THE EFFECTS OF NOREPINEPHRINE INFUSION
ON THE CIRCULATING LYMPHOCYTE COUNTS
OF POST OPEN HEART SURGERY PATIENTS

by

Wanda Nell Zziwambazza

A Thesis Submitted to the Faculty of the

COLLEGE OF NURSING

In Partial Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

In the Graduate College

THE UNIVERSITY OF ARIZONA

1998

STATEMENT BY AUTHOR

This thesis has been submitted in partial fulfillment of requirements for an advanced degree at The University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgment of source is made. Requests for permission of extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his or her judgment the proposed use of the material is in the interest of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: Wanda Guzman

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

Carrie Murkle PhD, RN   6-19-98
Signed   Date

ACKNOWLEDGMENTS

I would like to acknowledge my gratitude to all the professors at the University of Arizona College of Nursing who assisted me in the pursuit of my educational goal of an advanced practice nurse. I would also like to thank my committee members, Jean Davis, RN, Ph.D. and Ida Ki Moore, RN, Ph.D., for their patient assistance leading to the completion of this thesis. A very special thank you is reserved for my committee chairperson, Carrie Merkle, RN, Ph.D. The many hours of mentorship, which I received from Dr. Merkle, was the key to the successful completion of this research endeavor. Through the profound expertise of Dr. Merkle, I have gained an invaluable amount of knowledge regarding research conduction and interpretation. In addition, Dr. Merkle has helped develop my scholarly writing abilities through her insight and instruction on this thesis. I express my sincere gratitude for all your support leading to my success. I would also like to thank my family for their patience and support during my education.

DEDICATION

This thesis is dedicated to Elinor Irene Wilson, RN, an outstanding and compassionate nurse, a powerful mentor, a wonderful friend, and my beautiful mother. Through the growing years of my life, she offered me nurturing and love while exposing me to the joys and endless possibilities of helping others through her career in nursing. Throughout my academic endeavors, she shared her knowledge and expertise, enhancing my professional role development. She also offered support in every other possible way to ensure my success, not withstanding the many personal sacrifices she made in my shadows. Mom, I can attribute a great deal of success in my professional role and personal development to you. You truly are the wind beneath my wings.

TABLE OF CONTENTS

LIST OF TABLES	7
LIST OF ILLUSTRATIONS	8
ABSTRACT	9
I. INTRODUCTION	10
Rationale for Study.....	10
Significance.....	11
Purpose and Research Questions.....	11
Extraneous Variables.....	12
Definition of Terms.....	12
II. LITERATURE REVIEW	14
Overview of Beta-Adrenergic Immunosuppression.....	14
Neuro-Immune Pathways and Cellular Signaling Mechanisms.....	15
Norepinephrine Regulation of Lymphocytes	20
Norepinephrine Administration in Open Heart Surgery Patients.....	25
Dose of Norepinephrine	27
Inflammation and Infection.....	28
Summary	31
III. METHODOLOGY	34
Design.....	34
Selection Criteria.....	34
APACHE II Selection Tool.....	35
Description of tool.....	35
APACHE II score criteria	35
Sampling and Selection Process.....	37
Data Collection.....	38
Procedures	38
Measures.....	39
Norepinephrine Dose Determination	39
Analysis.....	40
IV. RESULTS	41
Sample Characteristics	41
Postoperative Characteristics of Sample	41
Norepinephrine Administration.....	46
Effect of Norepinephrine on Lymphocyte Counts	46

TABLE OF CONTENTS- *continued*

Effect of Preoperative Beta-Blocking Agents on Lymphocyte Counts	49
Effects of Extraneous Variables	56
Effects of Norepinephrine on Infection.....	56
V. DISCUSSION	59
Summary of Findings	59
Linked Variables	59
Possible Explanations.....	61
Differences in Lymphocyte Counts.....	61
Differences in Beta-Blocking Agent Recipients	62
Prevention of Infection.....	62
Implications for Nursing	63
Limitations	64
Methods of Differential Count	64
Endogenous Norepinephrine	65
Future Research Suggestions	65
APPENDIX A: DATA COLLECTION TOOLS	67
APPENDIX B: FORMULAS	70
APPENDIX C: HUMAN SUBJECTS APPROVAL	73
REFERENCES.....	75

LIST OF TABLES

Table 1. Criteria for Selection of Sample.....	36
Table 2. Description of the Sample by Group.....	42
Table 3. Preoperative Characteristics of the Sample by Group	44
Table 4. Pre and Postoperative Drug Therapy by Group	47
Table 5. Postoperative Norepinephrine Drug Therapy by Group	48
Table 6. Mean Circulating Lymphocyte Count by Group	49
Table 7. Mean % Change in Circulating Lymphocytes from Postoperative Day 1 to Day 2 with and without β -Blocking Agent by Group	57

LIST OF ILLUSTRATIONS

Figure 1. Summary of Possible Nervous/Immune System Interaction Patterns	32
Figure 2. Principle Admitting Diagnosis by Group	43
Figure 3. Postoperative Complications Excluding Infection by Group	45
Figure 4. Mean Circulating Lymphocyte Count by Group	51
Figure 5. Mean % Increase in Circulating Lymphocytes from Postoperative Day 1 to Day 2 by Group	52
Figure 6. Mean Circulating Lymphocyte Counts in Group 1 Subjects with and without Preoperative β -Blocking Agent.....	53
Figure 7. Mean Circulating Lymphocyte Counts in Group 2 Subjects with and without Preoperative β -Blocking Agent.....	54
Figure 8. Mean Circulating Lymphocyte Counts in Group 3 Subjects with and without Preoperative β -Blocking Agent.....	55
Figure 9. Number of Postoperative Infections by Group	58

ABSTRACT

In this retrospective study employing chart reviews, 75 open-heart surgery patients (OHSPs) were divided into three groups of 25 patients. Group 1 received no intravenous (IV) norepinephrine (NE) after surgery. Group 2 and Group 3 received a minimum of 0.028 mcg/kg/minute of IV NE for 6-24 hour and 24-36 hour respectively. In the three groups, preoperative lymphocyte counts were compared to counts obtained on postoperative days 1 and 2. The results showed lower lymphocyte counts on postoperative day 2 in group 3 subjects, who received NE for 24 hours or more, compared to subjects of the other groups who received no NE or 6-24 hours of NE ($p<0.05$). There was also evidence that preoperative use of beta-blocking agents significantly affected the change in lymphocyte counts from day 1 to day 2 in both groups receiving NE. Further, postoperative infections were more prevalent in group 3 than the other two groups ($p<0.05$). The lower lymphocyte counts and higher infection rate, however, may be linked to lower postoperative blood pressure and increased number of intensive care unit days in group 3. Further investigation is warranted to elucidate the effects of IV NE administration on the lymphocyte counts of OHSPs and to reduce infections in those receiving NE.

CHAPTER I

Introduction

Rationale for Study

The past few centuries of man's history have been influenced by the belief that the mind can impact one's health, sickness and recovery (Weil, 1995). Only, however, during the later half of the twentieth century have the studies scientifically linked the nervous and immune systems (Kiecolt-Glaser, Glaser, Gravenstein, Malarky, & Sheridan, 1996). It has been discovered that the immune system is linked to the nervous system by sympathetic innervation of the bone marrow, spleen, and lymph nodes (Felten, Felten, Bellinger, & Madden, 1993). The lymphocyte, itself, has been shown to have receptors for catecholamines released at the nerve synapse (Aarons, Neis, Gallo, & Hegstrand, 1980). One receptor, the beta-adrenergic receptor, binds the catecholamine norepinephrine (NE) (Carlson, Brooks, & Roszman, 1989).

Recently, investigative effects have focused on the effects of NE on the immune system and its primary effector cell, the lymphocyte. Studies have determined that NE is a negative regulator of the immune system. That is, NE exerts its regulatory effects by inhibiting lymphocyte proliferation in the lymph nodes and decreasing migration to the lymph nodes from lymphocyte storage tissues (Madden, Felten, Felten, Hardy, & Livnat, 1993). This is reflected in a lower circulating lymphocyte count as fewer lymphocytes enter the blood stream from the lymph nodes via efferent lymph vessels. Research on both endogenous and exogenous NE has supported these findings (Harris, Waltman, Carter, & Maisel, 1995). Research on the effect of endogenous NE on the lymphocyte in

open heart surgery patients (OHSPs) has been conducted (Smiley & Vulliemoz, 1992), however, no research to date has investigated the effects of IV NE administration on lymphocyte counts and infections in OHSPs, who frequently receive this drug.

Significance

Research on the effects of IV administration of NE on the immune status of OHSPs could have potential significance for the health care field. If links between NE administration, lower lymphocyte counts, and increased infection are found perhaps other cardiac drugs could be substituted especially in patients at high risk for infections. Further measures, such as prophylactic antibiotic use or extended use of sterile dressings, during NE administration may be needed to reduce postoperative infections.

Purpose and Research Questions

The purpose of this research was to examine the effects of IV NE infusion on immune status in postoperative cardiac surgery patients. The research specifically investigated the effects of postoperative NE administration on the total number of circulating lymphocytes at postoperative day 1 and day 2 compared to preoperative values. The specific research questions were:

1. Are the total number of circulating lymphocytes in post OHSPs reduced by NE administration of a mean dose of 0.028 micrograms per kilogram per minute (mcg/kg/min) for 6 to 24 hours or greater than 24 hours, compared to those OHSPs who did not receive NE?
2. What is the effect of preoperative beta-blocking agent administration on the total number of circulating lymphocytes in OHSPs?

3. What is the effect of NE administration on the rate of infection in OHSPs compared to OHSPs who did not receive NE?

Extraneous Variables

Other variables, with the potential to alter postoperative circulating lymphocyte counts, were also recorded. Anti-inflammatory drugs administered before or after surgery may affect the inflammatory response by limiting immune cell proliferation. Data on other variables that might affect immunity, such as age, allergic reactions, and other preexisting medical conditions, were collected and analyzed.

Definition of Terms

Open heart surgery patient: For this study, an OHSP is a patient in an acute clinical setting having had surgical repair of cardiac vessels or valves while supported by heart-lung bypass.

Norepinephrine: This is a hormone secreted by the adrenal medulla and categorized as a catecholamine. It is also a drug given exogenously to promote vasoconstriction and increase blood pressure.

Lymphocyte: This is a white blood cell that becomes activated in specific immune responses and normally consists of 20% to 40% of the total white blood cell population. These cells are further differentiated into B cells, which produce antibodies, and T cells, which contribute to cell-mediated immunity.

Infection: For this study, an infection is defined as documentation of a positive gram stain or culture and sensitivity from a specific culture site from the patient within six days of surgery.

Immunosuppression: For this study, immunosuppression is defined as a decrease in lymphocyte and overall white blood cell counts.

Lymphocytopenia: This is a deficiency in the number of mature, circulating lymphocytes characterized by a number less than 20% of total white blood cell count.

CHAPTER II

Literature Review

Overview of Adrenergic Immunosuppression

Behavioral scientists have spent much effort investigating the effects of stress on immune function in both animals and humans. Studies have shown that stressful situations can negatively impact the immune system with results ranging from susceptibility to the common cold to increases in tumor growth rate (Sklar & Anisman, 1979; Cohen, Tyrell, & Smith, 1991). In studies utilizing the rat, a positive correlation was found between the graded level of stress to which the animal was exposed and the suppression of the mitogen stimulation of the immune cells (Keller, Weiss, Schleiffer, Miller, & Stein, 1981). In human studies, the same immunosuppressive effect was found in individuals after prolonged episodes of bereavement and during academic stress experienced by medical students (Schleifer, Keller, Camerino, Thornton, & Stein, 1983; Malarkey, Pearl, Demers, Kiecolt-Glaser, & Glaser, 1995).

The results of these and other related psychosomatic studies have been applied to research attempting to alter the immune response in clinical situations in which suppression of the immune system is advantageous. For example, animal studies have used stress induction to decrease the delayed type hypersensitivity reaction to sheep red blood cells (SRBC) and contact sensitivity to the mitogen dinitrofluorobenzene (DNFB) (Blecha, Barry, & Kelley, 1982). In clinical studies, a state of stress was purposefully initiated in skin graft patients by modeling stressfully perceived situations. This resulted

in successful alteration of the disease process in graft versus host response (Bovbjerg, Ader, & Cohen, 1982).

Several studies have linked the physical and psychological stress response to an actual physiological event. One such study investigated the effects of heat exposure and repeated exercise of healthy male subjects on circulating stress hormones, including NE. The results revealed a significant increase in plasma NE, epinephrine, and human growth hormone levels when these subjects were exposed to either exercise or heat for prolonged periods of time (Brenner, Zamecnik, Shek, & Shephard, 1997).

A second study investigated the effects of psychological stress alone on plasma NE levels and immunity. In this study, the plasma cortisol and NE levels of Alzheimer patient care givers were measured and compared to scores on a life stress indicator tool. The results revealed a significant difference between low and high perceived stress levels and the serum NE levels with high stress reporters having higher serum NE levels. There was no significant difference in plasma cortisol levels among the sample. Further, the results showed an increase in the density of beta adrenergic receptors on the lymphocytes of the subjects with significantly higher serum NE levels. However, this was coupled with a decreased sensitivity of these receptors to adrenergic stimulation by isoproterenol. These results suggested a possible association between high psychological stress and mediation of the adrenergic pathway behind cellular immunity (Mills, Ziegler, Patterson, Dimsdale, Hauger, Irwin, & Grant, 1997).

Neuro-Immune Pathways and Cellular Signaling Mechanisms

Pioneering studies to identify the physiological mechanism by which the mind

and body communicate to mediate the immune system were first documented in academic journals in 1960. Initial identification of the pathway was reported in the discovery that nerve fibers of the autonomic nervous system are prevalent in both primary and secondary lymphoid organs. Further research revealed the bone marrow- a primary lymphoid organ responsible for production and differentiation of immune cells- to have significant innervation by sympathetic noradrenergic nerve fibers. High degrees of innervation in the spleen and lymphatic system- secondary lymphoid organs responsible for storage, maturation, and proliferation of immune cells- were also found (Calvo, 1968).

Further delineation of the nervous/immune systems' communication utilized the knowledge that the nervous system communicates via the release of catecholamines from the nerve synapse to the target cells or organs. Immunization of animals with the immune stimulating antigen or mitogen, SRBC, revealed a decrease in NE levels in the spleen during the immune response. This reduction of NE during immune activity inferred that the presence of NE might in fact inhibit the immune cell function. Further experimental results in this study did, in fact, reveal that low immune responders are found to have a significantly higher content of NE in the spleen than high responders to the mitogen. The data also revealed a significant increase in splenic weight in high responders, when compared to low responders, from the resultant increase in immune cell content of the spleen in the presence of decreased NE levels (del Rey, Besedovsky, Sorkin, de Prada, & Bondiolotti, 1982).

A second study, which measured the levels of NE in immune activated animals,

compared an antigen injected group to a saline injected control group. The experimental group was injected intraperitoneally with SRBC and sacrificed three days later. The control group was injected with the same volume of saline and sacrificed at the same time. The thymus, spleen and lymph nodes were harvested from each animal.

Examination of these selected lymphoid organs showed the experimental group to contain less NE than those of the control group (del Rey, Besedovsky, Sorkin, de Prada, & Arrenbrecht, 1981).

To test whether an increase in lymphocytes is directly related to the decrease in NE release from the nerve synapse, animal studies were conducted in which mice were chemically sympathectomized. That is, the neurotoxic drug 6-hydroxydopamine (6-OHDA) was administered systemically to mice. The noradrenergic nerve terminals were selectively destroyed by this drug, thereby causing depletion in the NE and other catecholamines released to the organs or cells. When the drug induced sympathectomized mice were compared to a saline injected control group, the NE content of the lymphoid organs in the sympathectomized group was less than 10% of the control group. The *in vivo* lymphocyte proliferation of the control and experimental groups was measured by the uptake of injected [^{125} I] deoxyuridine (IUDR) into the DNA of dividing cells. Upon harvest of the animals, the sympathectomized mice exhibited a significant increase in the number of new lymphocytes, measured by radioactivity of the isotope in the spleen, bone marrow, and lymph nodes suggesting increased cell proliferation in the absence of NE. The inguinal and axillary lymph nodes of the sympathectomized mice displayed an even larger significance of IUDR uptake, as much as 100 times greater than

controls, suggesting increased cell migration to these lymph nodes in the absence of NE. This study substantiated the theory that NE exerts a negative regulatory effect on the immune system (Madden et al., 1993).

The ability of NE to affect the lymphoid system was further investigated in research on the lymphocyte itself. Researchers identified the beta-adrenergic receptor (beta-receptor) that corresponds to the noradrenergic catecholamines on the surface of the lymphocyte by using a beta-receptor antagonist, which binds to the receptor sites. Different numbers of these beta-receptors were found on the various subpopulations of the human lymphocytes (Krawietz, Werdan, Schober, Erdmann, Kindfleisch, & Hannig, 1982). When the addition of the same volume and concentration of the beta-receptor antagonist was added to the various subsets, a difference in binding capacity, although not saturation, was exhibited. The distribution of the beta-receptors on the B cells, which is usually enhanced during bacterial infections, revealed a binding capacity of 400 to 600 sites per cell. The T cytolytic cell population, which is usually enhanced during viral infection, displayed approximately 200 receptors per cell. These studies suggested that NE might exert stronger control on specific populations of lymphocytes (Pochet, Delespesse, Gausset, & Collet, 1979).

Identification of the characteristic number of beta-adrenergic binding sites for all the lymphocyte subsets led to the ability to examine the precursor cells for each T subtype (cytolytic, suppressor, and helper). These studies revealed that the number and distribution of the beta-receptors on the immature precursor cells of each T cell type varied immensely, even between phenotypically distinct cells. These findings suggested

that the precursor cells could still be induced to modulate the expression of the beta-receptor while maturing to functional cells (Khan, Sansoni, Silverman, Engleman, & Melmon, 1986).

Mechanisms by which NE exerts its inhibitory effect on the lymphocyte after binding to the beta-receptors on the cell have continually been proposed. One tested hypothesis involved the use of isoproterenol, a beta-receptor agonist, to stimulate the effects of NE. When T cells were treated with isoproterenol and the mitogen phytohemagglutinin, there was a synergistic increase in cyclic adenosine monophosphate (cAMP) levels in the cells, which reduced cell responsiveness to the mitogen. When mitogen without isoproterenol was added to the T cells, there was no increase in cAMP but a high response to the mitogen. These data suggested that NE might exert its control through a "second messenger system," thereby inhibiting lymphocyte activation (Carlson et al., 1989).

The second messenger system was tested by increasing cAMP levels in activated T cells by isoproterenol stimulation. The results showed a drastic impairment of proliferation and function in activated cells. When the experiment was repeated in a calcium rich environment, there was an even larger increase in cAMP and impairment of lymphocyte activation. This suggested that there are additional controls beyond the beta-receptor involving calcium that inhibit lymphocyte proliferation (Carlson, Trauth, Brooks, & Roszman, 1994).

Further studies of the signaling involved between NE and lymphocyte inhibition suggested that NE is also regulated in the lymphoid tissue by other immune mediators,

such as the cytokines Interleukin-1 beta (IL-1 β) and Interleukin-2 (IL-2). IL-1 and IL-2 have been shown to regulate neuroendocrine release and neuronal activity. Animal experiments in this area revealed that the addition of exogenous IL-1 β and IL-2 inhibited NE release in highly vascularized areas of the spleen. This, in turn, caused an increase in splenic weight resulting from a significant increase in lymphocyte proliferation. These data suggested that the immune regulator NE, itself, is also regulated by a third party of cytokines (Bognar, Albrecht, Farasaty, & Fuder, 1995).

Norepinephrine Regulation of Lymphocytes

Having established a link between NE and the lymphocyte via the beta-receptor, further research was conducted which focused on the relationship of NE to the immune reaction. NE's actual effect on the lymphocyte was investigated through research involving lymphohematopoiesis, in vivo production and maturation of the lymphocyte. Animal experiments involving the bone marrow, the site of lymphohematopoiesis, revealed that intraosseous addition of NE to mice resulted in a dose dependent decrease in the proliferation and differentiation of leukocytes. The decrease in lymphocyte proliferation was evident using as low as 10^{-8} Molar concentrations of NE and resulted in almost a complete inhibition of lymphocyte differentiation and proliferation at concentrations of 10^{-4} Molar. This research further supported a regulatory effect of NE on immune cells (Maestroni & Conti, 1993).

In animal experiments with aged rodents, the 27 month old rodent exhibited a significant decrease in splenic and lymph node noradrenergic innervation when compared to the 3 month old rodent. Similar correlations between NE content of the spleen and age

was observed as well as protein content of the spleen and age reflecting a decrease in splenic white blood cell count. The weight of the spleen in the animals, however, reflected an increase in splenic weight with age resulting in splenomegaly by the age of 27 months. This weight increase was explained by a significant increase in red pulp within the spleen and a decrease in white pulp, the site of lymphocyte proliferation. This research suggested an immune dependence on the presence of NE for optimal functioning.

The findings also implied that a decrease in noradrenergic innervation of the spleen resulted in the deterioration of the white pulp compartments necessary for lymphocyte activity, thus resulting in a low splenic lymphocyte count. The lack of NE content was also associated with a decrease in IL-2 within the spleen. As stated earlier, IL-2 has been evident in NE regulation within the immune system as well as other immune function responsibilities (Bellinger, Ackerman, Felten, & Felten, 1992).

The effects of NE on lymphocyte production and proliferation were studied in depth with further research, which focused on the effects of NE on lymphocyte migration. Human studies involving in vitro research on the effects of anaesthetic agents on leukocyte migration showed the potentiating effect that NE had on inhibiting migration of lymphocytes. When blood samples were exposed to the anaesthetic agents bupivacaine and lignocaine there was a significant decrease in lymphocyte migration from one medium to the next. The exposure of these cells to NE had a similar effect on migration. However, when lymphocytes were exposed to both NE and anaesthetic agent, there was an increase in migration inhibition at even lower molar concentrations than the

initial inhibitory concentration (Smith, Edwards, Gowert, Ferguson, & Williams, 1992).

Further investigation on surgical procedures and how they affected the lymphocyte was conducted using the rabbit model. Experimental studies which compared an anaesthetized, surgically incised experimental group of rabbits to an anaesthetized only control group revealed a pronounced stress response in the experimental group following the surgery exhibited by increased serum cortisol and adrenaline levels. When the experimental group was compared to the control group, the experimental group demonstrated a significant increase in granulocytes but a significant decrease in lymphocytes in peripheral blood analysis. The radioactivity of presurgical lymphocytes, which were labeled with Indium-tropolone and injected systemically into the rabbit, revealed a drastic increase in lymphocyte activity in the areas of the lymph nodes in the experimental group. This suggested that the presence of NE in peripheral blood influenced a redistribution of lymphocytes to the lymphatic tissue from the circulating blood. The presence of lymphocytes in the area of the heart and lungs was especially reduced in the experimental group. This was probably due to the large number of beta-receptors in these organs providing an increased concentration of NE in these areas (Toft et al., 1992).

Research on animals exhibiting defects in the noradrenergic system provided a model to identify the effects of high endogenous NE on the immune system. Genetically epilepsy-prone rats presented with a splenic content of NE significantly greater than control rats. However, these epileptic rats also exhibited a 33% reduction of beta-receptors on the surface of the lymphocytes. The epileptic rats were also

documented as being immune compromised through diminished immune cell activation after immunization.

In comparing the splenic content of NE in the epileptic rat to the controls, it was shown that the epileptic rats' spleens contain 60 percent more NE than the controls. The splenic weight of the epileptic rat was significantly lower than that of the controls. However, upon analysis of lymphocyte count in the spleen, there was no difference in numbers between the controls and the epileptic rats.

Analysis of the lymphocyte, itself, revealed a decrease in beta-receptors on the surface of the lymphocyte of epileptic rats. This suggested that the continual exposure to elevated levels of NE result in a down regulation of the beta-receptors on the lymphocyte. The difference in the weight of the spleens was explained by a shift in subpopulations of lymphocytes from B cells to T cells. Since the B cells were documented to contain more beta-receptors on the surface than T cells, a reduction in NE binding sites of the T cell could have rendered the T cell completely unresponsive to the negative regulatory effects of NE. Thus, it was hypothesized that the proliferation of the cells within the spleen was primarily that of the T cells (Carr, Ortiz, Paxton, Saland, & Savage, 1993).

The human model for examining the effects of elevated levels of NE on the immune system for an extended period of time has been the congestive heart failure (CHF) patient. CHF patients have been documented as having elevated levels of circulating catecholamines due to the compensatory action of the autonomic nervous system for controlling blood pressure. These patients have also been documented as immune compromised, suggesting a correlation between these clinical manifestations.

To further examine this possible link, the animal model was studied by administering NE and other catecholamines for a period of four weeks to an experimental group of rats. The results of the NE infusion showed a dose dependent decrease in proliferation of T cells and an overall decrease in the total number of T cells. However, exposure of B cells to a high level of NE for a prolonged period of time actually led to an increase in B cell proliferation and count. This suggested that consistently high levels of NE might lead to immune compromise in CHF patients by down regulation of the beta-receptor leading to a block in the negative regulatory mechanisms of the cell. This may have caused an inhibition in differentiation of antibodies to specific mitogens due to premature proliferation of B cells (Harris et al., 1995).

The OHSP was also used as a model to investigate the effects of exposure to prolonged bursts of NE. The OHSP is exposed to extreme physiological stress while on cardiopulmonary bypass and under the surgical procedure itself. This physiological stress, along with anaesthetic and vasolytic drug use, can result in a very high level of circulating catecholamine levels. Studies to test the effects of this intraoperative stress on the OHSP were conducted. Blood samples from OHSPs were drawn before and after surgery. The samples were then stimulated with a beta-adrenergic agonist, isoproterenol. The cAMP production of the cells was measured to indicate beta-receptor binding and activation. The post surgical blood when compared to the presurgical blood showed significantly less cAMP when stimulated with isoproterenol reflecting a decrease in the binding of the receptor. In previous research, cAMP was indicated as a second messenger system of NE to negatively regulate the lymphocyte. Therefore, a decrease in

cAMP production, in this study, reflected a decrease in the presence or binding of beta-adrenergic receptors on the cells. The researchers in this study hypothesized this to be a desensitization of the beta-receptors on the lymphocyte surface (Smiley & Vulliemoz, 1992).

The number of existing beta-receptors on the lymphocyte was shown in research to be subject to up-regulation as well. Clinical investigation of the administration of beta-adrenergic blocking agents revealed that chronic exposure of the cells to the beta-blocking agent, propranolol, caused an increase in beta-adrenergic receptors of human lymphocytes. The up-regulation of the beta-receptors was measured at a 43 percent increase after only 5 days of the drug propranolol. Withdrawal of the drug resulted in a return of the beta-receptor count to normal within 24 hours (Aarons, Nies, Hegstrand, & Molinoff, 1980). However, it was hypothesized that patients with beta-receptor up-regulation from chronic beta-blockade may be highly susceptible to beta-receptor desensitization if the beta-blocking agent is removed and the patient experiences high levels of catecholamine levels from cardiac surgical stress. (Smiley & Vulliemoz, 1992). This hypothesis is extremely relevant to the OHSP, as the patient has probably been stabilized by cardiac drugs prior to surgery. However, no studies have been done to test this hypothesis.

NE Administration in Open Heart Surgery Patients

The process of open heart surgery requires that the patient be maintained with a nonpulsatile heart for a period of time while undergoing the valve replacement or coronary vessel grafting. For this, the OHSP is often anesthetized using a combination of

hypothermia, opioids, ketamine-benzodiazepines, and inhaled gas (Tuman, McCarthy, O'Connor, Holm,, & Ivankovich, 1995). The physiological effects of these agents, however, often result in profound hypotension requiring an exogenous adrenergic receptor agonist using IV medication, such as dopamine or NE. A clinical study of patients receiving open heart surgery and intravenous ketamine-benzodiazapine or opioids revealed a significant decrease in blood pressure following the administration of both drugs. There was also a significantly greater amount of hypotension in the ketamine-benzodiazapine group compared to the opioid group in the postoperative period (Plunkett, Reeves, Ngo, Bellows, Shafer, Roach, Howse, Herskowitz, & Mangano, 1995).

Animal studies on the physiological effects of isoflurane, an inhaled anesthetic gas, during open heart surgery have identified hypotension as one of the major side effects of this agent (Raner, Biber, Henriksson, Lundberg, Martner, & Winso, 1995). One such study, using the canine model, identified a dose dependent decrease in both left and right ventricular function, measured by a decrease in ventricular systolic pressure and stroke volume, following the administration of inhaled isoflurane. There was also a dose dependent decrease in systemic vascular resistance, measured by mean arterial pressure (MAP), coupled with an increase in pulmonary vascular resistance, measured by pulmonary artery wedge pressure, following administration of the agent (Purebi, 1987).

While the drug of choice to counteract the hypotensive effects of anesthesia and pain control is often dopamine (Raner et al, 1995), this drug at high doses of creates undesirable vasoconstrictive effects. Research focusing on the effects of dopamine and NE after open heart surgery has been done using the canine model. In this research, it

was found that IV administration of sufficient amounts of dopamine to raise the MAP by 10 mm Hg resulted in a significant increase in pulmonary vascular resistance compared to canines receiving NE to increase the MAP by the same degree. There was also a significant increase in renal artery pressure in the dopamine group resulting in a decreased blood flow to the kidneys compared to the NE group. For this reason, NE is sometimes preferred in the treatment of post open heart surgery hypotension over high doses of dopamine (Angle, Molloy, Penner, Jones, & Prewitt, 1989).

Human studies on open heart surgery patients support the use of IV NE postoperatively to increase MAP. One recent clinical study identified the use of low-dose dopamine in conjunction with NE as the optimal treatment for hypotension following open heart surgery. Administration of a mean dose of 0.118 mcg/kg/min of IV NE resulted in an increase in the MAP of 22 ± 2 mm Hg with a significant decrease in renal blood flow. The addition of 3 mcg/kg/min of IV dopamine restored the renal blood flow to normal values. It was hypothesized that while NE acted upon the sympathetic nervous system to increase cardiac output and systemic vascular resistance, dopamine counteracted the constrictive effects of NE on the renal artery resulting in an increase in renal blood flow. The addition of dopamine, however, did significantly decrease NE affect on the MAP resulting in a final MAP only 15 ± 4 mm Hg above original values (Richer, Robert, & Lebel, 1996).

Dose of Norepinephrine

In recent animals studies, the minimal NE dose of 0.15 mcg/kg/min was determined to raise the mean arterial blood pressure (MAP) of a nonseptic animal by 15

mm Hg. At this dose, there were no significant changes in baseline systemic vascular resistance index (SVRI), cardiac index (CI), heart rate (HR), arterial oxygen (PaO_2), systemic oxygen delivery, or systemic oxygen consumption. The minimal NE dose of 0.28 mcg/kg/min was shown to raise the MAP of these experimental animals by 32 mm Hg. This dose has also been shown to cause significant changes in SVRI, CI, HR, PaO_2 , DaO_2 , systemic oxygen delivery, and systemic oxygen consumption (Meyer, Booke, Waurick, Prien, & Van Aken, 1996). Further studies conducted on animals showed that the use of IV NE to raise the MAP by 30 mm Hg from baseline blood pressure resulted in the inhibition of bacterial clearance by animals injected with the bacteria *Escherichia coli* (Koch, Heller, van Ackern, Schiefer, & Neuhof, 1996). Therefore, it was hypothesized that a dose of 0.28 mcg/kg/min or more of IV NE is sufficiently high to cause an obvious failure in the immune response.

The ability for the human body to release larger amounts of endogenous NE, in addition to exogenous NE, when under intense stress must be considered when applying this research to humans. In clinical studies of OHSPs, a dose of only 0.020 mcg/kg/min of IV NE was shown to raise the MAP by 15 mm Hg (White & Leenen, 1997). This in contrast to the animal dose of 0.15 mcg/kg/min for the same increase in MAP (Meyer et al, 1996) reflects an intense difference in human and animal dose responses to NE. To date, there have been no studies to determine the effect of IV NE on lymphocyte counts or bacterial clearance in humans.

Inflammation and Infection

Early studies involving surgical interference with tissue was shown to produce a

local inflammatory response in the area of surgical trauma (Quin & Shannon, 1975). Inflammation in the OHSP was hypothesized, by other researchers, to originate from lung injury while on cardiopulmonary bypass. This hypothesis identified the lack of blood supply and expansion in the lung while on bypass to be the cause of injury to the lung tissue resulting in cytokine release by local neutrophils. It was further hypothesized that these cytokines, in turn, most likely activate the other local immune cells, including lymphocytes, to proliferate. The lymphocyte can then enter systemic circulation via efferent lymph vessels (De Backer, Amsel, Jorens, Bossaert, Hiemstra, van Noort, & van Overveld, 1996).

The cytokines, IL-2 and IL-6, were found in another study to be secreted by both B and T lymphocytes in response to their immune activation (Quigley, Caplan, Perkins, Arentzon, Alexander, Kuehn, Hoff, & Wallock, 1995). Studies on the blood serum levels of cytokines in OHSPs showed that IL-6 increased very rapidly following open heart surgery and was useful as an early detector of acute inflammation (Sakai, Mori, Suzuki, Katayam, & Matsuyama, 1994). IL-2 was detected in OHSPs in the first post operative day and declined by the third or fourth day (Lahat, Shtiller, Zlothnick, & Merin, 1993). Although no studies were done on the average rise in circulating lymphocyte counts after open heart surgery, these data suggested that at least some rise in lymphocyte count should be expected following open heart surgery based on the normal inflammatory response.

Several clinical studies have been conducted on the incidence of surgical wound infection in the OHSP. One multicenter study investigating the incidence of

postoperative infection in eight general surgical wards and one cardiothoracic surgical ward found a significantly higher rate of surgical wound infection among cardiac or vascular surgery patients compared to general surgery patients. The overall infection rate of general surgery patients was less than four percent compared to eight percent in the cardiac or vascular surgery patients (Moro, Carrieri, Tozzi, Lana, & Greco, 1996)

A separate study of 22,180 OHSPs alone identified a 1.9% incidence of sternal wound infection by coagulase-negative staphylococci, a common bacteria found on the skin. Of these patients with a staphylococci sternal wound infection, 14% had a secondary systemic (blood) infection by the same organism. The results of this study revealed an additional mean cost per sternal infection of \$20,000 and a total of 2,600 additional hospital days (Mossad, Serkey, Longworth, Cosgrove, & Gordon, 1997).

An additional study which focused on reducing hospital cost related to surgical site infection, identified an 7.3% incidence of surgical wound infections among 928 OHSPs (control group) at a single hospital over a eighteen month period. In this study, an experimental group of 868 OHSP was created over the subsequent eighteen months. The experimental group was administered mupirocin nasal ointment (antibiotic ointment) during surgery in an attempt to decrease the rate of surgical site infections originating from preexisting nasal bacteria. The results revealed a significant decrease in surgical wound infection of the experimental group, only 2.8%, when compared to the control group. This resulted in a savings of \$16,633 for each surgical site infection prevented in the experimental group (VandenBergh, Kluytmans, van Hout, Maat, Seerden, McDonnel, & Verbrugh, 1996).

Summary

The exact mechanisms by which the nervous and immune systems interact are very complex in nature. The detailed research in this area has identified many of the probable signaling methods and pathways. An overwhelming amount of literature on the subject has supported the hypothesis that NE is one of its major messengers and the beta-receptor on the lymphocyte is the target for the regulatory mechanism. The regulatory mechanism of the lymphocyte by NE appears to be a decrease in migration of lymphocytes to the lymph nodes as well as a decrease in proliferation of these lymphocytes. The result of this negative regulatory mechanism is a decrease in immune activity by lymphocytes in the presence of NE.

Clinical studies involving CHF patients have shown that chronic elevation of endogenous NE may lead to down regulation of the beta-receptors on the lymphocytes causing a decrease in immune regulation by NE. Prolonged bursts of both endogenous and exogenous NE have also been shown to effect NE regulation of the lymphocyte by beta-receptor desensitization. Further, beta-receptor blockade resulting from the use of beta-blocking drugs can also influence the beta-receptors on the lymphocyte by causing an up regulation of these receptors. It is hypothesized that once the beta-blocking agent is removed, the regulation of the lymphocytes by NE is enhanced due to a greater number of receptor sites. A brief summary of the relationship between the nervous and immune systems is illustrated in Figure 1.

The OHSP has been shown to have a higher rate of surgical wound infection than general surgery patients. The OHSP is also at risk for pulmonary infection related to lung

*From premature proliferation of lymphocytes

Figure 1. Summary of Possible Nervous/Immune System Interaction Patterns

injury while supported on cardiopulmonary bypass. These patients are also the recipients of IV NE postoperatively for hemodynamic and cardiovascular support. Endogenous NE has been shown to decrease immune function resulting in immunosuppression in both animal and human studies. Animal studies have shown that exogenous NE infusion also causes immunosuppression in animals related to a decrease in lymphocyte counts. However, no clinical study to date has investigated the effects of NE infusion on lymphocyte counts and infection rates on OHSPs and the influence that preoperative beta-blocking agent use has on these results.

CHAPTER III

Methodology

Design

This study was designed primarily to determine if NE administration was associated with reduced lymphocyte counts and an increased incidence of infections in OHSPs. The use of beta-blocking agents preoperatively was also considered when analyzing the final results. A retrospective chart review was performed in three groups of OHSPs:

- ◆ Group 1- records from patients who received no IV NE following complications of the surgical procedure and was identified as the NO NE group (No NE).
- ◆ Group 2- records from patients who received 6 to 24 hours of a minimal IV NE dose of 0.028 mcg/kg/min following complications of the surgical procedure and was identified as the less than 24 hour NE group (<24hr NE).
- ◆ Group 3- records from patients who received more than 24 hours of a minimal IV NE dose of 0.028 mcg/kg/min following complications of the surgical procedure and was identified as the greater than 24 hour NE group (>24hr NE).

Selection Criteria

To ensure that the groups were comparable, the APACHE II model, a morbidity and mortality tool, was used to ensure group similarity in physiological parameters/status

immediately prior to surgery. All patients were admitted to a cardiovascular ICU immediately postoperative for recovery and remained in the ICU for at least 24 hours. Further selection criteria as defined in Table 1 ensured similarities both within and between the three groups.

APACHE II Selection Tool

Description of tool. The APACHE II (acute physiology and chronic health evaluation) is a tool designed to provide a severity of disease prediction measure which has been revised from its original form, the APACHE I. The measure uses an interval scale to rank from one to four the physiological states of the organ systems through numerically labeled values such as temperature, blood pressure, PaO₂, serum pH, and eight other measurable values.

The age and chronic health status of the patient are also assigned interval values. A copy of the tool to be used can be seen in Appendix A. The APACHE II score is the sum of the physiological score plus the age and chronic health scores. The maximum score which can be obtained is 71, however there has been no recorded score greater than 55 (Knaus, Draper, Wagner, & Zimmerman, 1985).

APACHE II score criteria. Factors, which may have contributed to post operative infection or immune compromise, were limited by selecting the sample from a population, which possessed a preoperative APACHE II score of a limiting risk. The score of 17 or less was chosen as the limiting APACHE II score for selection. This value was derived by inserting a diagnostic category constant and the calculated APACHE II score for a diagnostic category into a formula which calculated the percent risk of death

Table 1.

Criteria for Selection of Sample

Variable	Observations and treatments
Cardiac Diagnosis (1 or more of the following diagnosis)	Acute myocardial infarction Unrelieved angina pectoralis Valvular insufficiency Valvular stenosis Atrial septal defect
Surgical Procedure (1 or more of the following nonemergency surgeries)	Cardiac Arterial Bypass Graft Valvular Replacement/Repair
Surgical Stabilization (All of the following)	Use of general anesthesia Endotracheal intubation Cardiopulmonary bypass for at least one hour
Postsurgical Drug therapy (1 of the following post operative day 1 or 2)	Use of IV NE for 6-24 hours Use of IV NE for > 24 hours No use of IV NE after surgery
Preoperative Glasgow Coma Score	15
APACHE II Score	Less than or equal to 17

after hospital admission. See Appendix B for formula and calculation. The score of 17 or less yielded a percent risk of death to be 19 or less for OHSPs.

The APACHE II also contains the variable of 15 minus the Glasgow Coma Scale, (see Appendix B), in the physiological measurements. The value of zero for this variable was assumed for the entire sample, as no patient with a Glasgow Coma Score of less than 15 will be selected for this study i.e., 15 minus Glasgow score of 15 equals the variable zero. This eliminated the inclusion of any emergent, unresponsive OHSPs, which could have experienced other recent complications as a result of prolonged sudden death. Please refer to the APACHE II collection tool in Appendix A.

Sampling and Selection Process

The sample population was selected from an OHSP population of a Level I hospital in Southern Arizona. The selected hospital was a regional medical center responsible for the performance of 35% of cardiac surgeries in the area. The total sample of records from 75 subjects was divided into the three groups based on the amount of NE received after surgery. Each group contained 25 individuals, as determined by a sample size power analysis, to produce a power level of 0.82.

For patient selection, a computer search of 1996/97 hospital records was completed using the appropriate diagnostic related group (DRG) codes for OHSPs. The computer records revealed a population of 987 patients between the ages of 35 and 75 who had elective open heart surgery and no pharmacy charge for the drug NE. A computer program designed for random sampling randomly selected 45 patients from this group. There were 28 complete charts available for review from the sample. None had

an APACHE II score greater than 17 or received NE therapy during their hospitalization. Manual random selection of 25 patients produced the sample population for group 1 (No NE).

A further computer search of the 1996/97 hospital records, which matched the appropriate DRG codes to a hospital pharmacy charge for NE, revealed 151 patients between the ages of 35 and 75 who had elective open-heart surgery and a pharmacy charge for NE. There were 127 complete charts available for review. Of these patients, 44 were eliminated due to procedures other than valvular repair/replacement or coronary artery bypass, death resulting from surgical complications, or lack of documented NE use. Of the remaining 77 patients, 34 met the selection criteria for group 2, 6 to 24 hours of NE infusion (<24hr NE), and 26 met the criteria for group 3, greater than 24 hours of NE infusion (>24hr NE). Of these patients, none had an APACHE II score greater than 17. Manual random selection of 25 patients for each group resulted in the sample population for groups 2 and 3. Hence, the final sample consisted of 75 patients with 25 patients in each group. Data were collected using the Data Collection Form (see Appendix A).

Data Collection

Procedures

Data for this research was obtained through retrospective, hospital chart reviews on selected patients who met the sampling criteria. This study was exempt from human subjects review, (see Appendix C), as no direct patient contact or experimental methods were used. Hospital admitting records were examined for patients' consent for

anonymous data collection from the chart. Protection of the patients' privacy included anonymous data listing and confidentiality by the researcher.

Measures

The chart reviews included data collection from the history and physical, anesthesia record, operatory report, laboratory records, physician's notes, nurse's notes, drug administration records, and nurse's flow sheets. The demographic data collection included age, sex, weight, diagnosis, surgical procedure, time on bypass, mortality and complications, (refer to Data Collection Form in Appendix A).

The research data for analysis to be collected on the Data Collection Form included preoperative complete blood count (CBC) with differential, first postoperative morning CBC with differential, and second post operative morning CBC with differential. Further data collection included length of NE infusion, dose of NE infusion, and other beta agonistic drugs, anti-inflammatory drugs, and post operative infections. See Data Collection Form in Appendix A for collection information.

Norepinephrine Dose Determination

The NE dose was recorded as a peak dose and a mean dose. The mean dose was calculated using the standard method of dosage administration of micrograms per minute and adjusting for variations in weight by dividing the recorded dose by the patients' preoperative weight in kilograms. The dosage data was recorded in one to sixty minute titrations. Each recorded dose was multiplied by the number of minutes which that dose was administered to obtain the total micrograms administered during the recorded period. The total dose administration was calculated by adding the total micrograms administered

for each titration and dividing by the total number of minutes the patient received the NE. The value was then divided by the documented preoperative weight to obtain a mean value of administration in mcg/kg/min.

Analysis

The total lymphocyte counts from the CBC with differential for each time period was calculated by multiplying the total WBC by the percentage of these cells, which were lymphocytes. Statistical analysis was conducted on the data utilizing ANOVA's Two Factor with Replication to identify differences within or between groups. Analysis of differences in the mean change in circulating lymphocyte counts between days 1, 2, and 3 between groups was done using ANOVA's Two Factor without Replication. Analysis to identify differences in preoperative APACHE II scores, age, and lymphocyte counts between groups was done using ANOVA's One Way Analysis of the variance. The probability for significance level was at $p < 0.05$.

Other data considered in the analysis included trends in lymphocyte counts without significance, documented post surgical infections prior to discharge, other complications (see Data Collection Form), and the influence of other beta agonistic drugs on the individual recipient's lymphocyte count. Possible associations between the total dose of NE and the lymphocyte count were also assessed for any obvious trends.

CHAPTER IV

Results

Sample Characteristics

Each of the three groups in this study, group 1 (No NE), group 2 (<24hr NE), and group 3 (>24hr NE) contained 25 patients which met the criteria for selection in Table 1. The demographic characteristics, APACHE II scores, and preoperative lymphocyte counts of each group are shown in Table 2. Preoperative diagnoses are shown in Figure 2. Some of the patients in each group had more than one admitting diagnosis. In this case, both diagnoses are included in the representation of each group in Figure 2. There was no significant difference between age and APACHE II scores between the groups ($p > 0.05$). There was, however, a significant difference in the male to female ratio in group 2 (<24hr NE) compared to the other groups ($p > 0.05$).

Post Operative Characteristics of Sample

The postoperative characteristics of each group are shown in Table 3 and Figure 3. There was a significant difference in the total number of ICU days between all three groups and a significant difference in total number of hospital days for group 3 (>24hr NE), ($p > 0.05$). Some patients had more than one surgical procedure or complication, and both are included in the representation of each group in the each group in the table and figure. Those patients assigned a complication for blood pressure included patients whose blood pressure was less than 90 or greater than 150 mm Hg systolic pressure despite drug therapy. Group 3 (>24hr NE) had a significantly greater number of low blood pressure complications, including shock, than the other two groups ($p > 0.05$).

Table 2.

Description of the Sample by Group

	Group 1 (No NE)	Group 2 (<24hr NE)	Group 3 (>24hr NE)	Significance
Sex				
Female	6†	2†‡	9‡	sign†‡*
Male	19	23	18	
Age				
Mean	62.5±8.4	62.6±9.9	64.8±6.8	ns*
Range	41 – 75	37 – 75	46 – 76	
APACHE II Score				
Mean	8.8±1.0	9.1±2.0	9.7±1.2	ns*
Range	7.0 – 11.0	6.0 – 16.0	7.0 – 12.0	
Preoperative Lymphocytes (cells/mcL)				
Mean	1890.8±744.8	1962.6±928.7	1744.7±525.6	ns*
Range	190.0 – 3649.0	270.0 – 4557.0	720.0 – 3171.0	

Note: Results are mean ± SD with 25 subjects in each group.

sign†‡- significant (One Way ANOVA: p value < 0.05)

ns*- not significant (Two Factor ANOVA with replication: p value > 0.05)

Figure 2. Principle Admitting Diagnosis by Group

Note: Twenty-five subjects in each group with multiple diagnoses included.
 Number in box is number of patients with diagnosis.

Table 3.

Preoperative Characteristics of the Sample by Group

	Group 1 (No NE)	Group 2 (<24hr NE)	Group 3 (>24hr NE)	Significance
Surgery				
CABG	22	21	18	ns*
MVR	3	2	3	
AVR	2	5	8	
ICU Days				
Mean	1.9±0.5†	3.2±2.4†	5.6±2.8†	sign†
Range	1.0 – 4.5	1.0 – 9.0	3.0 – 14.0	
Total Hospital Days				
Mean	6.5±2.8†	8.2±2.1†	12.6±3.8†	sign†
Range	4.0 – 19.9	6.0 – 15.3	7.0 – 19.1	
Postoperative Antibiotic Therapy (# of Days)				
Mean	2.2±0.5	2.1±0.4	2.4±1.0	ns*
Range	2.0 – 4.0	2.0 – 4.0	2.0 – 5.0	

Note: Results are mean ± SD with 25 subjects in each group.

sign†- significant (Two Factor ANOVA with replication: p value < 0.05)

ns*- not significant (One Way ANOVA: p value > 0.05)

Figure 3. Postoperative Complications Excluding Infection by Group

Note: Twenty-five subjects in each group with multiple complications included.
 Number in box is number of patients with complication.

Data collection also included the preoperative and post operative drug therapy for each patient. Patients receiving medical treatment for previous cardiac conditions may have been receiving beta-blocking agents, nonsteroidal anti-inflammatory drugs (NSAID), and steroids as well as calcium-channel blocking agents, diuretics, or nitroglycerine. The latter drugs were not included in the data collection, as there is no literature to support any role that they might play in lymphocyte regulation. The pertinent drugs for this study with the number of patients receiving each drug per group are identified in Table 4.

Norepinephrine Administration

The amounts of NE administered to patients in groups 2 and 3 are listed in Table 5. The basis for treatment with NE for patients in group 2 and group 3 was intraoperative or immediate postoperative hypotension that was unresponsive to initial documented use of dopamine. Once NE therapy was initiated, 21 patients from group 2 maintained systolic blood pressures greater than 90 mm Hg until discharged from the ICU. A significantly fewer number of patients ($p < 0.05$), in group 3, $N = 10$, were able to maintain systolic blood pressures greater than 90 mm Hg despite NE infusion. For this reason, the NE was continually increased in these patients resulting in large amounts of NE infusion. The hypotension was generally resolved by the second day post operative in this population.

Effect of Norepinephrine on Lymphocyte Counts

As stated previously, there was no significant difference in preoperative circulating lymphocyte counts between the three groups as shown in Table 2. There

Table 4.

Pre and Postoperative Drug Therapy by Group

	Group 1 (No NE)	Group 2 (<24hr NE)	Group 3 (>24hr NE)	Significance
NSAID*				
Preoperative	8	11	12	ns*
Postoperative	25	24	24	
Steroids				
Preoperative	1	3	1	ns*
Postoperative	0	0	0	
β -Blocking Agent				
Preoperative	7	9	10	ns*
Postoperative	3	3	1	

Note: Numbers are number of patients receiving drug.

ns*- no significant difference between groups (One Way ANOVA: p value > 0.05)

NSAID*- Nonsteroidal anti-inflammatory drug

Table 5.

Postoperative Norepinephrine Drug Therapy by Group

	Group 2 (<24hr NE)	Group 3 (>24hr NE)	Significance
Total Dose (mcg)			
Mean	1788±386	12196±1026	sign†
Range	806 – 2730	3124 – 1789	
Total mcg/kg			
Mean	21.1±3.2	138±10.1	sign†
Range	4.9 – 53.7	23.9 – 273.1	
Mean mcg/kg/min			
Mean	0.034±0.006	0.076±0.011	sign†
Range	0.028 – 0.070	0.031 – 0.104	

Note: Results are mean ±SD with 25 subjects in each group.

sign†- significant (Two Factor ANOVA with replication: p value < 0.05)

was an overall decrease in the mean circulating lymphocyte count in all three groups with no significant difference between groups on postoperative day 1. There was a significant difference in the mean circulating lymphocyte count on postoperative day 2 for group 3 when compared to groups 1 and 2, (see Table 6 and Figure 4). The percentage of increase in the mean circulating lymphocyte count from day 1 to day 2 was also significantly different in groups 1 and 2 when compared to group 3, (see Figure 5).

Effect of Preoperative Beta-Blocking Agents on Lymphocyte Counts

There was no significant difference between preoperative and postoperative lymphocyte counts within the groups when comparing those who received preoperative beta-blocking agents to those who did not, (see Figures 6, 7, & 8). However, there appeared to be some pattern in group 2 (<24 hr NE) and group 3 (>24hr NE) on postoperative day 1 when comparing the patients within these groups. There was a lesser decrease on day 1 in the mean circulating lymphocyte counts in the beta-blocking agent recipients of both groups, although not significant ($p > 0.05$). The statistical analysis was done using a Two Factor ANOVA without replication due to an unequal number of patients receiving beta-blocking agents compared to those who did not.

When taking in to consideration the effects of NE on both migration and proliferation, further investigation of the actual change in lymphocyte counts was conducted. Although the actual mean lymphocyte counts revealed no significant difference between beta-blocking agent and non beta-blocking agent recipients ($p > 0.05$), there was a significant difference in the percent change in the lymphocyte counts both within and between groups ($p < 0.05$). Patients in group 1 (No NE), revealed no

Table 6.

Mean Circulating Lymphocyte Count by Group

	Group 1 (No NE)	Group 2 (<24hr NE)	Group 3 (>24hr NE)	Significance
Preoperative (cells/mcL)				
Mean	1890.8±744.8	1962.6±928.7	1744.7±525.6	ns*
Range	190.0 – 3649.0	270.0 – 4557.0	720.0 – 3171.0	
Day 1 (cells/mcL)				
Mean	631.7±258.9	844.9±321.5	642.0±214.7	ns*
Range	65.0 – 1001.0	0.0 – 1450.0	0.0 – 1161.0	
Day 2 (cells/mcL)				
Mean	1126.1±328.5†	1178.4±341.0‡	718.8±198.9†‡	sign†‡
Range	328.0 – 2550.0	0.0 – 2770.0	0.0 – 1161.0	

Note: Results are mean ±SD with 25 subjects in each group.

ns*- not significant (Two Factor ANOVA with replication: p value > 0.05)

sign†‡- significant (Two Factor ANOVA with replication: p value < 0.05)

Figure 4. Mean Circulating Lymphocyte Count by Group

Note: Twenty-five subjects in each group.

*Significant Difference (Two Factor ANOVA with replication: p value <0.05)

Figure 5. Mean % Increase in Circulating Lymphocytes from Postoperative Day 1 to Day 2 by Group

Note: Twenty-five subjects in each group.

*Significant difference (Two Factor ANOVA with replication: p value <0.05)

Figure 6. Mean Circulating in Lymphocyte Counts in Group 1 Subjects with and without Preoperative β -Blocking Agent

Note: No significant difference (Two Factor ANOVA without replication: p value >0.05)

N= number of patients with or without β -Blocking agent.

Figure 7. Mean Circulating Lymphocyte Counts in Group 2 Subjects with and without Preoperative β -Blocking Agent

Note: No significant difference (Two Factor ANOVA without replication: p value >0.05)
N= number of patients with or without β -Blocking agent.

Figure 8. Mean Circulating in Lymphocyte Counts in Group 3 Subjects with and without Preoperative β -Blocking Agent

Note: No significant difference (Two Factor ANOVA without replication: p value (>0.05))
N= number of patients with or without β -Blocking agent.

significant difference within the group when comparing beta-blocking agent recipients to non beta-blocking agent recipients ($p > 0.05$). However, there was a significant difference within both NE groups in the percent increase in the mean lymphocyte counts from day 1 to day 2 (see Table 7, $p < 0.05$). Those who did not receive beta-blocking agents preoperatively had a significantly greater percent increase in the mean lymphocyte counts in both groups. The beta-blocking agent recipients in group 2 (<24 hr NE), had a minimal percent increase in the mean lymphocyte count while group 3 (>24 hr NE), had an actual decrease in the mean lymphocyte count. There was also a significant difference between both NE groups and the No NE group ($p < 0.05$), when comparing this change.

Effects of Extraneous Variables

Since there was no significant variation of NSAID, steroid, or postoperative beta-blocking agent use among the groups (One Way ANOVA, $p > 0.05$), the data was not presented. Additionally, there was no significant difference within or between the groups in lymphocyte counts when comparing the preoperative diagnosis of CHF to other diagnoses (One Way ANOVA, $p > 0.05$), therefore, these data were also not presented.

Effects of Norepinephrine on Infection

There was a significant difference (One Way ANOVA, $p < 0.05$) in documented postoperative infection between the group 3 (>24hr NE) and the other two groups, (see Figure 9). Postoperative infection was identified by a positive laboratory culture for bacterial presence within 5 days of surgery and documentation by the physician in the progress notes. The timeframe for infection was limited to allow for the earlier discharge time of the No NE group and other infectious exposure besides surgery.

Table 7.

Mean % Change in Circulating Lymphocytes from Postoperative Day 1 to Day 2 with and without β -Blocking Agent by Group

	Group 1 (No NE)	Group 2 (<24hr NE)	Group 3 (>24hr NE)
No β -Blocking Agent	45 $\pm$ 21 (N=18)	40 $\pm$ 17 [†] (N=16)	37 $\pm$ 17 [‡] (N=15)
Preoperative β -Blocking Agent	83 $\pm$ 39 ^{¶¶} (N=7)	11 $\pm$ 3 ^{†¶} (N=9)	-16 $\pm$ 4 ^{‡¶} (N=10)

Note: Results are mean percent $\pm$ SD.

N= number of subjects on drug.

^{†‡}- significant difference within groups (Two Factor ANOVA without replication: p value < 0.05)

^{¶¶}- significant difference between groups (Two Factor ANOVA without replication: p value < 0.05)

Figure 9. Number of Postoperative Infections by Group

Note: *Significant difference (One Way ANOVA: p value <0.05)

CHAPTER V

Discussion

Summary of Findings

The findings of this study are consistent with the hypothesis that NE infusion can alter the circulating lymphocyte counts of OHSPs. The results suggest beta-blocking drugs given preoperatively may affect both endogenous and exogenous regulation of lymphocytes. Also, these findings are consistent with increased infections in this population with NE infusion. However, the findings related to NE and lymphocyte counts causing a direct effect on increased infection rates in group 3 are not conclusive due to differences in linked variables in group 3 compared to the other two groups, namely, sex, low blood pressure complications, and ICU days.

Linked Variables

The extraneous variable which may have affected the results were attempted to be controlled by following the selection criteria in Table 1 and prescreening all subjects with the APACHE II tool (see Appendix A). However, other differences in the sample characteristics of each group may have affected some results. The fewer number of females in group 2 (<24hr NE) may have affect the mean lymphocyte counts in this group compared to the other two groups. Although there is no difference in the documented standard lymphocyte counts between men and women, there may be some differences in the immune reaction process between the sexes since hormones affect production, storage, and release of lymphocytes (Fischbach, 1995).

There was also a minor trend in the mean age and APACHE II score of the

groups, with group 3 (>24hr NE) being slightly higher in both areas than the other two groups, (see Table 2). The higher risk associated with age and APACHE II score may have contributed to both the increased rate of infection and the need for NE therapy. NE therapy was initiated in groups 2 and 3 for systolic blood pressures less than 90 mm Hg. While most of the patients in group 2, N = 21, were consistently maintained with a systolic blood pressure greater than 90 mm Hg while on NE, group 3 had a significantly fewer number of patients with this therapeutic response to the drug, N = 10. The remaining 15 patients exhibited hypotension which may have put them at a higher risk for infection due to decreased perfusion of immune and vital organs as well as surgical areas. Additionally, this hypotension may have caused the release of increased levels of endogenous NE and cytokines which may have affected lymphocyte counts.

The postoperative characteristics revealed a significant difference in both total ICU days and total hospital days. Since all documented infections were recorded by the sixth postoperative day, it is doubtful that the increased number of total hospital days contributed to a higher infection rate of group 3 (>24hr NE). However, an increased number of ICU days has been linked to higher infection rates (Nicholls, 1997) and may have had some influence within this group.

Since DM is also a factor in infection rates (Alexiewicz, Smorgorzewski, Kumar, & Massry, 1997), this preoperative diagnosis should be considered. However, the number of DM patients in all three groups was similar decreasing the likelihood of influence on results. The number of patients with preoperative CHF was also closely related between groups, limiting the effect of this diagnosis on results as well, (see Figure 2).

Possible Explanations

Differences in Lymphocyte Counts

The mean circulating lymphocyte count of all three groups was lower on both postoperative day 1 and day 2 than the preoperative value. This could be related to the postoperative inflammation process causing a migration of immune cells to the surgical sites and surrounding tissue (Quinn & Shannon, 1975) as well as increased cortisol levels from surgical stress causing a reduction in lymphocyte production (Smiley & Vulliemoz, 1992). The mean day 2 lymphocyte count was relatively higher in all three groups when compared to day 1, (see Figure 4). This relative increase in circulating lymphocytes may be explained by two distinct events:

- 1) An overall increase in the total number of lymphocytes due to proliferation
- 2) An influx of lymphocytes back into circulation from the tissue via lymph vessels after migration to the lymph nodes (Quinn & Shannon, 1975).

The mean percent increase in circulating lymphocytes from day 1 to day 2 was lower in both of the NE groups than the No NE group with group 3 (>24hr NE) being significantly lower than both groups, (see Figure 5). Since NE negatively regulates both proliferation and migration of lymphocytes, the administration of exogenous NE may have contributed to a decrease in these activities in both NE groups. The significant difference in group 3 (>24hr NE) could be due to the significantly higher dosage of NE used in this group along with the increased time that the drug was used.

Differences in Beta-Blocking Agent Recipients

When comparing the patients receiving beta-blocking agents preoperatively to those who did not, the trend in all three groups can be seen in Table 7. All patients receiving beta-blocking agents preoperatively had a significant difference in the percent change in circulating lymphocytes from day 1 to day 2 within the group when compared to the nonbeta-blocking agent patients. This smaller percent change was mainly caused by a higher mean circulating lymphocyte count on day 1 in the beta-blocking agent patients of each group, (See Figures 6, 7, & 8). This resulted in a relatively smaller increase in the mean circulating lymphocyte count of beta-blocking agent recipients in group 1 and 2 and an actual decrease in the mean lymphocyte count of beta-blocking agent recipients in group 3 on day 2.

Clinical studies have shown that patients receiving beta-blocking drugs up regulate the beta-receptors on their lymphocytes within five days of taking the drug, (see Figure 1). The higher mean lymphocyte count on day 1 in the patients receiving beta-blocking drugs in all three groups might possibly be related to a greater inhibition of migration to the surgical sites by endogenous NE in these patients due to a greater number of beta-receptors on the lymphocytes. The significant difference in the percent increase between the three groups might also be related to the addition of exogenous NE and the length of time for administration.

Prevention of Infection

The significantly higher rate of infection among patients in group 3 (>24hr NE), (see Figure 9), may have a positive correlation to the lower lymphocyte counts in these

patients. All of the patients in group 3 with a documented postoperative infection, $N = 20$, received only two days of antibiotic therapy postoperatively, (see Table 3). However, three of the patients in group 3, who had no documented postoperative infection before discharge from the hospital, received four or more days of prophylactic antibiotic therapy postoperatively. This intervention may have influenced the results.

Other variables, which may have prevented postoperative infection in group 3, may be related to NE dose, lymphocyte count, and ICU days as seen in the remainder of the patients in group 3 without documented postoperative infection. One of these patients received the smallest dose of NE in mcg/kg/min among the patients in this group, dose = 0.031, (see Table 5). The patients circulating lymphocyte count on day 2 was similar to the mean of the other two groups, circulating lymphocytes = 1182, (see Table 6). The last patient in group 3 with no documented postoperative infection received only 0.042 mcg/kg/min of NE and had a lymphocyte count on day 2 greater than the mean within the group, lymphocyte count = 820, (see Table 6). This patient also had no postoperative complications, and the least number of ICU days, $N = 3$, and hospital days, $N = 7$, of all the patients in group 3, (see Table 3).

Implications for Nursing

Applications of this research would require more in depth research to isolate the effects of NE dose, decreased blood pressure, and number of ICU days on lymphocyte counts and infection rates in OHSPs. However, the ICU nurse might positively affect the outcome of OHSPs by closely monitoring changes in lymphocyte counts and antibiotic therapy orders of patients exhibiting a profile of increased risk for infection. This study

has been beneficial in the identification of one such patient profile. Patients requiring IV NE doses of greater than 0.028 mcg/kg/min for more than 24 hours due to hypotension after open-heart surgery may indeed be at a higher risk for infection as determined by this study. When applying this study to the assessment of such a patient, the nurse may wish to consider a total circulating lymphocyte count of less than 1100 on postoperative day 2, (see Table 6) as an indicator of the increased risk of infection. Additionally, preoperative use of beta-blocking agents may also be considered in the patient with this profile. The ICU nurse may then discuss with the physician extending the prophylactic antibiotic therapy and initiate strict infection monitoring and precautions.

Limitations

Since the sample size was limited, $N = 75$, and the data were collected from only one hospital, caution should be taken before applying these results to all patients. Other limitations to this study include both the variation in preoperative differential counts and the inability to measure or control endogenous NE levels in these patients.

Methods of Differential Count

Two different methods were used for the preoperative lymphocyte counts. These were manual counts and automated counts. The method used depended on the laboratory site at which the CBC was performed. The onsite hospital laboratory routinely performs automated lymphocyte counts for presurgical screening. However, outside laboratories may perform either method depending on their resources. The automated lymphocyte count identifies cells based on appearance and size. This eliminates immature cells from inclusion into the total count. The manual count depends on microscopic examination of

the cells by the human eye. Although a general appearance and size are required for the identification of each cell type, the variance between laboratory sites and staff decreases the standardization of each count.

The variation in the methods of preoperative lymphocyte counts resulted in the inability to perform statistical analysis on premature lymphocyte in selected preoperative diagnoses. The postoperative lymphocyte counts were all done using the manual method for differentials, however, cells not meeting the criteria in size, shape, or granulation for classification in a WBC category were identified only as atypical cells in most cases. This also eliminated the ability for statistical analysis of premature cells among groups.

Endogenous NE

Since the amount of endogenous NE produced by each individual in the groups cannot be controlled or estimated, there was a wide range in the individual lymphocyte values in each group. However, the significant difference in both the total circulating lymphocyte count and the percent change of group 3 was identified using an ANOVA: Two factor with replication indicating a pattern of change for individuals in group 3 when compared to the other two groups. Therefore, the administration of the exogenous NE more than likely contributed to the inhibition of the lymphocytes.

Future Research Suggestions

Further research should be done to confirm this study using several hospitals in different regions for data collection and including a larger patient population. Additional research should be done limited to the effects of beta blocking agent therapy on lymphocyte counts of OHSPs receiving NE therapy with a larger population of patients.

If possible, the extraneous variables can be limited by comparing lymphocyte counts in only same sex patients. A comparison of infection rates based on therapeutic responses, as measured by blood pressure, to similar doses of NE should also be studied. In addition, infection rates among OHSPs receiving NE therapy should be assessed in groups of patients with similar numbers of ICU days. An individual unit based data collection of lymphocyte trends in patients on NE therapy may also give the ICU nurse at a given hospital an indication of the application of this research to their institution. This research along with future studies may help decrease the incidence of infection in OHSP receiving IV administration of NE.

APPENDIX A

Data Collection Tools

Data Collection Form

Demographics

Sex:	M	F		Patient#	Age	Weight
Group#	1	2	3	Diagnosis		APACHE II Score

Surgical Info

Procedure	CABG	MVR	AVR	TVR	Repair ASD
Bypass Time	>4hr	<4hr	<3hr	<2hr	at least 1hr

Outcome

#ICU days	<2	3	4	5	>6 days
Postop day					
Discharge	<6	7	8	9	>10
or Death	<1	2	3	4	5
	7	8	9	10	>10
Complications	Arrhy-	Hemo-	Pulm-	Coag-	Allergic
Shock	thmia	dynamic	onary	ulation	reaction

Postoperative Infection

Site:	Respiratory	Incisional	Urinary	Systemic
Source:				
Organism:				

Preop Meds

Beta specific:			
Name	Daily Dose	Length of Tx	

Anti-inflammatory:

Name	Daily Dose	Length of Tx

Time interval for NE Infusion

0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	hr
15	16	17	18	19	20	21	22	23	24	25	26	27	29	>30	

Peak dose:

Mean dose:

Total dose:

Complete Blood Count

Postop time	RBC	WBC	Neutro-	Eosino-	Baso-	Lympho-	Mono-
day			phils	phils	phils	cytes	cytes

0

1

2

APACHE II Score Collection and Calculation Sheet

A.

Variable	High abnormal				Low Abnormal				
	4+	3+	2+	1+	0	1+	2+	3+	4+
Temp.	>41	39- 40.9		38.5- 38.9	36- 38.4	34- 35.9	32- 33.9	30- 31.9	<30
MAP	>160	130- 159	110- 129		70- 109		50- 69		<49
HR	>180	140- 179	110- 139		70- 109		55- 69	40- 54	<39
RR	>50	35- 49		25- 34	12- 24	10- 11	6- 9		<5
Fio2>.5	>500	350- 499	200- 349		<200				
A-aDO2									
Fio2<.5					>70	61- 70		55- 60	<55
PaO2									
pH	>7.7	7.6- 7.69		7.5- 7.59	7.33 7.49		7.25 7.32	7.15 7.24	<7.15
Na	>180	160- 179	155- 159	150- 154	130- 149		120- 129	111- 119	<110
K+	>7	6- 6.9		5.5- 5.9	3.5- 5.4	3- 3.4	2.5- 2.9		<2.5
Creat	>3.5	2- 3.4	1.5- 1.9		0.6- 1.4		<0.6		
x2 for ARF									
Hct	>60		50- 59.9	46- 49.9	30- 45.9		20- 29.9		<20
WBC	>40		20- 39.9	15- 19.9	3- 14.9		1- 2.9		<1
Glascow 15-score									

Total Acute Physiology
Score

B. Age Points:

<44	0
45-54	2
55-64	3
65-74	5
>74	6

C. Chronic Health Points: If a person has a hx of severe organ system insufficiency or is immuno-compromised assign points as follows:

- a. for nonoperative or emergency postoperative pts. 5 points
b. for elective postoperative pts. 2 points

APACHE II SCORE =

Sum of A + B + C

APPENDIX B

Formulas

APACHE II Score Risk Calculation

Equation for Computation of the Risk of Hospital Death

Where R equals percent risk of death

$$\begin{aligned}\ln(R/1-R) = & -3.517 + (\text{APACHE II score} \times 0.146) \\ & + (0.603 \text{ if post emergency surgery}) \\ & + (\text{Diagnostic category weight below})\end{aligned}$$

Principle Diagnostic Category Leading to Postoperative ICU Admission

<u>System</u>	<u>Weight</u>
Neurological	-1.150
Cardiovascular	-0.797
Respiratory	-0.610
Gastrointestinal	-0.613
Metabolic/renal	-0.196

(Knaus et al, 1985).

For example: A patient admitted to the cardiovascular intensive care unit after open heart surgery (nonemergent) having an APACHE II score of 17 would have the following predicted death risk:

$$\begin{aligned}\ln(R/1-R) = & -3.517 + (17 \times 0.146) \\ & + (0 \times 0.603) - 0.797 \\ = & -3.517 + 2.482 + 0 - 0.797 \\ = & -1.832\end{aligned}$$

Since the inverse ln of -1.832 is 0.1601 then solving for (R/1-R) gives the predicted risk of death to be 0.19 or 19%.

Calculating the Glasgow Coma Score

<u>Best Verbal Response</u>		<u>Best Motor Response</u>		<u>Eye Opening</u>	
Oriented	5	Spontaneous	6	Spontaneous	4
Confused	4	Obeys Commands	5	To Voice	3
Inappropriate	3	Localizes Pain	4	To Pain	2
Incomprehensible	2	Flexion	3	None	1
None	1	Extension	2		
		None	1		

Choose best response from each category

Score is the total of all three categories.

(Teasdale & Jennett, 1974)

APPENDIX C

Approval for Research

Human Subjects Committee

1622 E. Mabel St.
P.O. Box 245137
Tucson, Arizona 85724
(520) 626-6721

14 July 1997

Wanda N. Zziwambazza, RN, BSN
c/o Carrie Merkle, Ph.D.
College of Nursing
PO BOX 210203

RE: THE EFFECTS OF NOREPINEPHRINE INFUSION ON THE CIRCULATING
LYMPHOCYTE COUNTS OF POST OPEN HEART SURGERY PATIENTS

Dear Ms. Zziwambazza:

We have received documents concerning your above cited project. Regulations published by the U.S. Department of Health and Human Services [45 CFR Part 46.101(b) (4)] exempt this type of research from review by our Committee.

Thank you for informing us of your work. If you have any questions concerning the above, please contact this office.

Sincerely yours,

A handwritten signature in cursive script that reads "William F. Denny".

William F Denny, M.D.
Chairman
Human Subjects Committee

WFD:js
cc: Departmental/College Review Committee

Works Cited

- Aarons, R., Nies, A., Gal, J., Hegstrand, L., & Molinoff, P. (1980). Elevation of beta-adrenergic receptor density in human lymphocytes after propranolol administration. Journal of Clinical Investigation, 65, 949-957.
- Alexiewicz, J., Smogorzewski, M., Kumar, D., & Massry, S. (1997). Elevated cytosolic calcium and impaired proliferation of B lymphocytes in type II DM. American Journal of Kidney Disease, 30, 98-104.
- Angle, M., Molloy, D., Penner, B., Jones, D., & Prewitt, R. (1989). The cardiopulmonary and renal hemodynamic effects of norepinephrine in canine pulmonary embolism. Chest, 95, 1333-1337.
- Bellinger, D., Ackerman, K., Felten, S., & Felten, D. (1992). A longitudinal study of age-related loss of noradrenergic nerves and lymphoid cells in rat spleen. Experimental Neurology, 116, 295-311.
- Blecha, F., Barry, R., & Kelley, K. (1982). Stress-induced alterations in delayed-type hypersensitivity to SRBC and contact sensitivity to DNFB in mice. Proc. Social and Experimental Biology in Medicine, 169, 239-246.
- Bognar, I., Albrecht, S., Farasaty, M., & Fuder, H. (1995). Inhibition by interleukin-1 beta of noradrenaline release in rat spleen: Involvement of lymphocytes, NO and opiod receptors. Naunyn-Schmeideberg's Archives of Pharmacology, 351, 433-438.
- Bovbjerg, D. H., Ader, R., Cohen, N. (1982). Behaviorally conditioned suppression of a graft-versus-host response. Proc. of the National Academy of Science USA, 79, 583-585.
- Brenner, I., Zamecnik, J., Shek, P., & Shephard, R. (1997). The impact of heat exposure and repeated exercise on circulating stress hormones. European Journal of Applied Physiology and Occupational Physiology, 76, 445-454.
- Calvo, W. (1968) The innervation of bone marrow in laboratory animals. American Journal of Anatomy, 123, 315-328.
- Carlson, S., Brooks, W., & Roszman, T. (1989). Neurotransmitter-lymphocyte interactions: dual receptor modulation of lymphocyte proliferation and cAMP production. Journal of Neuroimmunology, 24, 155-162.
- Carlson, S., Trauth, K., Brooks, W., & Roszman, T. (1994). Enhancement of β adrenergic cAMP in activated T-cells. Journal of Cellular Physiology, 161, 39-48.

Carr, J., Ortiz, K., Paxton, L., Saland, L., & Savage, D. (1993). Alterations in spleen norepinephrine and lymphocyte [H]Dihydroalprenolol binding site number in genetically epilepsy-prone rats. Brain, Behavior, and Immunity, 7, 113-120.

Cohen, S., Tyrrell, D. A. J., & Smith, A. P. (1991). Psychological stress and susceptibility to the common cold. New England Journal of Medicine, 325, 606-612.

De Backer, W., Amsel, B., Jorens, P., Bossaert, L., Hiemstra, P., van Noort, P., & van Overveld, F. (1996). N-acetylcysteine pretreatment of cardiac surgery patients influences plasma neutrophil elastase and neutrophil influx in bronchoalveolar lavage fluid. Intensive Care Medicine, 22, 900-908.

del Rey, A., Besedovsky, H., Sorkin, E., Prada, M., Bondiolotti, P. (1982). Sympathetic immunoregulation: Difference between high and low-responder animals. American Journal of Physiology, 241, R30-R35.

del Rey, A., Besedovsky, H., Sorkin, E., Prada, M., & Arrenbrecht, S. (1981). Immunoregulation mediated by the sympathetic nervous system, II. Journal of Cellular Immunology, 63, 329-334.

Felten, D., Felten, S., Bellinger, D., & Madden, K. (1993). Fundamental aspects of neural immune signaling. Psychotherapy and Psychosomatics, 60, 46-56.

Folch, H. & Waksman, B. H. (1974). The splenic suppressor cell I. Activity of thymus-dependent adherent cells: Changes with age and stress. Journal of Immunology, 113, 127-139.

Harris, T., Waltman, T., Carter, S., & Maisel, A. (1995). Effects of prolonged catecholamine infusion on immunoregulatory function: Implications in congestive heart failure. Journal of the American College of Cardiology, 26(1), 102-109.

Keller, S. E., Weiss, J. M., Schleiffer, S. J., Miller, N. E., & Stein, M. (1981). Suppression of immunity by stress effect of graded series of stressors on lymphocyte stimulation in the rat. Science, 213, 1397-1400.

Khan, M., Sansoni, P., Silverman, E., Engleman, E., & Melmon, K. (1986). Beta-adrenergic receptors on human suppressor, helper, and cytolytic lymphocytes. Biochemical Pharmacology, 35, 1137-1142.

Kiecolt-Glaser, J.K., Glaser, R., Gravenstein, S., Malarkey, W.B., & Sheridan, J. (1996). Chronic stress alters the immune response to influenza virus vaccine in older adults. Proceedings of the National Academy of Sciences of the United States of America, 97, 3043-3047.

Knaus, W., Draper, E., Wagner, D., & Zimmerman, J. (1985). APACHE II: A severity of disease classification system. Critical Care Medicine, 13, 818-829.

Koch, T., Heller, S., van Ackern, K., Schiefer, H., & Neuhof, H. (1996). Impairment of bacterial clearance induced by norepinephrine infusion in rabbits. Intensive Care Medicine, 22, 637-643.

Krawietz, W., Werdan, K., Schober, M., Erdmann, E., Rindfleisch, G.E., & Hannig, K. (1982). Different numbers of beta-receptors in human lymphocyte subpopulations. Biochemical Pharmacology, 31, 133-136.

Lahat, N., Shtiller, R., Zlotnick, A., & Merin, G. (1993). Early IL-2/sIL-2R surge following surgery leads to temporary immune refractoriness. Clinical & Experimental Immunology, 92, 482-486.

Madden, K. S., Felten, S. Y., Felten, D. L., Hardy, C. A., & Livnat, S. (1993). Sympathetic nervous system modulation of the immune system. II. Induction of lymphocyte proliferation and migration in vivo by chemical sympathectomy. Journal of Neuroimmunology, 49, 67-75.

Maestroni, G. & Conti, A. (1993). Noradrenergic modulation of lymphohematopoiesis. International Journal of Immunopharmacology, 16(2), 117-122.

Malarkey, W.B., Pearl, D.K., Demers, L.M., Kiecolt-Glaser, J.K., & Glaser, R. (1995). Influence of academic stress and season on 24-hour mean concentrations of ACTH, cortisol, and beta-endorphins. Psychoneuroendocrinology, 20, 499-508.

Meakins, J.L. (1991). Surgeons, surgery, and immunomodulation. Archives of Surgery, 126, 494-498.

Meyer, J., Booke, M., Waurick, R., Prien, T., & Van Aken, H. (1996). Nitric oxide synthase inhibition restores vasopressor effects of norepinephrine in ovine hyperdynamic sepsis. Anesthesia and Analgesia, 83, 1009-1013.

Mills, P., Ziegler, M. Patterson, T., Dimsdale, J., Hauger, R., Irwin, M., & Grant, I. (1997). Plasma catecholamine and lymphocyte beta 2-adrenergic receptor alterations in elderly Alzheimer caregivers under stress. Psychosomatic Medicine, 59, 251-256.

Moro, M., Carrieri, M., Tozzi, A., Lana, S., & Greco, D. (1996). Risk factors for surgical wound infections in clean surgery: A multicenter study. Annali Italiani di Chirurgia, 67, 13-19.

Mossad, S., Serkey, J., Longworth, D., Cosgrove, D., & Gordon, S. (1997).

Coagulase-negative staphylococcal sternal wound infections after open heart operations. Annals of Thoracic Surgery, 63, 395-401.

Murray, D., Irwin, M., Reardon, C., Ziegler, M., Motulsky, H., & Maisel, A. (1992). Sympathetic and immune interaction during dynamic exercise: Mediation via a beta-adrenergic independent mechanism. Circulation, 86, 203-213.

Nicholls, T. (1997). Nosocomial infection in Auckland Healthcare Hospitals. New Zealand Medical Journal, 110, 314-316.

Plunkett, J., Reeves, J., Ngo, L., Bellows, W., Shafer, S., Roach, G., Howse, J., Herskowitz, A., & Mangano, D. (1997). Urine and plasma catecholamine and cortisol concentrations after myocardial revascularization. Modulation by continuous sedation. Multicenter study of Perioperative Ischemia (McSPI) Research Group and the Ischemia Research and Education Foundation (IREF). Anesthesiology, 86, 785-796.

Pochet, R., Delespesse, G., Gausset, P., & Collet, H. (1979). Distribution of beta-adrenergic receptors on human lymphocyte subpopulations. Clinical and Experimental Immunology, 38, 578-584.

Purebi, H. (1987). Differential effects of isoflurane on regional right and left ventricular performances and/or coronary, systemic, and pulmonary hemodynamics in the dog. Anesthesiology, 66, 262-272.

Quigley, R., Caplan, M., Perkins, J., Arentzon, C., Alexander, J., Kuehn, B., Hoff, W., & Wallock, M. (1995) Cardiopulmonary bypass with adequate flow and perfusion pressures prevents endotoxaemia and pathologic cytokine production. Perfusion, 10, 127-131.

Quin, J. & Shannon, A. (1975). The effect of anesthesia and surgery on lymph flow, protein, and leukocyte concentration in lymph of the sheep. Lymphology, 8(4), 126-135.

Raner, C., Biber, B., Henriksson, B., Lundberg, J., Martner, J., & Winso, O. (1995). Are the cardiovascular actions of dopamine altered by isoflurane? Acta Anaesthesiologica Scandinavica, 39, 678-684.

Rem, J., Brandt, M., & Kehlet, H. (1980). Prevention of postoperative lymphopenia and granulocytosis by epidural analgesia. Lancet, 9, 283-285.

Richer, M., Robert, S., & Lebel, M. (1996). Renal hemodynamics during norepinephrine and low-dose dopamine infusions in man. Critical Care Medicine, 24, 1150-1156.

Sakai, H., Mori, K., Suzuki, K., Katayama, Y., & Matsuyama, T. (1994). The clinical significance of interleukin-6 as an inflammatory marker (the studies in patients with open heart surgery). Rinsho-Byori-Japanese Journal of Clinical Pathology, 42, 1151-1157.

Schleifer, S. J., Keller, S. E., Camerino, M., Thornton, J. C., & Stein, M. (1983). Suppression of lymphocyte stimulation following bereavement. Journal of the American Medical Association, 250, 374-377.

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

Smiley, R. & Vulliemoz, Y. (1992). Cardiac surgery causes desensitization of the beta-adrenergic receptor system of human lymphocytes. Anesthesia Analg, 74, 212-218.

Smith, C., Edwards, E., Gowert, D., Ferguson, B., & Williams, C. (1992). Leukocyte migration: effects of in vitro exposure of anaesthetic agents: possible potentiation effects by adrenaline. European Journal of Anaesthesiology, 9, 463-472.

Teasdale, G. & Jennet, B. (1974). Assessment of coma and impaired consciousness. Lancet, 2, 81-84.

Tennesen, E., Christensen, N., & Brinklov, M. (1987). Natural killer cell activity during cortisol and adrenaline infusion in healthy volunteers. European Journal of Clinical Investigation, 17, 497-503.

Toft, P., Svendsen, P., Tonnesen, E., Rasmussen, J., & Christensen, N. (1993). Acta Anaesthesiology Scandinavica, 37, 245-249.

Tuman, K., McCarthy, R., O'Connor, C., Holm, W., & Ivankovich, A. (1995). Angiotensin-converting enzyme inhibitors increase vasoconstrictor requirements after cardiopulmonary bypass. Anesthesia and Analgesia, 80, 473-479.

VandenBergh, M., Kluytmans, J., van Hout, B., Maat, A., Seerden, R., McDonnel, J., & Verbrugh, H. (1996). Cost-effectiveness of perioperative mupiricin nasal ointment in cardiothoracic surgery. Infection Control and Hospital Epidemiology, 17, 786-792.

Weil, A. (1995). Spontaneous healing: How to discover and enhance your body's natural ability to maintain and heal itself. New York: Knopf.

White, M. & Leenen, F. (1997). Effects of age on cardiovascular responses to adrenaline in man. British Journal of Clinical Pharmacology, 43, 407-414.