EFFECTS OF GLUCOCORTICOIDS ON CANINE MONONUCLEAR PHAGOCYTES

by

Linda Joan DeBowes

D.V.M., Washington State University, 1981

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Surgery and Medicine

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1985

Approved by:

Neil V. Anderson

Co-Major Professor

Co-Major Professor

TABLE OF CONTENTS

LIST OF TABLES

ACKNOWLEDGEMENTS

I would like to express my most sincere thanks and appreciation to my co-major professors, Dr. Neil Anderson and Dr. Jacob Mosier and the other committee members, Dr. Kerry Keeton and Dr. Dan Upson for their guidance in the development and execution of my research project. I especially would like to acknowledge and thank Dr. Neil Anderson who gave generously of his time and was always available and willing to provide guidance and encouragement through every aspect of the project.

I would like to give my heartfelt thanks to my husband, Rick, for his constant love, patience, support and faith in me.

Lori Scimeca and Carol King, from Dr. Anderson's lab, provided valuable assistance in the collection and processing of the samples and their help was greatly appreciated. I would also like to acknowledge the assistance provided by the student workers in Dr. Anderson's lab.

Dr. Mike Clem assisted in the surgical procedures required for sample collection and I would like to express my sincere thanks for his help.

Dr. Dayton and Dr. Boyer provided the statistical analysis of the data.

Funds for this project were provided by the Squibb Company.

CHAPTER I

Introduction

Cells of the mononuclear phagocyte system include blood monocytes and tissue macrophages. These cells function in the development of both humoral and cell mediated immunity and also serve as one of the major phagocytic cell systems of the body.

Glucocorticoids and their effect on cells of the mononuclear phagocyte system have been studied in species other than the canine. Glucocorticoids are used in canine medicine for the treatment of a number of disorders including allergic disorders, immune-mediated diseases, neoplasms and shock.

The objectives of this project were to evaluate and partially characterize blood monocytes, alveolar macrophages and peritoneal macrophages: 1) in normal dogs, 2) in the same dogs following a shock dose of glucocorticoids and 3) in the same dogs following immunosuppressive doses of glucocorticoids.

CHAPTER II

Literature Review

The mononuclear phagocyte system, composed of blood monocytes and tissue macrophages, is one of the major immune defense systems of the body. Macrophages have phagocytic capability (immune- and nonimmune-mediated) and are intimately involved with induction of the immune response (humoral and cell mediated).[1,2,3]

Genesis, Morphology and Function

All the formed elements of the blood are derived from a common pluripotential stem cell in the bone marrow, which gives rise to myeloid and lymphoid stem cells.[4] The myeloid stem cells differentiate further into erythroid precursors, megakaryocyte precursors and colony-forming units-granulocyte, monocyte (CFU-G, M). The CFU-G, M gives rise to the granulocytic and monocytic series. Granulocyte and monocyte colonies require a hormone, referred to as colony-stimulating factor (CSF), to grow in culture media.[4] The primary sources of human CSF are blood monocytes, tissue macrophages and activated T lymphocytes.[4] Stimulation of T lymphocytes by lectins or antigens and stimulation of circulating monocytes and tissue macrophages with a number of materials such as endotoxin may result in release by these cells of an increased amount of CSF, which may cause an increased proliferation of monocyte precursors.[4] Monocytes and macrophages produce prostaglandins of the E series that have a direct inhibitory effect on hematopoietic colony-forming cells committed to macrophage differentiation.[5] Low concentrations of prostaglandin E_2 (PGE_2) selectively suppress macrophage colony formation without altering granulocyte colony

formation. If macrophages are exposed to high levels of CSF, they increase their PGE_2 synthesis which then may act as a negative feedback mechanism to limit excessive monocyte production. Granulocytes produce lactoferrin, which supresses CSF production by mononuclear phagocytes.[5] Thus, monocyte production is regulated by a number of factors, including CSF which stimulates production, and by PGE_2 which directly inhibits production, and by lactoferrin which indirectly inhibits production by suppressing CSF production.[4,5] There probably are additional as yet unidentified factors which also play a role in regulation of monocyte and neutrophil production.

Morphologic and functional characteristics of monocytes and macrophages can be identified in vitro by nonspecific esterase stains, phagocytosis of heat-killed Candida albicans and the formation of rosettes with sensitized sheep red blood cells.[6,7,8] Over fifty products have been identified as being secreted from macrophages, including components of the clotting system and of the complement system; lysozyme, plasminogen activator, elastases, collagenases, interleukin-1 (IL-1), interferons, fibronectin and prostaglandin E_2.[1,3,9] Depending upon the state of macrophage activation and the products they release, macrophages can function in a variety of different capacities in the inflammatory response and in regulation of cellular function.[1] Substances that are chemotactic for macrophages include C5a (anaphylatoxin), bacterial products, products from stimulated B and T lymphocytes, fragments of collagen and elastic, and denatured proteins.[1] Activated T cells produce macrophage chemotactic factor (MCF), specific macrophage-arming factor (SMAF) and macrophage stimulating factor (MSF).[10] Lymphokines (macrophage migration inhibition factor, macrophage aggregating factor) and proteolytic enzymes produced by

activation of the complement and fibrinolytic systems inhibit the random migration of macrophages, thereby increasing their number in sites of inflammation.[1,10]

Particle ingestion (phagocytosis) by macrophages is accompanied by an increased consumption of oxygen and a series of biochemical events that produce highly reactive oxygen metabolites. This increase in oxygen consumption with the resultant generation of oxygen metabolites is referred to as the respiratory burst. The reactive oxygen metabolites include hydrogen peroxide, superoxide anion, singlet oxygen, hydroxyl radicals, chloramines and aldehydes. These metabolites are toxic to microorganisms and are important in macrophage killing of intracellular pathogens.[1,11]

Cells of the mononuclear phagocyte system have receptors for IgG and the third component of complement (C3b) in their plasma membrane.[12,13] Mononuclear phagocytes that are capable of antigen-processing for presentation to lymphocytes have Ia antigen present in their plasma membrane. The Ia antigen is a major histocompatibility antigen.[3]

The receptors for IgG are specific for the Fc portion of the IgG molecule.[13] Fc-receptors and complement component (C3) receptors function in the antibody and complement binding capacity of macrophages, in antigen presentation to lymphocytes (B and T) and in immune mediated phagocytosis.[12,13] Fc-receptors are capable of binding IgG and initiating phagocytosis while complement (C3) receptors bind particles but do not initiate phagocytosis.[1,14] Inactive C3-receptors can become activated by specific lymphokines to respond to molecules on the substrate to which macrophages are attached in vitro.[14] The in vivo activation of macrophage C3-receptors requires the presence of immune complexes and T lymphocytes.[14]

When particles are phagocytosed via Fc-receptor mechanisms, the macrophages release large amounts of reactive oxygen metabolites (superoxide anion, free hydroxyl group and hydrogen peroxide) and arachidonic acid metabolites (prostaglandins and leukotrienes). In contrast to Fc-receptor mediated phagocytosis, neither active or inactive C3-receptor activity result in the release of hydrogen peroxide or arachidonic acid metabolites.[14] This observed difference in the secretion of inflammatory mediators during the phagocytic process suggests that activated C3-receptor-mediated phagocytosis may be a mechanism to clear opsonized particles without initiating an increased inflammatory response.[14]

Macrophages are heterogeneous in their affinity for IgG.[12] Guinea pig peritoneal macrophages have an affinity for IgG that is 3 1/2 times greater than that of alveolar macrophages.[12] Peritoneal exudate macrophages that are elicited by injecting an irritant into the peritoneal cavity have a six fold increase in the proportion of cells with a high affinity for IgG.[12] Alveolar macrophages have a broader range of cellular affinity for IgG than do peritoneal macrophages, which may be related to the aerobic environmental conditions for the alveolar macrophages.[12] A greater proportion of canine alveolar macrophages have Fc-receptors than do canine blood monocytes, and canine alveolar macrophages also have greater phagocytic ability than do canine blood monocytes.[15]

Macrophage populations differ in their expression of the Ia antigen depending upon the compartment in which the macrophages reside.[16] The percent of cells (monocytes, macrophages) expressing the Ia antigen in bone marrow is 20-80%, peritoneal exudate 30-50%, thymic macrophages 40-60%, alveolar macrophages 5-80% and splenic macrophages 40-60%.[16] The local

concentration of prostaglandin in each tissue controls the amount of Ia-positive macrophages within that tissue due to prostaglandin E_2 inhibiting the induction of Ia-positive macrophages.[17] When Ia-negative macrophages are compared to Ia-positive macrophages, the former have an increased number of Fc- and C3-receptors, equal bacterial binding and latex phagocytosis, and equal IL-1 secretion following endotoxin activation. Ia-negative macrophages do not secrete IL-1 following T cell-macrophage interaction while Ia-positive macrophages do.[17]

Traffic of Mononuclear Phagocytes

Monocytes leave the bone marrow within 24 hours of completing their last division, thus, unlike the bone marrow granulocytes, there is no marrow reserve pool of monocytes.[4] There is no substantial marginated pool of monocytes; thus, the blood monocyte pool is composed almost entirely of circulating elements. Monocytes leave the circulation at random and the half-time of disappearance of labeled monocytes from the circulation appears to be about 71 hours in humans.[1,4] Once having emigrated from capillaries, tissue macrophages are believed to be incapable of re-entering the circulation.

Tissue macrophages are present in a number of organs and tissues and are referred to as alveolar macrophages in the lungs, Kupffer cells in the liver, Langerhans cells in the skin, epithelioid cells and giant cells in granulomatous reactions, as synovial macrophages in joint cavities, and as peritoneal and pleural macrophages in those spaces, and as osteoclasts in the bone.[4]

Maturational Characteristics of Cells of the Mononuclear Phagocyte System

The promonocyte is the earliest morphologically identifiable mononuclear phagocyte progenitor in the bone marrow.[4] It has poorly developed phagocytic capacity, secretes small quantities of lysozyme and displays few Fc-receptors.[1,4] Promonocytes, precursors of circulating monocytes, are myeloperoxidase positive and esterase positive.[4]

Monocytes, as compared to promonocytes, have decreased myeloperoxidase staining and an increase in lysosomes, Fc-receptors, lysozyme secretion and phagocytic ability. Monocytes usually remain viable when they leave the circulation and mature into tissue macrophages characteristic of the compartment into which they enter.[4]

Mature tissue macrophages, such as pulmonary alveolar macrophages, do not stain for myeloperoxidase. Compared to their less differentiated precusors (promonocytes, monocytes), they have increased lysosomes, phagocytic ability, numbers of Fc-receptors and of complement receptors, and have increased lymphocyte interactions.[1] Monocytes and tissue macrophages are nonspecific esterase positive as are all the cells of the mononuclear phagocyte system. The intensity of esterase staining is somewhat variable, with alveolar macrophages staining with the most intensity followed by blood monocytes and then by peritoneal macrophages. This property is used as a marker for identifying cells of the mononuclear phagocyte system.[8,18]

Macrophages are morphologically and functionally different between sites (i.e. alveolar versus peritoneal) and within an organ or tissue.[19,20] Peritoneal and alveolar macrophages differ in their cellular metabolism in that alveolar macrophages utilize aerobic metabolism and peritoneal macrophages utilize anaerobic metabolism.[19] The population of resident and

paraffin-induced, stimulated guinea pig peritoneal macrophages includes 5 to 15% with low esterase activity.[20] Human peritoneal macrophages were predominantly perioxidase positive in one study.[21] A population of macrophages may respond differently to the same stimulus with respect to an array of functions. A stimulus such as bacteria may induce a subpopulation of peritoneal macrophages to increase phagocytic ability without altering its Fc-receptor activity, and a second subpopulation in the same subject may exhibit the opposite results. This has been referred to as dissociation of function.[22] The heterogeneity of macrophages may be the result of local environmental influences, separate subpopulations of macrophages or the state of activation that the macrophage is in.[19]

Pulmonary Alveolar Macrophages

Pulmonary alveolar macrophages are the primary (first-line) defense mechanism of the gas-exchange region of the lung.[4] Resident alveolar macrophages are derived from blood monocytes which, in turn, originated in bone marrow.[4] Under normal steady state conditions, approximately 15% of the monocytes leaving the circulation enter the lungs to become pulmonary macrophages.[23] Alveolar macrophages have a very limited proliferative capability.[4] The life span of human alveolar macrophages is unknown.[4] The pulmonary alveolar macrophage population in mice is reported to have a turnover time of one month.[24] Activation of resident macrophages and recruitment of circulating monocytes into alveoli are part of the response of the lung to an irritant. The influx of macrophages derived from the peripheral blood provides the main source of macrophages for pulmonary defense, while resident macrophages act briefly as sentinels to call in

neutrophils and blood monocytes. When resident alveolar macrophages are stimulated by specific or nonspecific immune stimulants, the number and affinity of Fc-receptors and C3-receptors increases.[4] Hamster alveolar macrophages exhibited increased immune phagocytosis (Fc- and C3b-receptor-mediated), as well as increased nonimmune yeast phagocytosis, when incubated in supernatant fluid from Concanavalin A (Con A)-stimulated lymphocytes. This leads to the conclusion that lymphokines are capable of enhancing both immune and nonimmune phagocytosis by alveolar macrophages.[25] In addition to this, alveolar macrophages exposed to specific or nonspecific immune stimulants have increased numbers of mitochondria and lysosomes (the latter having increased enzyme activities) and increased metabolic activities.[4] Resident alveolar macrophages produce chemotactic factors that attract inflammatory cells (neutrophils and monocytes) to the lung and also produce CSF which increases the production of polymorpho nuclear and mononuclear phagocytes in the bone marrow.

Peritoneal Macrophages

Peritoneal macrophages are derived from blood monocytes.[26] The turnover time in mice has been reported to be approximately 19 days.[23] Once in the peritoneal cavity, macrophages retain the ability to synthesize DNA for less than 24 hours. In mice during steady state conditions, 7.5% of the monocytes leaving the circulation enter the peritoneal cavity.[27] The fate of peritoneal macrophages is unknown, however, they may be removed from the peritoneal cavity via lymphatic drainage and die in local lymph nodes.[23]

Afferent Limb of Immune System

Macrophages are important in the induction of both antibody and cell-mediated immune responses.[1,2] Macrophages that have the Ia antigen upon their surface membrane present antigens to T and B lymphocytes that respond with their characteristic antibody or cell mediated immune response.[2] Macrophages appear to have two functions in antibody formation: 1) processing and presentation of antigens and 2) viability promotion in spleen cells.[2] Macrophages with Ia antigen on their surface membranes are capable of responding to antigens by phagocytosis of the antigens followed by incorporation of the antigen on their surface membrane. Once the antigen is associated with the macrophage membrane, it can bind to receptor sites on T or B lymphocytes and elicit responses such as B cell differentiation into plasma cells and IL-2 production from T lymphocytes. Macrophages are required for the primary humoral immune response (antibody formation) to T-cell dependent antigens. The secondary humoral immune response is much less dependent upon macrophages and only a small number are required for antibody formation.[2]

The viability-promoting function of macrophages for spleen cells is illustrated by the removal of macrophages from spleen cell suspensions, which results in the inability of splenic lymphocytes to form antibody against sheep RBC's (sRBC). When macrophages were added to whole spleen cell suspensions, the antibody forming ability is restored.[2]

Macrophages have an active role in cell-mediated immunity.[2] The primary sensitization of T cells *in vitro* requires antigen presentation by macrophages. Stimulated monocytes and macrophages release interleukin-1 which previously has been referred to as lymphocyte-activating factor

(LAF).[9] Interleukin-1 is a major regulator of T lymphocyte proliferation.[9] Activated T cells produce Interleukin-2 (Il-2), which also plays a role in T lymphocyte proliferation.[28] Macrophages are required for in vitro culture of T cells and Interleukin-2 production. Cytotoxic T cells are involved in allograft rejection and tumor immunity and require the presence of macrophages in their induction phase.[2]

Effector Limb of Immune System

Macrophages act as effector cells of the immune system and as such they can ingest and digest microorganisms, kill tumor cells and participate in other immunopathologic processes.[2] Macrophages are the predominant cell type present in chronic inflammatory reactions. Macrophage migration inhibition factor (MIF) is a soluble mediator released from antigen-stimulated lymphocytes, which inhibits the random movement of macrophages in vitro. Macrophage chemotactic factor is another lymphokine that may be partially responsible for the accumulation of macrophages in reactive sites in CMI responses.[2]

In canine lungs, the alveolar macrophages suppress lymphocyte proliferation.[29] This suppression may be due to the production of prostaglandins which have been shown to suppress lymphocyte proliferation. Studies have also shown that canine bronchoalveolar lymphocytes have an inherent hyporesponsiveness to mitogenic stimulation.[29]

Glucocorticoids and the Mononuclear-Phagocyte System

Glucocorticoids are used in small animal medicine in the treatment of a number of inflammatory, allergic, immune mediated and neoplastic diseases

as well as in the treatment of shock.[30] The primary allergic disorders in small animal medicine treated with glucocorticoids are dermatological disorders (i.e. atopy, flea allergy dermatitis). Glucocorticoids play a very significant role in treatment of immune mediated diseases such as autoimmune hemolytic anemia, immune mediated thrombocytopenia and systemic lupus erythematosus. Neoplasms of the lymphoid system (i.e. lymphosarcoma, multiple myeloma) are among the neoplasms that are glucocorticoid responsive. The role of glucocorticoids in the treatment of shock remains a controversial issue. However, a beneficial effect of glucocorticoids has been demonstrated in treatment of endotoxic and septic shock.

Glucocorticoids are effective in inhibiting humoral and cellular immunity, but have a greater effect on cellular immunity than on humoral immunity.[31,32] The glucocorticoid effect on immunoglobulin synthesis and complement metabolism is not felt to be clinically important.[32] The precise mechanisms involved in glucocorticoid suppression of the immune system are not completely understood but some hypotheses have been advanced.

The mechanism of action of glucocorticoids at the cellular level may be initiated by the interaction of glucocorticoids with specific steroid-binding protein receptors.[32] The steroid-receptor complex may then interact with nuclear DNA to signal the production of mRNA, which directs the production of new proteins. These new proteins, many of which are enzymes, determine the cellular response to the glucocorticoids.[32] In animal models it has been shown that the loss of steroid receptors results in loss of the response to steroid.

Glucocorticoids also affect the total and differential leukocyte counts in canine peripheral blood.[33] The response to endogenous or exogenous glucocorticoids by canine blood leukocytes is an increase in absolute numbers

(leukocytosis) primarily due to a mature neutrophilia. There also is an accompanying lymphopenia, eosinopenia, and monocytosis.[33]

Monocytes and macrophages in mouse, rabbit and man have glucocorticoid receptors.[34] Mononuclear phagocytes at all stages of maturation are sensitive to glucocorticoids.[35] In a study performed to evaluate the biochemical actions of glucocorticoids on macrophages in culture, it was shown that lysozyme secretion was not affected by glucocorticoids.[35] However, the secretion of elastase, collagenase, and plasminogen activator was inhibited by the addition of glucocorticoids to macrophage cultures.[35] Glucocorticoids exert an anti-inflammatory effect by blocking the production of plasminogen activator.[32] Dexamethasone has been shown to inhibit the rate of formation of macrophage colonies from mouse bone marrow stem cells and to alter differentiation of monocytes to macrophages in persons.[35,36] Interaction of glucocorticoids and specific monocyte cytoplasmic receptors was suggested as the mechanism by which monocyte differentiation is altered.[36] A six hour pulse exposure of glucocorticoids was shown to have no effect on monocyte to macrophage differentiation in man, however, a sustained plasma concentration did affect differentiation.[36] Glucocorticoids decrease the number of lysosomes within macrophages and inhibit the development of macrophage tumoricidal activity and prevent/inhibit increased lysosomal enzyme content.[36]

One of the most important effects of glucocorticoid administration is a decrease in the number of neutrophils and monocyte-macrophages at sites of inflammation.[32] This effect on the accumulation of phagocytic cells at inflammatory sites is probably the major mechanism of the anti-

inflammatory effect of glucocorticoids. It is also the probable cause for decreased immune function in patients on daily glucocorticoid therapy.[32]

In vitro studies have suggested that glucocorticoids reduce the number of macrophages in sites of inflammation by suppressing lymphokine-mediated processes.[37] Glucocorticoids inhibit MMF (Macrophage Mitogenic Factor) and MIF (Macrophage Migration Inhibition Factor) generation at physiological concentrations.[37] Pharmocological concentrations of glucocorticoids were necessary to inhibit MMF-induced macrophage proliferation.[37] Other studies have reported that glucocorticoids do not affect MIF production although they do inhibit the macrophages response to MIF.[32] Glucocorticoids do not suppress lymphocyte production of lymphokines, but they do inhibit the ability of the mediator to recruit cells necessary for the expression of the response.[32]

Treating mice with glucocorticoids prior to macrophage activation decreased Interleukin-1 production by macrophages.[38] Glucocorticoid inhibition of Interleukin-1 release from stimulated macrophages may result in decreased lymphokine production.[37]

Glucocorticoids exert their maximum activity on the immune response if they are present prior to antigenic challenge.[31] Glucocorticoids have as one of their primary actions the lysis of lymphocytes.[31] The transient lymphopenia observed following glucocorticoid administration is, however, not due to lympholysis but rather due to lymphocyte redistribution.[31]

Hydrocortisone suppresses interferon-induced macrophage cytotoxicity in vitro.[39] Prostaglandin production in vitro by phagocytic cells of the thymus is inhibited by the presence of dexamethasone.[40] The blocking

of Fc-receptors by glucocorticoids has been observed in guinea pig macrophages and it may be an important mechanism of action of glucocorticoids.[32] In vitro glucocorticoids inhibit blood monocyte Fc-receptor activity.[41] The blocking of macrophage membrane receptors (Fc-receptor, C3-receptor) by glucortocoids may play a role in the observed decrease in macrophage clearance of antibody-coated and antibody-complement-coated erythocytes in steroid-treated animals.[32] Glucocorticoid administration has been associated with a dose-dependent decrease in monocyte Fc-receptor numbers in normal persons and in patients with auto-immune hemolytic anemia.[42] In vitro incubation of mouse peritoneal macrophages with glucocorticoids yielded no change in the basal values for Fc-mediated phagocytosis. Incubation with glucocorticoids plus interferon (gamma) resulted in increased Fc-mediated phagocytosis and binding to a much greater extent than interferon (gamma) alone.[43] Glucocorticoids inhibited resting levels of Ia antigen and induction of Ia antigen by interferon.[43] Topical and systemic glucocorticoids in mice result in a dose-dependent reduction in the number of Ia-bearing epidermal Langerhans cells and when incubated with mouse peritoneal macrophages, glucocorticoids inhibited their Ia antigen expression and Interleukin-1 production.[44,45] Glucocorticoids may result in an interference with the antigen-presenting cell function of macrophages and T cell stimulation, and this may be one of the mechanism that result in glucocorticoid suppression of the T cell-dependent immune response.[44,45]

Hydrocortisone inhibits prostaglandin synthesis by rat peritoneal macrophages that are phagocytosing killed bacteria and interferes with

peritoneal macrophage activation.[46,47] Hydrocortisone resulted in normal macrophages becoming unresponsive to lymphokine and it also prevented macrophages from responding to T. gondii ingestion with an enhanced oxidative respiratory burst. Hydrocortisone readily interferes with macrophage activation by lymphokines but only if present before or during stimulation.[47] The ability of the macrophages to inhibit intracellular toxoplasma replication was also decreased.[47]

The effect of glucocorticoids on alveolar macrophages in species other than dogs has been studied. It has been demonstrated that glucocorticoid administration in guinea pigs results in a dose-dependent reduction in pulmonary defenses to intracellular pathogens and that suppression of both acquired local immunity as well as nonimmune defense mechanisms occur.[48] Additional studies utilizing guinea pigs showed that cortisone acetate treatment appeared to adversely influence certain chemotactic activities of pulmonary alveolar macrophages.[49] A brief and severe monocytopenia with no change in the number of pulmonary macrophages followed a single dose of dexamethasone.[50] In the same study, hydrocortisone acetate caused a severe monocytopenia for more than 144 hours and a decrease of pulmonary macrophage population to two-thirds of normal within 48 hours.

A study with mice showed that the number of alveolar macrophages remained within normal limits during intensive glucocorticoid therapy despite the presence of peripheral leukopenia.[51] From that study, the authors suggest that a mechanism in the lung was able to maintain the resident alveolar population and this was possible due to proliferation of resident alveolar macrophages.

Glucocorticoids may alter cell mediated immune function by affecting the mononuclear phagocyte system. It has been suggested by several investigators that the most important effect is the decreased number of macro-macrophages will result in a decreased number available for interaction with other cells of the immune system. There will also be a decreased number available to perform functions such as phagocytosis of foreign particles and debris from damaged tissues, resisting intracellular proliferation of infectious organisms, and exhibiting cytotoxic reactions towards foreign materials. Thus, the effect of glucocorticoids on cellular immunity is of more importance in altered resistance than its effect on humoral immunity. However, when the population of macrophages that are able to process and present antigen are adversely affected, the humoral system will also be affected, especially in the formation of antibody to a T cell-dependent antigen presented to the immune system for the first time.

CHAPTER III

Materials and Methods

A. Animals

Seven male and five female adult mixed breed dogs weighing between 13.2 and 33.6 kgs were obtained from and housed by the Kansas State University Animal Resource Facility (ARF). These dogs were vaccinated against canine distemper, leptospira, hepatitis, parainfluenza and canine parvo virus with a modified live vaccine and against rabies. All dogs were heartworm negative as determined by a modified Knott test. Fecal flotations were performed and three dogs had Trichuris vulpis and two had Ancylostoma canimum/Uncinaria stenocephala. All dogs were medicated with pyrantel pamoate.[a]

B. Isolation of Blood Monocytes

Blood monocytes were harvested from thirty mls of heparinized blood collected from each dog. The blood was diluted 1:1 (10-12 ml of each) with sterile phosphate buffered saline solution (PBSS). Eight ml of Ficoll-sodium diatrizoate solution[b] was added to 50 ml siliconized glass centrifuge tubes and 10 ml of diluted blood was layered on top. Differential migration due to differences in cell densities occurs with centrifugation.[52] Following centrifugation (1500 g, 20°C, 20 min), monocytes, lymphocytes and thrombocytes are located at the interface between the plasma and the Ficoll-Paque.® The erythrocytes and granulocytes are found in a layer at the bottom of the tube. The monocytes were harvested with the use of

---

[a]Nemex: Pfizer Inc., New York, NY 10017

[b]Ficoll-Paque, Pharmacia Fine Chemicals, Piscataway, NJ

sterile pasteur pipettes from the plasma-Ficoll-Paque [R] interface and were transferred to a 50 ml siliconized glass centrifuge tube. The cells were washed with PBSS, centrifuged (1000 g, 5°C, 10 min.) and the resultant pellet of cells was resuspended in 2 mls of PBSS. The red blood cells (RBCs) were lysed by the addition of ten mls of cold distilled water (for 30 seconds) followed by 10 ml of 1.7% NaCl.

Following RBC lysis, the sample was centrifuged and the pellet was resuspended in 10 ml RPMI 1640[c] medium with 10% Bovine Fetal Serum[d]. A 1:10 dilution of cells was made using 0.1 ml of the cell suspension, 0.8 ml RPMI 1640 with 10% Bovine Fetal Serum (BFS), and 0.1 ml of 0.4% Trypan Blue. A total cell and viable cell count was performed. Viability was assessed using Trypan Blue exclusion. The cells were resuspended to 4 x 10^6 cells/ml using RPMI 1640 with 10% BFS.

C. Isolation of Peritoneal Macrophages

The dogs were anesthetized and maintained under anesthesia with sodium thiamylal[e] administered via a cephalic vein catheter. Ventral midline celiotomies were performed using sterile surgical techniques. Five hundred mls of sterile PBSS was instilled into the peritoneal cavity. The lavage fluid was recovered using a 60 ml syringe attached to tubing placed into the peritoneal cavity with a fenestrated syringe case surrounding it. The abdomen was closed in two layers using a simple interrupted suture

---

[c]RPMI 1640: Grand Island Biological Co., Grand Island, NY 14076

[d]Bovine Fetal Serum: KC Biological, Inc., Lenexa, KS 66215

[e]Bio-tal: Bio-Ceutic Division, St. Joseph, MO 64502

pattern in the linea alba and simple interrupted or continuous pattern in the skin. The lavage fluid was filtered through 4" x 4" gauze sponges and transferred to 50 ml silconized glass centrifuge tubes. Following centrifugation (1000 g, 4°C, 10 min.) the pellet was resuspended in 2 mls of PBSS. The RBCs were lysed as with the isolation of blood monocytes. The remaining steps were identical to those followed for the blood monocytes except the cells were resuspended to 4×10^5 cells/ml.

D. Isolation of Bronchoalveolar Macrophages

Following collection of the peritoneal macrophages, the dogs were placed in left lateral recumbency for bronchoalveolar lavage. A polyethelene tube (PE-320) was passed through the endotracheal tube into the trachea and advanced until resistance was met and then slightly withdrawn. The lavage was performed by instilling 20 ml increments of PBSS (maximum volume 10 ml/kg) and immediately aspirating to recover the lavage fluid. The lavaging procedure was repeated until the total volume of PBSS had been used or a sufficient volume of fluid (100 ml) had been recovered. The lavage fluid was filtered through 4" x 4" gauze sponges and transferred to 50 ml siliconized glass centrifuge tubes. The isolation procedure was identical to that followed for the isolation of the peritoneal macrophages. The cells were resuspended to 4×10^5 cells/ml.

E. Formation of Monocyte and Macrophage Monolayers

One ml of the cell suspensions (blood monocytes, bronchoalveolar macrophages or peritoneal macrophages) were added to each of four wells on two previously prepared tissue culture plates.[f] Plastic tissue culture

[f]Costar Tissue Culture Plates: Costar, Cambridge, MA

coverslips[g] had been placed in each of the wells prior to the cells being added. The plates were incubated (37°C, w/5% CO_2) for one hour to allow for adherence of the cells to the coverslips. Following the incubation period, the cells in each well were washed three times using RPMI 1640 with 10% BFS to remove nonadherent cells. Plates were viewed under the inverted microscope to check for monolayer formation. After the cells had been washed, they were ready for either the EA-rosetting or yeast phagocytosis assay. Cytospin preparations[h] of each of the cell suspensions were made for nonspecific esterase staining (outlined in the following section) and Diff Quik[i] staining for differential counts.

F. Fc-Receptor Rosette Assay

One ml of 1% sensitized sRBCs[j] was added to wells with blood monocytes, bronchoalveolar macrophages and peritoneal macrophages. RPMI 1640 with 10% BFS was added to control wells. The plates were incubated for 20 minutes (37°C, w/5% CO_2) and then washed with fresh RPMI 1640 with 10% BFS. Fresh RPMI 1640 with 10% BFS was added to each well and they were viewed under the inverted microscope. The monolayers were stained with Diff-Quik ^{R}, air dried and the coverships were mounted onto glass slides. A total of 200 cells per preparation were counted and any cell with three or more sRBCs attached to the cell membrane was considered a rosetting cell.

---

[g]Thermanox Coverslips: Miles Scientific, Naperville, IL 60566

[h]Cytospin 2, Shandon Southern Instruments Inc., Sewickley, PA

[i]Harleco Diff-Quik: American Scientific Products, N. Kansas City, MO 64116

[j]Rabbit anti-sheep erythrocyte immunoglobulin G, Cordis Laboratories Inc., Miami, FL

G. Phagocytosis of Yeast

One ml of heat killed, new methylene blue stained Candida albicans suspension (RPMI 1640 with 10% BFS with 2 X 10^7 yeast cells per ml) was added to each well. Control wells contained macrophages and RPMI 1640 with 10% BFS. The plates were incubated (37^oC, w/5% CO_2) for forty-five minutes, and were then washed two times. Fresh RPMI 1640 with 10% BFS was added and the wells were viewed under the inverted microscope. The cells were stained, air dried and the coverslips were mounted onto glass slides. A total of 200 cells per preparation were counted and cells with three or more ingested yeast were considered positive for phagocytosis.

H. Non-specific Esterase Stain

Cytospin preparations of blood monocytes, peritoneal macrophages and bronchoalveolar macrophages were prepared for staining alpha naphthyl acetate esterase activity as has been previously described.[8] Two hundred cells per preparation were counted. The cells that were esterase positive had a dark red to reddish brown cytoplasm. The stain faded quickly therefore the cell counts were performed within a few days of the staining procedure.

I. Data Collection

The project was designed to obtain data from dogs prior to treatment and following two different treatments of glucocorticoids. Samples were collected from the 12 dogs prior to treatment for isolation of blood monocytes, peritoneal macrophages and bronchoalveolar macrophages. The resultant monolayer preparations were used for the previously outlined procedures. In addition, blood was collected for a complete blood count (CBC)

and serum chemistry profile (SMA-12). The data obtained from this collection is referred to as Phase I data.

The second set of samples were collected six and 12 hours following the intravenous administration of prednisolone sodium succinate[k] (20 mg/kg) and dexamethasone sodium phosphate[l] (6 mg/kg). These drugs are referred to as treatment 1 (Rx-1). Blood only was collected six hours following Rx-1 for blood monocyte isolation, CBC, and serum chemistry profile. At 12 hours following Rx-1, samples were collected from CBC, serum chemistry profile, and for isolation of blood monocytes, peritoneal macrophages and bronchoalveolar macrophages, as described above. The data obtained from samples collected at six hours is referred to as Phase II data and the data from the 12 hour samples is referred to as Phase III data.

The last set of samples were collected after the dogs had been administered prednisone (2 mg/kg BID) orally for seven days. This treatment was treatment two (Rx-2). Samples were collected approximately 10-12 hours following the last dose, for CBC, serum chemistry profile and isolation of blood monocytes, peritoneal macrophages and bronchoalveolar macrophages. The data obtained was referred to as Phase IV data.

A minimum of 30 days time lapse between procedures was allowed for recovery from surgery and experimental treatments.

---

[k]Solu-Delta-Cortef: The Upjohn Co., Kalamazoo, MI 49001

[l]Dexamethasone sodium phosphate: Invenex Laboratories, Chagrin Falls, OH 44022

CHAPTER IV

Results

The data was analyzed statistically by analysis of variance and a randomized block design. Results were considered to be statistically significant if the P value was ≤ 0.05.

Phagocytosis (Table 1)

The percentage of blood monocytes that were phagocytosis positive was significantly decreased 12 hours following treatment one (Rx-1). No significant change was observed following treatment two (Rx-2). There was an increased percentage of phagocytosis-positive pulmonary alveolar macrophages at 12 hours following Rx-1, but no change following Rx-2. Peritoneal macrophage phagocytosis was not significantly altered following Rx-1, but following Rx-2, the percentage of phagocytosis-positive macrophages was increased.

Fc-Receptor Activity (Table 1and 2)

The percentage of blood monocytes with Fc-receptor activity was unchanged following Rx-1 and increased following Rx-2. The percentage of pulmonary alveolar macrophages that exhibited Fc-receptor activity increased 12 hours following Rx-1 and was unchanged following Rx-2. Peritoneal macrophage Fc-receptor activity was unchanged following Rx-1 and Rx-2.

There were no significant changes observed in blood, lung or peritoneal neutrophils in their Fc-receptor activity following either treatment.

Adherence

The adherence of pulmonary alveolar macrophages to plastic cover slips

was not changed following the treatments. The adherence of peritoneal macrophages to plastic cover slips was significantly decreased 12 hours following Rx-1 and following Rx-2.

Non-Specific Esterase Activity (Table 1 and 3)

The percentage of esterase-positive blood monocytes, pulmonary alveolar macrophages and peritoneal macrophages increased six and 12 hours following Rx-1 and following Rx-2.

The percentage of esterase-positive blood lymphocytes increased at six and 12 hours following Rx-1 and following Rx-2. The percentage of esterase-positive pulmonary lymphocytes increased following Rx-1 and was unchanged following Rx-2. The percentage of esterase-positive peritoneal lymphocytes was unchanged following both treatments.

Differential Counts of Cytospin Preparations (Table 1,2,3 and 4)

Blood monocytes were significantly increased following Rx-1 (six and 12 hours) and Rx-2. Pulmonary macrophages were unchanged following Rx-1 and were significantly increased following Rx-2. Peritoneal macrophages were significantly decreased following Rx-1 and were not significantly decreased following Rx-2.

Blood and lung neutrophils were not significantly changed following Rx-1 or Rx-2. Peritoneal segmented neutrophils were significantly increased following Rx-1 and Rx-2.

Blood and peritoneal lymphocytes were decreased following Rx-1 and Rx-2. Pulmonary lymphocytes were not significantly changed following either treatment.

Blood and peritoneal eosinophils were decreased following Rx-1 (six and 12 hours) and Rx-2. Pulmonary eosinophils were significantly decreased after Rx-2.

Complete Blood Counts (Table 5)

All the parameters measured were significantly altered by at least one treatment with the exception of MCH, MCHC, band neutrophils and fibrinogen. The total white blood cell counts and absolute neutrophil counts were increased following Rx-1 (six and 12 hours) and Rx-2. Monocytes remained unchanged following Rx-1 and increased following Rx-2. Eosinophils and lymphocytes decreased following Rx-1 (six and 12 hours) and Rx-2. The red blood cell count and hemoglobin concentration decreased 12 hours following Rx-1 and following Rx-2. The hematocrit remained unchanged except at 12 hours following Rx-1 where it decreased. The plasma total protein increased after Rx-2.

Serum Chemistries (Table 6)

All parameters were significantly changed by one or more treatments except glucose and blood urea nitrogen. The SGPT, SAP and total serum protein were elevated after Rx-2 and were not significantly altered following Rx-1. Six hours following Rx-1, creatinine and calcium were decreased and chloride and sodium were increased. Twelve hours following Rx-1, creatinine, inorganic phosphorus, calcium and potassium were decreased and chloride was increased. Following Rx-2, the creatinine and chloride were decreased. Albumin was decreased 12 hours following Rx-1 and increased following Rx-2.

TABLE 1. Cytologic and market data for blood, lung and peritoneal monocytes and macrophages from 12 dogs prior to and following glucocorticoid administration.

		Monocytes/Macrophages			
Source	Assay	Phase 1	Phase 2	Phase 3	Phase 4
Blood	Differential Count[a]	61.08 ± 6.91	126.92 ± 6.91*	134.33 ± 6.91*	141.50 ± 6.91*
	Esterase Stain (%)	83.15 ± 2.12	95.68 ± 2.12*	94.46 ± 2.12*	98.61 ± 2.12*
	Phagocytic Cells (%)	0.88 ± 0.21	1.05 ± 0.34	-0.05 ± 0.34*	0.57 ± 0.34
	EA-Rosetting Cells (%)	22.05 ± 3.20	18.78 ± 5.24	28.75 ± 5.24	39.51 ± 5.24*
Lung	Differential Count[a]	133.33 ± 8.19	ND	135.50 ± 8.19	169.08 ± 8.19*
	Esterase Stain (%)	91.29 ± 1.12	ND	95.62 ± 1.12*	99.39 ± 1.12*
	Phagocytic Cells(%)	5.92 ± 1.61	ND	16.81 ± 2.62*	9.91 ± 2.62
	EA-Rosetting Cells (%)	42.85 ± 4.22	ND	65.70 ± 6.90*	52.20 ± 6.90
Peritoneal	Differential Count[a]	70.75 ± 6.97	ND	37.25 ± 6.97*	50.67 ± 6.97
	Esterase Stain (%)	71.73 ± 4.09	ND	83.77 ± 4.09*	92.03 ± 4.09*
	Phagocytic Cells (%)	6.43 ± 2.47	ND	7.04 ± 2.47	15.93 ± 2.47*
	EA-Rosetting Cells (%)	42.07 ± 4.20	ND	31.92 ± 4.20	38.51 ± 4.20

[a]Number of cells per 200 total cell count
EA - Erythrocyte-antibody
* $P \leq 0.05$
Phase 1 = control; Phase 2 = 6 hours following treatment one, blood only; Phase 3 = 12 hours following treatment one; Phase 4 = 10 - 12 hours following treatment two.

TABLE 2. Cytologic and marker data for blood, lung and peritoneal neutrophils from 12 dogs prior to and following glucocorticoid administration.

		Segmented Neutrophils (Bands)			
Source	Assay	Phase 1	Phase 2	Phase 3	Phase 4
Blood	Differential Count[a]	19.92 ± 7.17 (0.33 ± 0.28)	15.75 ± 7.17 (0.25 ± 0.28)	29.67 ± 7.17 (0.33 ± 0.28)	20.75 ± 7.17 (1.25 ± 0.28)
	Esterase Stain (%)	0	0	0	0
	Phagocytic Cells (%)	0.32 ± 1.52	-0.24 ± 2.40	3.93 ± 2.40	-0.24 ± 2.40
	EA-Rosetting Cells (%)	18.31 ± 3.34	6.71 ± 5.24	7.96 ± 5.23	6.96 ± 5.45
Lung	Differential Count[a]	7.75 ± 1.94 (0 ± 0.10)	ND	13.00 ± 1.94 (-0.00 ± 0.10)	6.00 ± 1.94 (0.17 ± 0.10)
	Esterase Stain (%)	0	ND	0	0
	Phagocytic Cells(%)	1.25 ± 2.39	ND	5.18 ± 3.66	-1.83 ± 4.14
	EA-Rosetting Cells (%)	5.21 ± 1.18	ND	6.32 ± 1.87	6.37 ± 1.95
Peritoneal	Differential Count[a]	105.58 ± 8.02 (1.00 ± 0.39)	ND	156.33 ± 8.02* (0.83 ± 0.39)	141.83 ± 8.02* (0.50 ± 0.39)
	Esterase Stain (%)	0	ND	0	0
	Phagocytic Cells (%)	0.57 ± 0.30	ND	0.28 ± 0.30	0.33 ± 0.30
	EA-Rosetting Cells (%)	15.28 ± 3.87	ND	20.71 ± 3.87	13.26 ± 3.87

[a]Number of cells per 200 total cell count
EA - Erythrocyte-antibody
* $P \leq 0.05$
Phase 1 = control; Phase 2 = 6 hours following treatment one, blood only; Phase 3 = 12 hours following treatment one; Phase 4 = 10 - 12 hours following treatment two.

Table 3. Cytologic and marker data for blood, lung and peritoneal lymphocytes from 12 dogs prior to and following glucocorticoid administration.

		Lymphocytes			
Source		Phase 1	Phase 2	Phase 3	Phase 4
Blood	Differential Count[a]	96.50 ± 7.01	54.17 ± 7.01*	35.08 ± 7.01	36.17 ± 7.01*
	Esterase Stain (%)	79.36 ± 3.00	93.12 ± 3.00*	95.11 ± 3.0*	97.84 ± 3.00*
	Phagocytic Cells (%)	0	0	0	0
	EA-Rosetting Cells (%)	0	0	0	0
Lung	Differential Count[a]	27.25 ± 3.53	ND	20.83 ± 3.53	18.00 ± 3.53
	Esterase Stain (%)	52.70 ± 10.22	ND	83.19 ± 10.22	62.90 ± 10.22
	Phagocytic Cells (%)	0	ND	0	0
	EA-Rosetting Cells (%)	0	ND	0	0
Peritoneal	Differential Count[a]	18.00 ± 2.58	ND	4.83 ± 2.58*	6.83 ± 2.58*
	Esterase Stain (%)	75.34 ± 9.07	ND	73.61 ± 9.07	79.17 ± 9.07
	Phagocytic Cells (%)	0	ND	0	0
	EA-Rosetting Cells (%)	0	ND	0	0

[a]Number of cells per 200 total cell count
EA - Erythrocyte-antibody
* $P \leq 0.05$
Phase 1 = control; Phase 2 = 6 hours following treatment one, blood only; Phase 3 = 12 hours following treatment one; Phase 4 = 10 - 12 hours following treatment two.

Table 4. Cytologic and marker data for blood, lung and peritoneal eosinophils from 12 dogs prior to and following glucocorticoid administration.

		Eosinophils			
Source		Phase 1	Phase 2	Phase 3	Phase 4
Blood	Differential Count[a]	22.17 ± 2.33	2.92 ± 2.33*	0.58 ± 2.33*	0.33 ± 2.33*
	Esterase Stain (%)	0	0	0	0
	Phagocytic Cells (%)	0	0	0	0
	EA-Rosetting Cells (%)	0	0	0	0
Lung	Differential Count[a]	31.66 ± 6.20	ND	30.66 ± 6.20	6.75 ± 6.20*
	Esterase Stain (%)	0	ND	0	0
	Phagocytic Cells (%)	0	ND	0	0
	EA-Rosetting Cells (%)	0	ND	0	0
Peritoneal	Differential Count[a]	4.66 ± 1.00	ND	0.75 ± 1.00*	0.17 ± 1.00*
	Esterase Stain (%)	0	ND	0	0
	Phagocytic Cells (%)	0	ND	0	0
	EA-Rosetting Cells (%)	0	ND	0	0

[a]Number of cells per 200 total cell count
EA - Erythrocyte-antibody
* $P \leq 0.05$
Phase 1 = control; Phase 2 = 6 hours following treatment one, blood only; Phase 3 = 12 hours following treatment one; Phase 4 = 10 - 12 hours following treatment two.

Table 5. Hematology data for 12 dogs prior to and following glucocorticoid administration.

Complete Blood Counts				
	Phase 1	Phase 2	Phase 3	Phase 4
WBC x 10^3	12.41 ± 0.79	17.27 ± 0.79*	19.69 ± 0.79*	19.73 ± 0.79*
RBC x 10^6	6.81 ± 0.12	6.89 ± 0.12	6.30 ± 0.12*	6.30 ± 0.12*
Hgb (g/dl)	15.87 ± 0.35	16.27 ± 0.35	14.84 ± 0.35*	14.79 ± 0.35*
HCT (%)	44.93 ± 0.90	46.39 ± 0.90	42.34 ± 0.90*	42.65 ± 0.90
MCV (fl)	66.33 ± 0.47	68.08 ± 0.47*	67.75 ± 0.47*	68.17 ± 0.47*
MCH (pg)	23.12 ± 0.19	23.28 ± 0.19	23.29 ± 0.19	23.25 ± 0.19
MCHC (g/dl)	35.23 ± 0.18	34.87 ± 0.18	34.92 ± 0.18	34.60 ± 0.18
Segs x 10^3	8.03 ± 0.68	15.40 ± 0.68*	18.23 ± 0.68*	16.11 ± 0.68*
Bands x 10^3	0.03 ± 0.04	0.09 ± 0.04	0.10 ± 0.04	0.14 ± 0.04
Lymphs x 10^3	2.87 ± 0.12	0.86 ± 0.12*	0.62 ± 0.12*	0.80 ± 0.12*
Mono's x 10^3	0.80 ± 0.16	0.99 ± 0.16	0.88 ± 0.16	2.82 ± 0.16*
Eos. x 10^3	0.79 ± 0.10	0.12 ± 0.10*	0.03 ± 0.10*	0.02 ± 0.10*
T.P. (gm%)	6.28 ± 0.07	6.29 ± 0.07	6.23 ± 0.07	6.84 ± 0.07*
Fibronogen (mg%)	200.00 ±25.22	200.00 ±25.22	250.00 ±25.22	141.67 ±25.22

EA - Erythrocyte-antibody
* $P \leq 0.05$
Phase 1 = control; Phase 2 = 6 hours following treatment one; Phase 3 = 12 hours following treatment one; Phase 4 = 10-12 hours following treatment two.

Table 6. Serum Chemistry data for 12 dogs prior to and following glucocorticoid administration.

	Chemistry Profiles			
	Phase 1	Phase 2	Phase 3	Phase 4
SGPT (I.U.)	32.71 ± 28.67	41.25 ± 27.07	42.83 ± 27.07	171.50 ± 27.07*
Creat (mg%)	1.03 ± 0.05	0.73 ± 0.05*	0.73 ± 0.05*	0.88 ± 0.50*
Glu (mg%)	106.83 ± 3.02	116.03 ± 3.02	105.17 ± 3.02	108.83 ± 3.02
I Phos (mg%)	4.83 ± 0.18	5.29 ± 0.18	3.78 ± 0.18*	4.44 ± 0.18
Ca (mg%)	10.11 ± 0.11	9.68 ± 0.11 *	9.17 ± 0.11*	9.98 ± 0.11
Alb (gm%)	3.26 ± 0.04	3.23 ± 0.04	3.13 ± 0.04*	3.43 ± 0.04*
T.P. (gm%)	6.18 ± 0.09	6.18 ± 0.09	5.94 ± 0.09	6.54 ± 0.08*
CL (meq/L)	112.17 ± 0.71	118.83 ± 0.71*	114.83 ± 0.71*	104.50 ± 0.71*
Alkaline Phos. (I.U.)	66.75 ± 21.08	46.58 ± 21.08	46.42 ± 21.08	238.83 ± 21.08*
BUN (mg%)	19.75 ± 1.45	22.75 ± 1.45	16.92 ± 1.45	19.42 ± 1.45
K^+ (meq/L)	4.57 ± 0.08	4.35 ± 0.08	4.14 ± 0.08*	4.47 ± 0.08
Na^+ (meq/L)	148.50 ± 0.74	154.75 ± 0.74*	149.75 ± 0.74	146.67 ± 0.74

EA - Erythrocyte-antibody
* $P \leq 0.05$
Phase 1 = control; Phase 2 = 6 hours following treatment one; Phase 3 = 12 hours following treatment one; Phase 4 = 10-12 hours following treatment two.

CHAPTER V

Discussion

The discussion is divided into two sections with the first section covering aspects of the mononuclear phagocyte system and its response following glucocorticoid administration. The goal of the research project was oriented toward evaluation of the effects glucocorticoids have on monocyte and macrophages. The second section covers the effect glucocorticoids had on hematologic values and on serum chemistries.

I. Effects of Glucocorticoids on Blood Monocytes, Alveolar Macrophages and Peritoneal Macrophages

The monocytes and macrophages were evaluated morphologically (percentage of cells that were non-specific esterase positive), functionally (percentage of cells demonstrating Fc-receptor activity, percentage of cells phagocytosing heat killed Candida albicans) and by differential count (a total of 200 cells were counted).

Treatment one (Rx-1) was a shock dose of prednisolone sodium succinate (20 mg/kg) and dexamethasone sodium phosphate (6 mg/kg). Treatment two (Rx-2) was an immunosuppressive dose of prednisone (2 mg/kg every 12 hours) administered for seven days.

Specific cytoplasmic glucocorticoid receptors have been identified in species other than the canine. The presence of specific glucocorticoid receptors and their interaction with glucocorticoids is considered to be an important initial step in the cellular mechanism of action of glucocorticoids. The presence of specific glucocorticoid receptors in the

canine species has not been domonstrated, however, it is reasonable to consider that they exist.

Blood Monocytes

The increase in the differential count of blood monocytes following Rx-1 may have been due to an increased release of bone marrow monocytes into the circulation and/or a decreased efflux of monocytes leaving the circulation. The percentage of monocytes that were phagocytosis-positive was decreased 12 hours following Rx-1, which suggests that the population was comprised largely of younger cells with less phagocytic ability. This conclusion would propose the release of bone marrow monocytes ("young cells") as the reason for an increased differential monocyte count. An alternative possibility is glucocorticoid inhibition of blood monocyte phagocytosis. The decrease of blood monocyte phagocytosis following Rx-1 may not be clinically significant, since blood monocytes do not exhibit a high proportion of phagocytosis, under normal conditions and decreased phagocytosis by tissue macrophages was not observed.

The increase in the monocyte differential count following Rx-2 was probably due to increased release of bone marrow-derived monocytes and a decrease in the number of monocytes leaving the circulation. A steady state of monocytes entering and leaving the circulation may thus have been reached at day seven of prednisone therapy, with the population of cells having a normal distribution at that time. This is supported by the percentage of phagocytic cells not being significantly different from the control group. The percentage of cells with Fc-receptor activity, which was increased following Rx-2, may have been due either to an increased number of receptors per cell or an increased affinity of the receptors

for the Fc portion of the IgG molecule. This observation conflicts with data from studies in mice and humans where glucocorticoids decrease the number of Fc-receptors and inhibits the Fc-receptor activity. It would appear that canine monocytes respond differently than do human monocytes to glucocorticoids and the dog may therefore not be a good experimental animal for use in comparisons of glucocorticoid effects on human monocytes.

The decrease in phagocytosis-positive monocytes following Rx-1 and the increased monocyte Fc-receptor activity following Rx-2 may have been due to the different drugs used. Dexamethasone may affect phagocytosis and not Fc-receptors and "long-term" prednisone may not alter phagocytosis but rather alter Fc-receptor function in blood monocytes.

Alveolar Macrophages

The differential count of the alveolar macrophages did not change following the shock dose of glucocorticoids. The percentage of phagocytosis-positive and Fc-receptor positive alveolar macrophages increased following Rx-1. The alveolar macrophage population may have been stable due to decreased influx and efflux and the resident alveolar macrophages may have been altered in their functional capacity by the single dose of dexamethasone and prednisolone. Glucocorticoid inhibition of the presence of pulmonary alveolar macrophage plasma membrane associated Ia antigen may result in an increase in the population of Ia antigen negative macrophages, which have an increase in the number of Fc-receptors per cell. This is a possible mechanism for the increase in Fc-receptor activity. Another possible mechanism is that the pulmonary alveolar macrophages are altered in such a way that they secrete an increased amount of interferon.

Glucocorticoids and interferon, when incubated in vitro with macrophages, increase Fc-receptor mediated phagocytosis. Phagocytosis and Fc-receptor activity was unaltered following Rx-2 and this suggests that the increased number of alveolar macrophages following Rx-2 may have been due to an increased influx of functionally unaltered blood monocytes. The difference in the percentages of phagocytosis and Fc-receptor positive alveolar macrophages between treatments may have been due to the different glucocorticoid preparation used.

Peritoneal Macrophages

The decrease in the differential count for peritoneal macrophages following Rx-1 may have been due to a decrease in monocytes entering the peritoneal cavity, with the normal number leaving or dying. The differential count of peritoneal neutrophils was increased following Rx-1, therefore an increase in the total number of peritoneal neutrophils may have contributed to the decreased percentage of peritoneal monocytes following Rx-1. The normal differential count for peritoneal macrophages following Rx-2 may have been due to: 1) prednisone may not alter the population of peritoneal macrophages as dexamethasone does, or 2) prednisone may have an early effect on the differential count of the peritoneal cells and over time, the distribution of cells returns to normal. The increased percentage of phagocytosis-positive peritoneal macrophages following Rx-2 supports a decreased efflux of macrophages as a reason for a normal macrophage differential count, because more mature cells have an increased phagocytic ability. Fc-receptor function was not altered after Rx-2 which again may be due to dissociation of function, i.e. the glucocorticoids inhibited the Fc-receptor

activity but not phagocytic ability. The decreased adherence of the peritoneal macrophages may have been the result of glucocorticoid-induced membrane changes.

Non-specific Esterase Activity

The increase in percentage of non-specific esterase-positive monocytes and macrophages (alveolar, peritoneal) following both treatments may have been due to glucocorticoid induction of the esterase enzyme or other alterations affecting enzymatic function.

Summary

The shock dose of glucocorticoids enhanced the phagocytic and Fc-receptor activity of alveolar macrophages and decreased blood monocyte phagocytic activity, while having no effect on the peritoneal macrophages in their phagocytic or Fc-receptor activities. The treatment of prednisone (7 days) enhanced blood monocyte Fc-receptor activity and peritoneal macrophage phagocytic activity while not altering either Fc-receptor or phagocytic activity of alveolar macrophages.

Alveolar and peritoneal macrophages and blood monocytes differ in their response to glucocorticoids. Alveolar macrophages have enhanced Fc-receptor and phagocytic activity following a shock dose of glucocorticoids, while peritoneal macrophage phagocytic activity is enhanced following seven days of prednisone. Blood monocyte response differed between treatments, the shock dose decreased phagocytic activity, while the prednisone (7 days) increased Fc-receptor activity. The differential counts varied between treatments also. Blood monocytes increased in percentage with both treatments while the percentage of alveolar macrophages only increased following

the prednisone (7 days) and the percentage of peritoneal macrophages decreased with the shock dose.

II. Effects of Glucocorticoids on Hematologic Values and on Serum Chemistries

Hematologic Values

A well known classic hematologic response to glucocorticoids in the canine species is neutrophilia, monocytosis, lymphopenia and eosinopenia. All were observed following one or both of the treatments. The observed peripheral neutrophilia and monocytosis is most likely due to an increased number of cells entering the circulation from the bone marrow and a decreased number leaving the circulation. Lymphopenia is due to a redistribution of cells out of the circulation following Rx-1 and probably due to redistribution and lympholysis following Rx-2. Eosinopenia is due to a decrease in cells entering from the bone marrow and a redistribution of peripheral cells.

The observed decrease in RBC count, hemoglobin concentration and hematocrit may be the result of redistribution of the red cell mass within the circulation and/or secondary effects from sodium retention due to the mineralocorticoid properties of prednisone and prednisolone. The slight increase in mean corpuscular volume may be due to older, smaller cells being redistributed or removed with younger cells remaining, or more young cells in the circulation. There were statistically significant alterations in these blood cell parameters, however, the values were all within the normal ranges. These changes would be most significant in evaluating clinical cases of anemia where glucocorticoids have been administered.

Serum Chemistries

The increased serum alkaline phosphatase (SAP) and serum alanine aminotransferase (ALT) (formerly known as serum glutamic pyruvic transaminase; SGPT) are consistent with previous reports. The increase in SAP is due to the induction of the steroid-inducible alkaline phosphatase isoenzyme. There may also be an increase in hepatic alkaline phosphatase isoenzyme secondary to glucocorticoid-induced hepatic injury. The elevated SALT is due partially to enzyme induction and probably partially due to altered membrane permeability secondary to glucocorticoid administration. The hepatic enzymes were not significantly elevated six or 12 hours following glucocorticoid administration, which is useful information for the clinical setting. This would help in evaluating chemistry results following glucocorticoid administration and the recommendation could be made to collect blood within 12 hours of therapy to avoid drug induced alterations in the hepatic enzymes.

The decrease in creatinine is probably due to an increase in renal clearance secondary to the effects of glucocorticoids on urine production.

The decreased inorganic phosphorus at 12 hours following Rx-1 was probably due to a translocation from extracellular to intracellular loci and/or an increase in phosphaturia. Infusion of glucose and administration of insulin result in translocation of inorganic phosphorus into cells. Following the shock dose of glucocorticoids there was a mild increase in glucose at six hours that was not statistically significant; however, a mild increase in glucose may have stimulated insulin production and the combination of these two may have resulted in a lowering of the serum

inorganic phosphorus. The mild decrease in serum calcium may have stimulated parathyroid hormone production, with a result of increased phosphorus excretion in the urine. The serum phosphorus values were within normal limits for the laboratory even though statistically significant changes were observed.

Glucocorticoids affect serum calcium by decreasing intestinal calcium absorption, enhancing renal excretion of calcium and decreasing mineralization of bone. Of these mechanisms, the one most likely to have resulted in the decreased calcium following the shock dose of glucocorticoids is the enhanced renal excretion. The other mechanisms would be of more importance in long-term therapy.

The increased sodium and chloride following Rx-1 was most likely due to increased sodium (and chloride) retention due to the mineralocorticoid effect of the prednisolone.

Glucocorticoids increase the release of potassium from muscle and other cellular stores which in turn result in increased renal potassium excretion Insulin increases potassium movement from the extracellular to the intracellular spaces. The mineralocorticoid effect of the prednisone may have resulted in increased potassium excretion. These mechanisms may have played a role in the observed potassium decrease 12 hours following Rx-1.

The decreased albumin 12 hours following the shock dose may have been due to a dilutional effect secondary to fluid retention from the mineralocorticoid activity of prednisolone. Following seven days of prednisone therapy, the albumin and total protein were elevated most likely due to increased hepatic synthesis of globulins and albumin.

CHAPTER VI

Conclusions

1. Glucocorticoids alter alveolar and peritoneal macrophage and blood monocyte function (Fc-receptor activity and phagocytosis of heat killed Candida albicans).
   a. A single shock dose of prednisolone sodium succinate and dexamethasone sodium phosphate altered blood monocyte and alveolar macrophage function.
   b. An immunosuppressive dose of prednisone administered for seven days altered blood monocyte and peritoneal macrophage function.
2. Alveolar and peritoneal macrophages and blood monocytes differ in their response to glucocorticoids.
   a. Twelve hours following the shock dose of glucocorticoids, the percentage of phagocytosis-positive blood monocytes decreased and the alveolar macrophages showed increases in the percentage of phagocytosis-positive cells and Fc-receptor activity.
   b. Following the immunosuppressive treatment, the Fc-receptor activity of the blood monocytes increased and the percentage of phagocytosis-positive peritoneal macrophages increased.
3. The single shock dose of glucocorticoids and the seven day treatment of an immunosuppressive dose of glucocorticoid differed in their effect on alveolar and peritoneal macrophages and blood monocytes.
   a. The phagocytic activity of the blood monocytes was altered following the shock dose, whereas the Fc-receptor activity was altered following the immunosuppressive treatment.

b. The Fc-receptor and phagocytic activity of the alveolar macrophages was increased following the shock dose, but were unaltered following the immunosuppressive doses of glucocorticoids.

c. The peritoneal macrophages were not altered in their phagocytic or Fc-receptor activity following the shock treatment and only the phagocytic ability was altered following the immunosuppressive dose.

4. The observed changes in Fc-receptor activity and phagocytosing of heat killed Candida albicans occurred independently of each other.

CHAPTER VII

Bibliography

1. Werb, Z., Goldstein, I.M. Phagocytic Cells: Chemotaxis and Effector Functions of Macrophages and Granulocytes. In: Stites, D.P., Stobo, J.D., Fudenberg, H.H., Wells, J.V., Ed. Basic and Clinical Immunology, 109-123, Lange Medical Pub., Los Altos, CA, 1982.

2. Yoshida, T., Ward, P.A. Macrophage Participation in the Physiopathology of the Immune Response and Inflammation. In: Atrura, B.M., Saba, T.M., ed. Pathophysiology of the Reticuloendothelial System, 31-46, Raven Press, New York, 1981.

3. Tizard, I.R. "Trapping and Processing of Foreign Material", In: An Introduction to Veterinary Immunology, 14-28, W.B. Saunders Co., Philadelphia, 1982.

4. Golde, D.W., Hocking, W.G. Monocyte and Macrophage Development. In: Gallin, J.I., Fauci, A.S., ed. Advances in Host Defense Mechanisms, Vol. I: 13-30, Raven Press, New York, 1982.

5. Pelus, L.M., Broxmeyer, H.E., Kurland, J.I., et.al. Regulation of Macrophage and Granulocyte Proliferation. J. of Exp. Med., 150: 277-291, 1979.

6. Cohen, A.B., Cline, M.J. The Human Alveolar Macrophage: Isolation, Cultivation. in vitro, and Studies of Morphologic and Fuctional Characteristics. J. Clin. Investig., 50: 1390-1398, 1971.

7. Ho, C.K., Babiuk, L.A. Isolation of Various Canine Leukocytes and Their Characterization by Surface Marker Analysis. Immunology, 35: 733-740, 1978.

8. Osbaldiston, G.W., Sullivan, R.J., Fox, A. Cytochemical Demonstration of Esterases in Peripheral Blood Leukocytes. Am. J. Vet Res., 39(4): 683-685, 1978.

9. Mayernik, D.G., Haq, A., Rinehart, J.J. Interleukin 1 Secretion by Human Monocytes and Macrophages. J. Leukocyte Biol., 36: 551-557, 1984.

10. Tizard, I.R. "The Cellular Basis of the Immune Response", In: An Introduction to Veterinary Immunology, 74-92, W.B. Saunders Company, Philadelphia, 1982.

11. Murray, H.W., Cohn, Z.A. Macrophage Oxygen-Dependent Antimicrobial Activity, J. Exp. Med., 152: 1596-1609, 1980.

12. Rhodes, J. Macrophage Heterogeneity in Receptor Activity: The Activation of Macrophage Fc Receptor Function in vivo and in vitro. J. of Immunology, 114: 976-981, 1975.

13. Griffin, F.M., Jr. Mononuclear Cell Phagocytic Mechanisms and Host Defense. In: Gallin, J.I., Fauci, A.S., ed. Advances in Host Defense Mechansisms, Vol. I: 31-55, Raven Press, New York, 1982.

14. Wright, S.D., Griffin, F.M., Jr. Activation of Phagocytic Cells' C3 Receptors for Phagocytosis. J. Leukocyte Biol., 38: 327-339, 1985.

15. Shaw, S.E., Anderson, N.V. Isolation and Functional Analysis of Normal Canine Blood Monocytes and Resident Alveolar Macrophages. Am. J. Vet. Res., 45(1): 87-90, 1984.

16. Erb, P., Stern, A.C., Cecka, M.J. Ia Determinants on Macrophages: Significance and Role in the Immune Response. In: Normann, S.J., Sorkin, E., ed. Macrophages and Natural Killer Cells: Regulation and Function, Vol. 155: 579-590, Advances in Experimental Medicine and Biology, Plenium Press, New York, 1982.

17. Unanue, E.R. Symbiotic Relationships Between Macrophages and Lymphocytes. In: Normann, S.J., Sorkin, E., ed. Macrophages and Natural Killer Cells: Regulation and Function, Vol. 155: 49-63, Advances in Experimental Medicine and Biology, Plenium Press, New York, 1982.

18. Miller, G.A., Morahan, P.S. Use of Nonspecific Esterase Stain. In: Adams, D.O., Koren, E., ed. Methods for Studying Mononuclear Phagocytes, Chap. 40: 367-374, Academic Press, Inc., 1981.

19. Walker, W.S. Macrophage Functional Heterogeneity. In: Normann, S.J., Sorkin, E. ed. Macrophages and Natural Killer Cells: Regulation and Function, Vol. 155: 435-452, Advances in Experimental Medicine and Biology, Plenium Press, New York, 1982.

20. Raffael, A., Valet, G. Distinction of Macrophage Subpopulations: Measurement of Functional Cell Parameters by Flow Cytometry. In: Normann, S.J., Sorkin, E. ed. Macrophages and Natural Killer Cells: Regulation and Function, Vol. 155: 453-459. Advances in Experimental Medicine and Biology, Plenium Press, New York, 1982.

21. Haskill, S., Becker, S., Halme, J. Flow Cytometric Characterization of Macrophages: Fluorescent Substrates as Markers of Activation and Differentiation. In: Normann, S.J., Sorkin, E. ed. Macrophages and Natural Killer Cells: Regulation and Function, Vol. 155: 461-468, Advances in Experimental Medicine and Biology, Plenium Press, New York, 1982.

22. Wing, E.J., Gardner, I.D., Ryning, F.W., et. al. Dissociation of Effector Functions in Populations of Activated Macrophages. Nature, 268: 642-644, 1977.

23. Van Furth, R., Blusse van Oud Alblas, A. The Current View on the Origin of Pulmonary Macrophages. Path. Res. Pract., 175: 38-49, 1982.

24. Sawyer, R.T. Proliferation of Resident Pulmonary Alveolar Macrophages in Splenectomized-89Sr Monocytopenic Mice. Abstract 121, J. Leuk. Biol., 38(1): 103, 1985.

25. Widen, R.H., Paradise, L.F. Enhancement of Hamster Alveolar Macrophage Phagocytic Activity by Lymphokines. J. of Leukocyte Biol., 37: 349-358, 1985.

26. Van Furth, R., Cohn, Z.A. The Origin and Kinetics of Mononuclear Phagocytes. J. Exp. Med., 128: 415-433, 1968.

27. Van Furth, R., Diesselhoff-Den Dulk, M.C., Mattie, H. Quantitative Study on the Production and Kinetics of Mononuclear Phagocytes During an Acute Inflammatory Reaction. J. of Exp. Med. 138: 1314-1330, 1973.

28. Webb, D.R., Jr., Winkelstein, A. Immunosuppression, Immunopotentiation and Anti-inflammatory Drugs. In: Stites, D.P., Stobo, J.D., Fudenberg, H.H., Wells, J.V., ed. Basic and Clinical Immunology, 277-292, Lange Medical Publ, Los Altos, CA, 1982.

29. Ansfield, M.J., Kaltreider, H.B., Caldwell, J.L., et. al. Hyporesponsiveness of Canine Bronchoalveolar Lymphocytes to Mitogens: Inhibition of Lymphocyte Proliferation by Alveolar Macrophages. J. of Immunology, 122: 542-548, 1979.

30. Ferguson, D.C. Rational Glucocorticoid Therapy in Small Animals. Mod. Vet. Practice, 175-179, 1985.

31. Koller, L.D. Chemical-induced immunomodulation. J.A.V.M.A., 181: 1102-1106, 1982.

32. Parrillo, J.E., Fauci, A.S. Mechanisms of Glucocorticoid Action on Immune Processes. Ann. Rev. Pharmacol. Toxicol., 19: 179-201, 1979.

33. Schalm, O.W., Jain, N.C., Carroll, E.J. "The Leukocytes: Structure, Kinetics, Function and Clinical Interpretation", in: Veterinary Hematology, 471-538, Lea and Febiger, Philadelphia, 1975.

34. Werb, Z., Foley, R., Munck, A. Interaction of Glucocorticoids with Macrophages. J. Exp. Med., 1684-1694, 1978.

35. Werb, Z. Biochemical Actions of Glucocorticoids on Macrophages in Culture. J. Exp. Med., 1695-1709, 1978.

36. Rinehart, J.J., Wuest, D., Ackerman, G.A. Corticosteroid Alteration of Human Monocyte to Macrophage Differentiation. J. of Immunology, 129: 1436-1440, 1982.

37. Duncan, M.R., Sadlik, J.R., Hadden, J.W. Glucocorticoid Modulation of Lymphokine-Induced Macrophage Proliferation. Cellular Immunology, 67: 23-36, 1982.

38. Staruch, M.J., Wood, D.D. Reduction of Serum Interleukin-1-Like Activity After Treatment with Dexamethasone. J. of Leukocyte Biol., 37: 198-207, 1985.

39. Schultz, R.M., Chirigos, M.A., Stoychkov, J.N., et. al. Factors Affecting Macrophage Cytotoxic Activity with Particular Emphasis on Corticosteroids and Acute Stress. J. of Res., 36(1): 83-91, 1979.

40. Homo-Delarche, F., Duval, D., Papiernik, M. Prostaglandin Production by Phagocytic Cells of the Mouse Thymic Reticulum in Culture and its Modulation by Indomethacin and Corticosteroids. J. Immunol., 135(1): 506-512, 1985.

41. Schreiber, A.D., Parsons, J., McDermott, P., et. al. Effects of Corticosteroids on the Human Monocyte IgG and Complement Receptors. J. Clin. Investig., 56: 1189-1197, 1975.

42. Fries, L.F., Brickman, C.M., Frank, M.M. Monocyte Receptors for the Fc Portion of IgG Increase in Number in Autoimmune Hemolytic Anemia and Other Hemolytic States and Are Decreased by Glucocorticoid Therapy. J. of Immunology, 131: 1240-1245, 1983.

43. Warren, M.K., Vogel, S.N. Opposing Effects of Glucocorticoids on Interferon-Induced Murine Macrophage Fc Receptor and Ia Antigen Expression. J. Immunol., 134(4): 2462-2469, 1985.

44. Aberer, W., Stingl, L., Pogantsch, S. et. al. Effect of Glucocorticosteroids on Epidermal Cell-Induced Immune Responses. J. Immunol., 133(2): 792-797, 1984.

45. Snyder, D.S., Unanue, E.R. Corticosteroids Inhibit Murine Macrophage Ia Expression and Interleukin 1 Production. J. of Immunology, 129: 1803-1805, 1982.

46. Di Rosa, M., Persico, P. Mechanism of Inhibition of Prostaglandin Biosynthesis by Hydrocortisone in Rat Leukocytes. Br. J. Pharmac., 66: 161-163, 1979.

47. Masur, H., Murray, H.W., Jones, T.C. Effect of Hydrocortisone on Macrophage Response to Lymphokine. Infect and Immunity, 709-714, 1982.

48. Blackwood, L.L., Pennington, J.E. Dose Dependent Effect of Glucocorticosteroids on Pulmonary Defenses in a Steroid-Resistant Host. Am. Rev. Respir. Dis., 126: 1045-1049, 1982.

49. Pennington, J.E., Harris, E.A. Influence of Immunosuppression on Alveolar Macrophage Chemotactic Activities in Guinea Pigs. Am. Rev. Respir. Dis., 123: 299-304, 1981.

50. Van Oud Alblas, A., van der Linden-Schrever, R., Mattie, H., et. al. The Effect of Glucocorticoids on the Kinetics of Pulmonary Macrophages. J. of Res., 30: 1-14, 1981.

51. Lin, H., Kuhn, C., Chen, D. Effects of Hydrocortisone Acetate on Pulmonary Alveolar Macrophage Colony-forming Cells. Am. Rev. Respir. Dis., 125: 712-715, 1982.

52. Boyum, A. Isolation of Mononuclear Cells and Granulocytes from Human Blood. Scand. J. Clin. Lab. Invest. (Suppl.)., 97: 77-89, 1968.

EFFECTS OF GLUCOCORTICOIDS ON CANINE MONONUCLEAR PHAGOCYTES

by

Linda Joan DeBowes

D.V.M., Washington State University, 1981

AN ABSTRACT OF A MASTER'S THESIS

Submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Surgery and Medicine

Kansas State University

Manhattan, Kansas

1985

ABSTRACT

The objectives of this study were to collect and partially characterize blood monocytes, bronchoalveolar macrophages and peritoneal macrophages in the canine prior to glucocorticoid administration, again following a shock dose of prednisolone sodium succinate/dexamethasone sodium phosphate, and again following seven days of an immunosuppressive dose of prednisone. Twelve clinically healthy, mixed breed, adult dogs were used in the study. Functional assays included 1) evaluation of the percentage of cells that phagocytosed heat-killed Candida albicans and 2) the percentage of cells that had Fc-receptor activity for IgG. Morphologic evaluation included differential counts and a non-specific esterase stain.

Statistically significant results for each treatment included[a]:

1) Shock Dose

   a. Blood Monocytes

      - percent phagocytosis positive decreased at 12 hours
      - percent esterase positive increased at 6 and 12 hours
      - differential count: increased percentage at 6 and 12 hours

   b. Alveolar Macrophages

      - percent phagocytosis positive increased at 12 hours
      - percent Fc-receptor activity increased at 12 hours
      - percent esterase positive increased at 12 hours

---

[a]Shock dose: Intravenous injection of 20 mg/kg prednisolone sodium succinate and 6 mg/kg dexamethasone sodium phosphate.
Prednisone (7 days): 2 mg/kg orally every 12 hours
$P \leq 0.05$

c. Peritoneal Macrophages

- percent esterase positive increased at 12 hours
- differential count: decreased percentage at 12 hours

2. Prednisone (seven days)

a. Blood Monocytes

- percent Fc-receptor activity increased
- percent esterase positive increased
- differential count: increased percentage

b. Alveolar Macrophages

- percent esterase positive increased
- differential count: increased percentage

c. Peritoneal Macrophages

- percent phagocytosis positive increased
- percent esterase positive increased

The shock dose of glucocorticoids enhanced the phagocytic and Fc-receptor activity of alveolar macrophages and decreased blood monocyte phagocytic activity, while having no effect on the peritoneal macrophages in their phagocytic or Fc-receptor activities. The treatment of prednisone (7 days) enhanced blood monocyte Fc-receptor activity and peritoneal macrophage phagocytic activity while not altering either Fc-receptor or phagocytic activity of alveolar macrophages.

Alveolar and peritoneal macrophages and blood monocytes differ in their response to glucocorticoids. Alveolar macrophages have enhanced Fc-receptor and phagocytic activity following a shock dose of glucocorticoids, while peritoneal macrophage phagocytic activity is enhanced following seven days of prednisone. Blood monocyte response differed

between treatments, the shock dose decreased phagocytic activity, while the prednisone (7 days) increased Fc-receptor activity. The differential counts varied between treatments also. Blood monocytes increased in percentage with both treatments while the percentage of alveolar macrophages only increased following the prednisone (7 days) and the percentage of peritoneal macrophages decreased with the shock dose.