16 may include several false positives, a cut-off score of 18 was used on the day of data collection (Hammen, 1980; Kendall, Hollon, Beck, A., Hammen & Ingram, 1987). Even using the higher cut-off score it was necessary to reject data from six subjects, due to CES-D scores over 18. As the CES-D scores were not analyzed till after data collection was complete, these subjects participated in the project, but data from these subjects were not included in data analysis. Secondary analysis of data from these six subjects indicates that they did not have significantly different pre-NK or NK change scores, compared with the subjects retained for data analysis. In future studies, the CES-D could be used as a measure of depression, to determine the effects of depressive symptoms on baseline immune function. This would avoid the need to exclude subjects based on CES-D scores. However, if excluding subjects with depressive symptoms is necessary based on the results of the CES-D, a higher cut-off score might be used, as other studies have found the CES-D to lack specificity, particularly in younger female subjects (Kendall, Hollon, Beck, A., Hammen & Ingram, 1987). Despite the above noted problems, the CES-D appears to be one of the better self-report screening tools for depression. Other commonly used depression screening tools are not designed for use in a community setting and also suffer from problems with specificity (Hammen, 1980; Santor, Zuroff, Ramsay & Cervantes, 1995).

Finally, it should be noted that while some subjects who were exposed to the humorous video did not respond with mirthful laughter and did not have increased NK

cytotoxicity, this may not really represent the normal humor responses of these individuals. It must be acknowledged that a controlled research project, which involves monitoring and invasive testing, can never adequately duplicate the humor responses that may take place when a subject is naturally exposed to a humorous stimulus in real life. Watching a humorous video with friends and family in a familiar setting would probably produce more laughter than watching the same video with a group of strangers in an invasive experimental setting. A more natural setting for movie viewing, such as a darkened movie theater, could have helped stimulate a more natural laughter response. Unfortunately this would have made observation of humor responses very difficult. Having a larger number of people watching the humorous video at the same time could have led to increased laughter responses (Aiello, Thompson & Brodzinsky, 1983). Still, the use of small video viewing groups was necessary due to the need to limit numbers of NK samples conducted at one time. Therefore, while the humor methodology used in this study probably limited natural humor response, these limitations were mostly necessary in order to conduct the data collection.

CONCLUSION

The results of this study support a number of basic PNI assumptions. The negative effects of stress and arousal upon immune function were supported, as persons with increased arousal levels had lower levels of NK cytotoxicity at baseline, and persons with increased stress levels had less positive NK change following the interventions. In addition, the beneficial effects of exposure to a humorous stimulus on self-reported stress were supported, as persons viewing the humorous video reported significantly decreased stress following the video, compared with the participants in the distraction group. Taken with previous studies which document the effect of laughter on

stress, it appears that a brief period of mirthful laughter can be a quick and effective method to reduce stress levels. However, more research is needed to determine the duration of the stress reduction effect and if the effect can be cumulative over time.

The major finding of this study was the relationship between mirthful laughter and improved NK cell activity. Because of the role of NK cells in viral illness and various types of cancer, being able significantly to increase NK activity in a brief period of time using a non-invasive method could be clinically important. The use of humor to stimulate laughter could be an effective complementary therapy to decrease stress and improve NK activity in persons with viral illness or cancer. However, more research is needed to determine the clinical significance of these findings.

Need for Further Humor Research

Humor has a natural appeal to both patients and nurses alike. It seems almost intuitive that laughter can make you feel better, and therefore might help you get well. Humor workshops are being produced and marketed around the country, often in connection with a stress reduction or self-healing focus (Killeen, 1991; Martinez, 1989; Metcalf, 1987; Ruxton, 1988; Smith, 1992). There are multiple articles about humor in the health care and lay literature alike. These articles often suggest that the physiological effects of humor have been documented by empirical research and are therefore commonly accepted (Davidhizar 1992; Gilligan, 1993; Halley, 1991; Killeen, 1991; Leidy, 1992; MacHovec, 1991; Metcalf, 1987; Morreall, 1991, Saper, 1988; Sullivan, 1988; Williams, 1986). The work of Cousins (1979), or the new field of PNI research in general, are frequently cited as supporting the role of humor in healing. However, as the review of research literature noted, there are really very few studies that adequately document the effects of laughter on the immune system, and no controlled studies that document the effect of laughter on health outcomes. Therefore, more

research is needed before broad claims for the effect of humor on healing can be made (Friedman et al., 1993; Groves, 1991; Long, 1987; Trent, 1990).

In conclusion, while humor is an exciting new intervention for use in clinical nursing practice, we should continue to investigate the phenomenon. The effects of humor and laughter upon the immune system and physical health need further documentation (Groves, 1991; Long, 1987; Svebak, 1987; Trent, 1990). Are the effects of mirthful laughter on the immune system just temporary, or are they maintained over a long duration? Are the effects of laughter cumulative? The use of humorous stimuli and how best to elicit mirthful laughter in a given population also need more study. Finally, improved methods to capture the effects of the humor response and ways to measure sense of humor are needed. The profession of nursing is based on the foundation of nursing science, which is enlarged and solidified by the process of nursing research. Nursing interventions must be based on this body of knowledge, to maintain credibility and to address clinical concerns. Research into the use of humor needs to continue, to provide the foundation of nursing science needed to support humor as a viable nursing intervention.

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APPENDIXES

Appendix A

Office Use Only: CODE _____

Pre-Test Participant Screening Tool for Humor and Immune Function Testing
To be completed by the researcher during a phone interview with potential participants.

Name _____
Phone: Day _____ Phone: Evenings _____

Address _____
E-Mail (if applicable) _____

1. Are you a male?	No	Yes
2. Are you under the age of 18 years old?	No	Yes
3. Have you been sick or had any minor surgery in the past two weeks?	No	Yes
4. Have you had major surgery in the last 6 months	No	Yes
5.* Do you have any chronic illness, - if yes, what illness	No	Yes
6. Have you lost or gained more than 5 pounds in the last month?	No	Yes
7. Are you currently taking diet supplements (other than regular multivitamins) or herbal remedies?	No	Yes
8. Do you currently smoke?	No	Yes
9.* Do you drink alcohol, and if so, what kind and how many drinks in a week? (More than the equivalent of 10 ounces of alcohol in a week?)	No	Yes
10. Are you currently using street drugs?	No	Yes
11.* Are you having any problems sleeping? - describe Sleeping less than usual	No	Yes
12.* What medications are you taking on a regular basis? (On immunosuppressant medications or medications for depression?)	No	Yes
13. Are you pregnant?	No	Yes
14.* How many hours a week do you exercise? (Exercise more than 7 hours a week?)	No	Yes
15.* Have you had any problems when having your blood drawn for lab tests? (Are you afraid of having your blood drawn for laboratory testing?)	No	Yes
16. Will you work on the 11-7 shift during the preceding three days before your date to participate in this project?	No	Yes

*Questions will be asked in a format other than yes/no, but yes/no answers will be used to determine if subjects meet the inclusion/exclusion criteria. This will ease scoring, while allowing the researcher to explain any item that is not clear to a subject, and will avoid leading the subject to make a certain type of response.

Office Use Only: CODE _____

Participant Screening Tool for Humor and Immune Function Testing

Name _____

To be completed by the participant the day of the study.**Please circle yes or no to answer the following questions:**

1. Have you been sick or had any surgery in the past two weeks?	No	Yes
2. Have you lost or gained more than 5 pounds in the last month?	No	Yes
3. Did you sleep less than usual in each of the preceding 3 nights?	No	Yes
4. Have you worked the 11-7 shift in the past three days?	No	Yes

Appendix B

Office Use Only: CODE _____

Participant Information Sheet and Informed Consent

You are being asked to participate in a study of the effect of watching videos on stress and immune function. In order to participate in this research project you must be female, above the age of 18, non-pregnant, non-smoking, and generally healthy.

Before the study starts, you will be asked some questions about your current health status during a phone interview. During this phone interview, you will also be asked to chose from a list of available videos, and to pick a date to participate in the study. On the day of the study, you will be asked to complete some questionnaires about stress and life events. Then a sample of your blood will be drawn by an experienced technician, in order to determine your baseline immune function. Then you and the other participants will watch a video.

Following the movie, a second blood sample will be drawn, and you will be asked to complete one more questionnaire. This will complete the data collection part of the study. Following the data collection, refreshments will be served, and you will have a chance to ask questions about the research, and to talk with the researchers and other participants. It is estimated that your part in this study will take about three hours.

Participation in this study is entirely voluntary. Your identity will not be revealed at any time. You may withdraw from this study at any time. Risks to participants of this study are no greater than those encountered in a routine blood test at a medical lab. There are no costs to volunteers for participation in this study. Volunteers in this study are helping researchers learn more about the effect of positive emotions and stress on the body. Your immune function results and other test results will be made available to you at the end of the study. Volunteers will also be paid a \$30.00 fee for participation.

If you would like your test and/or the study results mailed to you, please print the following information.

Name _____
 Address _____
 Phone Number _____

Please feel free to ask questions as they arise. If you have questions concerning this research, you are encouraged to contact the primary investigator, Mary Bennett RN MSN, at Indiana State University, School of Nursing [REDACTED] I can also be reached via E-Mail - [REDACTED] or feel free to call me at home [REDACTED]

I have read and understand the above information. I have received a copy of this information for my own records. I have volunteered to participate based on this information.

Signature of Participant: _____
 Signature of Witness: _____
 Date: _____

Appendix C-Instruments

Humor Response Scale

Observe each subject in rotation for 2 minute time intervals at each checkpoint. Rate each subject's response using the scale below. Checkpoints should start 5 minutes after the beginning of the film, and proceed at 10 minute intervals throughout the video.

- 1 = No humor response
- 2 = Occasional Smile
- 3 = Nearly Continuous Smile
- 4 = Rare Laughter or Chuckle
- 5 = Intermittent Laughter
- 6 = Nearly Continuous Laughter
- 7 = Nearly Continuous Laughter plus belly laughter or other spontaneous body movements.

Subject Code #				
Time 1				
Time 2				
Time 3				
Time 4				
Time 5				
Time 6				
Total Score				

Subject Demographic Sheet

Office Use Only CODE _____

Age _____ Sex _____ Race _____

Marital Status S M W D

Number of children living at home _____

Currently a student _____

If you are a student - Number of hours Enrolled _____

Employment - Number of Hours per week. _____

Number of hours per week spent on employment and/or school work _____

Please list any medications that you take on a regular basis.Please list any medications you took this morning.

Following the Video, please rate how funny you found this movie:

_____ Not funny at all

_____ Somewhat funny

_____ Moderately Funny

_____ Very Funny

Please rate how you think you responded to the film.

_____ I did not laugh or smile.

_____ Smiled some.

_____ Laughed once or twice.

_____ Laughed Intermittently.

_____ Laughed nearly continuously.

Equipment and Materials for Modified 4-hour NK Assay

Lymphocyte Separation Procedure

1. Lymphocyte separation medium (LSM; Ficoll-Hypaque from Bioneteic Laboratory, or equivalent): Specific gravity 1.076
2. Pasteur pipets, 9 inch, disposable, sterile (Fisher)
3. Pipets, 10 ml, disposable, sterile, individually wrapped (Baxter)
4. Sterile plastic centrifuge tubes 15 and 50 cc (Corning)
5. Phosphate buffered saline solution (PBS) pH 7.2 w/o calcium chloride, w/o magnesium chloride - Cat. # 20012-027 Lot # 1206642 (Gibco)
6. Hemocytometer or automated cell counter
7. Laminar flow hood
8. Suction
9. Centrifuge
10. Gloves

Chromium Release Assay of NK cells

1. RPMI 1640 with 25 mM HEPES buffer Cat. # 22400-055 Lot # 17N6049 (Gibco)
2. Fetal Bovine Serum (FBS) (Gibco Laboratories)
3. L-Glutamine 200 mM (29.2 mg/ml) Cat. # 2503-81 Lot # 24N8153 (Gibco)
4. Penicillin/Streptomycin (Pen/Strep) Penicillin 5,000 units/ml and 5,000 µg/ml Streptomycin (Gibco)
5. Sodium Chromate (⁵¹Cr) 50-400 mCi/mg (Amersham Corp or New England Nuclear)
6. Hanks' balanced salt solution (HBSS) 1x w/o calcium chloride, w/o magnesium chloride, w/o magnesium sulfate - Cat# 14170-021 Lot # 14N3146 (Gibco)
7. 10 ml sterile disposable pipets
8. 6 channel pipettor and pipet tips (100 µl) for use with radioactive materials (Skatron)
9. Single channel pipettors and pipet tips 100-1000 µl
10. Skatron harvesting frames (filters) for use with Corning or Costar microplates (Skatron)
11. 96 well u-bottom microtiter plates (Corning or Costar)
12. 16 x 100 mm culture tubes for serial dilution (Baxter)
13. 12 x 75 mm culture tubes for gamma counter (Baxter)
14. Centrifuge and 37 °C waterbath approved for radioactive samples
15. Gamma Counter
16. K562 cells (American Type Culture Collection, Rockville, MD)
17. Microscope and hemocytometer or automated cell counter
18. Skatron Harvesting Press (Skatron)
19. Tuberculin syringe to remove chromium from vial (can also use sterile micropipettor if you remove the rubber stopper from vial)

Cryopreservation of Cells

1. Complete Medium - (see assay procedure for instructions)
2. Dimethyl Sulfoxide (DMSO) (Sigma)
3. 10 ml sterile disposable pipets
4. Single channel pipettors and pipet tips 100-1000 µl
5. Hemocytometer or automated cell counter
6. Nalgene Cryo 1° C Freezing Container Cat # 5100-0001 (Nalgene)
7. Sterile 2 ml screw-top Cryopreservation tubes (Corning)
8. -70° C freezer
9. Liquid nitrogen storage facilities
10. Isopropyl alcohol

Gibco Life Technologies Inc. Grand Island NY 14072
Sigma Chemical PO box 14508 St. Louis MO 62178 Phone 314-771-5750

Modified 4-Hour Chromium Release Natural Killer Cell Assay

(Brunner, Maunel, Cerottini & Chaupis, 1968; Miller, Ludek, Peacock & Tomar, 1991; Ortaldo, Bonnard & Herberman, 1977)

Note: for the entire NK assay procedure, all centrifuges and fluids are at room temperature unless otherwise specified. CDC precautions concerning the handling of blood and body fluids are maintained at all times. All tissue culture work is conducted using sterile technique. Radioactive materials are handled in a laminar hood, using double gloves, fluid proof lab coat, eye protection, and equipment labeled for use with radioactive materials.

Directions for Making Complete Medium

For complete description of stock FBS, L-Glutamine, and Penicillin/Streptomycin, see equipment and materials list.

Starting with 500 cc of RPMI 1640 with HEPES, remove 60 cc from the bottle and add the following:

1. 50 ml of heat inactivated FBS (final concentration 10% FBS)
2. 5 ml L-Glutamine (final concentration 2 mM L-Glutamine)
3. 5 ml Penicillin/Streptomycin (final concentration 50 u/ml Penicillin and 50 ug/ml Streptomycin)

K562 Preparation using Frozen Target Cell Procedure.

Grow K562 cells in a 37° C 5% CO₂ in air incubator. Split cells 1:1 every 48 hours and feed using complete media. K562 can be obtained from the American Type Culture Collection. Sufficient K562 cells are already in long term storage at Terre Haute Center for Medical Research).

Grow up 40 x 10⁶ cells K562 (enough for entire study) to be frozen into eight 5 x 10⁶ cell lots using the standard cell freezing method (described below).

Cell Freezing Procedure

1. Freeze Medium:
80% complete medium, 20% DMSO. Place on ice.
2. Remove 40 x 10⁶ K562 cells from culture (enough for 8 separate assays). K562 culture was split and fed 48 hours before freezing. Wash once with 10 ml complete medium.
3. Resuspend target cells in 8 ml of freeze medium (8 batches of 1 ml each; approximately 5 x 10⁶ cells/ml).
4. Immediately pipette samples into cryogenic freezer vials.

5. Place vials in room temperature isopropyl alcohol bath in cryogenic freezer container. Hold at -70°C for 24-28 hours.
6. Move cells to liquid nitrogen (liquid phase) for long term storage.
7. Procedure will yield eight (1 ml) vials. This will allow enough target cells for eight separate NK assay days.

Cell Thawing Procedure

1. Thaw one vial of target cells 72 hours before each experimental day by warming vial in hand just until pellet is moveable within vial, then dump contents from vial into 10 cc of complete medium which has been warmed to 37°C .
2. Wash the cells once 150 g (1600 RPM) x 5 minutes at room temperature and resuspend in 10 ml complete medium.
3. Place in vented tissue culture bottle on side and incubate in 37°C 5% CO_2 in air incubator for 48 hours.
4. Split target cells by removing 5 cc of cells and adding 5 cc of complete medium 24 hours before assay procedure. Target cells are now ready to be used in the modified NK assay procedure.

Target Cell Labeling Procedure

1. Cell count K562 prepared as described above, remove 5×10^6 cells and place in centrifuge tubes.
2. Centrifuge 150 g x 5 minutes (all centrifuges are at room temperature).
3. Remove supernatant by suction flask (in hood). Add 10 ml HBSS.
4. Spin 150 g x 5 minutes (while waiting on centrifuge, calculate the next step).
5. Calculate volume of ^{51}Cr to be used. Use 250 microcuries ($250 \mu\text{Ci}$) of ^{51}Cr .

For example:

On day 8 the factor is 0.819 (See radioactive decay sheet for decay factors)
Take $250/0.819$ to determine the amount of chromium needed, and divide by the concentration of the chromium solution. $250/0.819 = 305$. If solution is $1 \mu\text{Ci}/\mu\text{l}$, use 305 μl . If solution is $5 \mu\text{Ci}/\mu\text{l}$ use $305/5 = 61 \mu\text{l}$.

6. Remove tubes from centrifuge, suction off supernatant.
7. Observing radiation precautions, remove appropriate amount of ^{51}Cr from bottle.
8. Add enough HBSS to ^{51}Cr to make total volume equal 500 μl and mix gently.

9. Add ^{51}Cr -HBSS mixture to K562 pellet and mix gently. Cap tube and place in 37°C water bath for 1 hour if ^{51}Cr is "hot" (day 1-10), or 1-1/2 hours if on day 11 or later. Agitate tube gently every 15 minutes during incubation time.
10. After incubation in warm water bath, wash x 2 with 10 ml of HBSS at 150 g X 5 minutes.
11. Gently resuspend cells with 10 ml of complete medium and incubate at room temperature for 40 minutes to allow any excess radioactivity to dissipate from the cells.
12. After 40 minute incubation period, wash K562 x 2 (with 10 ml of complete medium at 150 g X 5 minutes).
13. Resuspend in 10 ml of complete medium.
14. Adjust final count to 1×10^5 /ml or 1 million cells in 10 ml. K562 cells are now radiolabeled and ready to be used in assay.

Lymphocyte Preparation

1. Draw blood - 10 ml per subject, using 10 cc vacutainer tubes (200 units preservative free heparin).
2. Working under hood, dilute each sample with phosphate buffered saline solution (PBS) at a 2:1 ratio (for example, 20 ml PBS and 10 ml blood), and place in a 50 cc capped centrifuge tube.
3. Underlayer with 10 ml of LSM by placing tip of pipette at bottom of centrifuge tube and slowly adding LSM.
4. Carefully, don't disturb layers, balance and place in centrifuge. Turn the BRAKE OFF. Centrifuge at 400 g x 30 minutes (2000 RPM in Centra 8 centrifuge).
5. Add 30 ml PBS to labeled 50 cc centrifuge tube.
6. Remove blood from centrifuge, will be in layers. Carefully suction off platelet rich plasma without disturbing WBC layer. Using sterile plastic bulb pipette, place tip into WBC layer and gently remove cells by skimming tip through layer. Place WBC's into the centrifuge tube with PBS, prepared as in step 5.
7. Spin 5 minutes x 1600 RPM in Centra 8.
8. Remove supernatant by pouring off top to leave WBC pellet.
9. Resuspend pellet in 10 ml complete medium.
10. Cell count and remove 4×10^6 cells.

11. Pellet and resuspend in 1 ml of complete medium.
12. Cells are now ready for serial dilution.

Serial Dilution for Microtiter Plate

Using two 96 well u bottom microtiter plates.

First Row (will need one row for each donor sample)

1. Place 100 μ l of WBC solution (4×10^6 /ml) into first 3 wells (40:1 effector-to-target).

Serial Dilution

2. Place 500 μ l of complete medium in each of three test tubes. Add 500 μ l of WBC solution (4×10^6 /ml) into first tube, and mix well. Place 100 μ l of this solution into each of the next 3 wells (20:1 effector-to-target).
3. Into next test tube of complete medium, place 500 μ l of the 20:1 solution and mix well. Place 100 μ l of this solution into each of the next 3 wells (10:1 effector-to-target).
4. Into third test tube of complete medium, place 500 μ l from of the 10:1 solution and mix well. Place 100 μ l of this solution into the last 3 wells (5:1 effector-to-target). Repeat above procedure for each donor sample.

Last Row - Spontaneous Release Wells.

5. Place 100 μ l of complete medium into wells 1-6 on last row to be used as a measure of spontaneous release.
The plate is now ready for the addition of the target cells.

Microtiter Plate Completion and Maximum Release Procedure

1. Add 100 μ l of radiolabeled K562 to each well in the assay plate.
2. Place 100 μ l of labeled K562 in each of three 12 x 75 mm gamma counter tubes to use as the maximum release measure for the assay. These tubes should be left in the radioactive holding area until after the incubation period. These tubes are placed in the gamma counter as is. The cells are not centrifuged, nor filtered. This allows all radioactivity present in 100 μ l of K562 to be read as the maximum activity value (Maximum Procedure from Dr Gebel's Lab RUSH Medical Center, Chicago, IL). This procedure is different than total release procedures which use detergent to lyse target cells. Detergent procedures usually obtain only 80-90% of the total radioactivity present in the cells, while the maximum activity procedure used in this assay accounts for the total radioactivity present (Trinchieri, 1989).
3. Cover assay plate, incubate in a 37° C CO₂ in air incubator for 4 hours.
4. During incubation period, label and rack 12 x 75 mm tubes (one for each well).

Harvester/Gamma Count

1. After incubation time is completed, centrifuge microtiter plates (5 minutes x 150 g) using plate rack.
2. Use Skatron Harvesting Press and filters (one filter per well) to harvest supernatant.
3. Remove filters from wells and place in the appropriately labeled tubes.
4. Count subject samples, spontaneous release samples, and maximum release samples in Gamma counter. Set gamma counter for ^{51}Cr and count for 2 minutes.

Calculation of Percent Cytotoxicity

1. Raw data from the gamma counter are used to calculate mean experimental values for each of the four effector-to-target ratios.
2. Mean experimental values for each of the four effector-to-target ratios (40:1, 20:1, 10:1, and 5:1) are used to calculate the percent cytotoxicity.
3. For each of the four effector-to-target ratios, use the following formula to calculate percent cytotoxicity.

$$\frac{\text{Experimental} - \text{Spontaneous}}{\text{Maximum Activity} - \text{Spontaneous}} \times 100$$

Calculation of Lytic Units

The percent cytotoxicity values for each of the four effector-to-target ratios are entered into Expfit Version 3, which calculates a single lytic unit value for each subject sample. Variables to be entered into Expfit are number of cells per well (1000), mean cytotoxicity (20%), number of data points to be used (4), number of unknowns to calculate (1), and fixed value of A (100).

Radioactive Decay for ^{51}Cr

Day	Factor	Day	Factor
2	0.951	48	0.302
4	0.905	50	0.287
6	0.861	52	0.273
8	0.819	54	0.260
10	0.779	56	0.247
12	0.741	58	0.235
14	0.705	60	0.224
16	0.671	62	0.213
18	0.638	64	0.203
20	0.607	66	0.193
22	0.578	68	0.183
24	0.550	70	0.174
26	0.523	72	0.166
28	0.497	74	0.158
30	0.473	76	0.150
32	0.450	78	0.143
34	0.428	80	0.136
36	0.407	82	0.129
38	0.388	84	0.123
40	0.369	86	0.117
42	0.351	88	0.111
44	0.334	90	0.106
46	0.317		

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